Adjuvant Colorectal Cancer Treatment Strategies

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The following table categorizes suggested adjuvant measures for colorectal cancer control with respect to their likely utility as retardants of cancer growth and spread, and as adjuvants to chemotherapy. Suggested dose schedules are provided for many of these agents; these are provisional and may change in light of future research. This is intended as a menu of options, from which patients can select to create a personal cancer control program, preferably with the guidance of a sympathetic physician or health scientist; no one could be expected to use all of these measures, and it is unlikely that all of them will ultimately prove to confer important benefit. Moreover, every cancer is unique, and measures which help some patients with colorectal cancer may not help others. Abstracts of research pertinent to each of these suggested measures are appended below.

“Low-insulin lifestyle” refers to lifestyle measures which promote good insulin sensitivity and keep insulin levels relatively low throughout the day; exercise training, and diets low in both saturated fat and glycemic index can be helpful in this regard. A wholly plant-based (vegan) diet can provide the ancillary benefit of lowering circulating levels of the cancer growth factor IGF-I. Metformin and berberine have very similar activities (activation of the enzyme AMPK), so one or the other can be used; metformin is a prescription drug, whereas berberine is a nutraceutical. I.v. ascorbate can be used alone or as an adjuvant to chemotherapy; so far, little published literature pertains to its use in colorectal cancer. Cimetidine has been found to improve survival outcomes if used for several weeks immediately following surgery, as it seems to reduce risk for post-surgical metastasis and immunosuppression. High-dose biotin and tadalafil, although they have not been directly tested against colorectal cancer, are suggested because they have the potential to raise tumor levels of cGMP, a cellular signaling molecule which often aids control of colorectal cancer.

Note that many of these agents are prescription drugs, and hence require the active cooperation and approval of your doctor. Doctor approval of low-dose aspirin is also wise, and use of spirulina at the same time as chemotherapy or i.v. ascorbate is not recommended. GcMAF (macrophage activating factor) must be administered by subcutaneous injection; although it does not have drug approval, it can be obtained by mail-order from Europe or Japan. Importantly, these measures should be considered as adjuvants to, not substitutes for, recommended surgery, chemotherapy regimens, or other therapies employed by your doctor.

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<th>Growth Control</th>
<th>Chemo Adjuvant</th>
<th>Prescription Drug</th>
<th>Suggested Dose</th>
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<td>Low-Insulin Lifestyle</td>
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<td>Metformin or Berberine</td>
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<td>500 mg 3 times daily</td>
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<td>Fish Omega-3</td>
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<td>Green Tea Catechins</td>
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<td>Grape Seed Extract</td>
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<td>Silibinin (as Siliphos)</td>
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<td>Met Cyclophosphamide</td>
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250 mg twice daily
200 mg twice daily
100 mg daily
15 mg twice daily
10-20 mg at bedtime
81 mg daily
5,000-10,000 IU daily
15-30 g daily
12-24 mg daily
10 mg daily
800 mg daily
PectaSol, 5 g, 3 times daily
1500 mg 2-3 times daily
1 g or more daily
300 mg twice daily
125 mg twice daily
3-5 mg before bedtime
100 ng i.m. once weekly
5-10 mg daily
600 mg 4 times daily
50 mg daily

Low-Insulin Lifestyle – Exercise, Diet Low in Saturated Fat and Glycemic Index
Abstract: BACKGROUND: Few studies have investigated the impact of body mass index (BMI) and physical activity (PA) on mortality among colorectal cancer (CRC) patients and the results are inconsistent. We aimed to examine the impact of these lifestyle factors on all-cause and disease-specific mortality. METHODS: Population-based longitudinal study followed 1,825 patients diagnosed with stages I to III primary CRC during 2003 to 2004 in Queensland, Australia for 5 years. Sociodemographics and clinical characteristics were obtained via questionnaires and medical records. RESULTS: Participants with some level of PA following diagnosis had 25% to 28% lower risk of all-cause mortality within 5 years of diagnosis than sedentary participants [insufficiently active: HR = 0.72, 95% CI = 0.57-0.91; sufficiently active: HR = 0.75 (0.60-0.94)]; however, the differential for CRC-specific mortality was not significant. Increases in PA from five to 12 months postdiagnosis was associated with reduced CRC-specific mortality by 32% to 36% (increase </= 2 hour per week: HR = 0.68 (0.48-0.97); increase > 2 hour per week: HR = 0.64 (0.44-0.93) and 31% for all-cause mortality (increase >2 hour per week: HR = 0.69 (0.50-0.94). Compared with participants with healthy BMI, significant higher mortality risk was observed in underweight patients (all-cause: HR = 2.29 (1.47-3.59); CRC: HR = 1.74 (1.00-3.04), although lower risk in overweight (all-cause: HR = 0.75 (0.61-0.94); CRC: HR = 0.75 (0.59-0.97) and no difference in obese. Excessive weight loss was associated with increased mortality risk by three-fold but no difference in those who gained weight. CONCLUSIONS: Protective effects of being physically active and increasing that activity underlines the importance of interventions to increase activity levels among people being diagnosed with CRC. IMPACT: Increased mortality risks associated with being underweight or having weight loss over time is an important indicator for which clinicians, patients, and support personnel can monitor


Abstract: CONTEXT: Alterations of the WNT signaling pathway and cadherin-associated protein beta 1 (CTNNB1 or beta-catenin) have been implicated in colorectal carcinogenesis and metabolic diseases. OBJECTIVE: To test the hypothesis that CTNNB1 activation in colorectal cancer modifies prognostic associations of body mass index (BMI) and level of postdiagnosis physical activity. DESIGN, SETTING, AND PATIENTS: Two US prospective cohort studies (Nurses' Health Study and the Health Professionals Follow-up Study) were used to evaluate CTNNB1 localization by immunohistochemistry in 955 patients with stage I, II, III, or IV colon and rectal cancer from 1980 through 2004. A Cox proportional hazards model was used to compute the hazard ratio (HR) for mortality, adjusting for clinical and tumor features, including microsatellite instability, CpG island methylator phenotype, level of long interspersed nucleotide element 1 methylation, mutations in KRAS, BRAF, or PIK3CA, and tumor protein p53. MAIN OUTCOME MEASURES: Colorectal cancer-specific mortality and overall mortality through June 30, 2009. RESULTS: In obese patients (BMI >/=30), positive status for nuclear CTNNB1 was associated with significantly better colorectal cancer-specific survival (adjusted HR, 0.24 [95% confidence interval {CI}, 0.12-0.49], P <.001 for interaction; 5-year survival: 0.85 for patients with positive nuclear CTNNB1 status vs 0.78 for those with negative status) and overall survival (adjusted HR, 0.56 [95% CI, 0.35-0.90], P = .03 for interaction; 5-year survival: 0.77 for patients with positive nuclear CTNNB1 status vs 0.74 for those with negative status), while CTNNB1 status was not associated with prognosis among nonobese patients (BMI <30). Among patients with negative status for nuclear CTNNB1 and cancer in stages I, II, or III, postdiagnosis physical activity was associated with better colorectal cancer-specific survival (adjusted HR, 0.33 [95% CI, 0.13-0.81], P = .05 for interaction; 5-year survival: 0.97 for >/=18 vs 0.89 for <18 metabolic equivalent task hours/week), while postdiagnosis physical activity was not
associated with colorectal cancer-specific survival among patients with positive status for nuclear CTNNB1 (adjusted HR, 1.07 [95% CI, 0.50-2.30]). CONCLUSIONS: Among obese patients only, activation of CTNNB1 was associated with better colorectal cancer-specific survival and overall survival. Postdiagnosis physical activity was associated with better colorectal cancer-specific survival only among patients with negative status for nuclear CTNNB1. These molecular pathological epidemiology findings suggest that the effects of alterations in the WNT-CTNNB1 pathway on outcome are modified by BMI and physical activity.


Abstract: BACKGROUND: Colorectal carcinoma is the most common type of tumor in Western countries. The risk of developing colorectal carcinoma depends both on genetic factors (familial predisposition) and on lifestyle-related factors such as body-mass index, level of physical activity, and nutritional behavior. Regular physical activity is important in primary prevention, and there is also evidence that the prognosis after treatment of a colorectal carcinoma can be improved by exercise. METHODS: The PubMed database was searched for relevant articles that appeared in the last 10 years, and selected articles were evaluated. RESULTS: Cross-sectional studies have shown that regular physical activity (ca. 7 hours of brisk walking per week) lowers the risk of colon carcinoma by 40%. Physical activity also improves the outcome of patients already diagnosed with colorectal carcinoma: for example, patients with advanced disease (UICC stage II or III) have been found to survive significantly longer if they perform 4 hours of brisk walking per week, or the equivalent degree of physical exercise. CONCLUSIONS: Cross-sectional studies show that physically active persons are less likely to develop colorectal carcinoma than physically inactive persons, and that they have better outcomes in the event that they do develop the disease. The positive findings with respect to secondary prevention still need to be confirmed in interventional trials, but in primary prevention, at least, physical activity should be actively promoted, along with other beneficial lifestyle habits and screening measures.


Abstract: Many studies have demonstrated the effects of exercise on both primary and secondary prevention of colon cancer. Exercise appears to have a dose-response reduction in the rate of colon cancer. The mechanism by which exercise provides this benefit is not known, but increase in insulin-like growth factor-binding protein and reduction of prostaglandins appear to be the likely cause. Once a person develops colon cancer the benefits of exercise appear to continue both by increasing quality of life and reducing cancer-specific and overall mortality.


Abstract: PURPOSE: Physically active individuals have a lower risk of developing colorectal cancer but the influence of exercise on cancer survival is unknown. PATIENTS AND METHODS: By a prospective, observational study of 573 women with stage I to III colorectal cancer, we studied colorectal cancer-specific and overall mortality according to predefined physical activity categories before and after diagnosis and by change in activity after diagnosis. To minimize bias by occult recurrences, we excluded women who died within 6 months of their postdiagnosis physical activity assessment. RESULTS: Increasing levels of exercise after diagnosis of nonmetastatic colorectal cancer reduced cancer-specific mortality (P for trend = .008) and overall mortality (P for trend = .003). Compared with women who engaged in less than 3 metabolic equivalent task [MET] -hours per week of physical activity, those engaging in at least 18 MET-hours per week had an adjusted hazard ratio for colorectal cancer-specific
mortality of 0.39 (95% CI, 0.18 to 0.82) and an adjusted hazard ratio for overall mortality of 0.43 (95% CI, 0.25 to 0.74). These results remained unchanged even after excluding women who died within 12 and 24 months of activity assessment. Prediagnosis physical activity was not predictive of mortality. Women who increased their activity (when comparing prediagnosis to postdiagnosis values) had a hazard ratio of 0.48 (95% CI, 0.24 to 0.97) for colorectal cancer deaths and a hazard ratio of 0.51 (95% CI, 0.30 to 0.85) for any-cause death, compared with those with no change in activity. CONCLUSION: Recreational physical activity after the diagnosis of stages I to III colorectal cancer may reduce the risk of colorectal cancer-specific and overall mortality.


Abstract: PURPOSE: Regular physical activity reduces the risk of developing colon cancer, however, its influence on patients with established disease is unknown. PATIENTS AND METHODS: We conducted a prospective observational study of 832 patients with stage III colon cancer enrolled in a randomized adjuvant chemotherapy trial. Patients reported on various recreational physical activities approximately 6 months after completion of therapy and were observed for recurrence or death. To minimize bias by occult recurrence, we excluded patients who experienced recurrence or died within 90 days of their physical activity assessment. RESULTS: Compared with patients engaged in less than three metabolic equivalent task (MET) -hours per week of physical activity, the adjusted hazard ratio for disease-free survival was 0.51 (95% CI, 0.26 to 0.97) for 18 to 26.9 MET-hours per week and 0.55 (95% CI, 0.33 to 0.91) for 27 or more MET-hours per week. The adjusted P for trend was .01. Postdiagnosis activity was associated with similar improvements in recurrence-free survival (P for trend = .03) and overall survival (P for trend = .01). The benefit associated with physical activity was not significantly modified by sex, body mass index, number of positive lymph nodes, age, baseline performance status, or chemotherapy received. Moreover, the benefit remained unchanged even after excluding participants who developed cancer recurrence or died within 6 months of activity assessment. CONCLUSION: Beyond surgical resection and postoperative adjuvant chemotherapy for stage III colon cancer, for patients who survive and are recurrence free approximately 6 months after adjuvant chemotherapy, physical activity appears to reduce the risk of cancer recurrence and mortality.


Ref ID: 39111

Abstract: BACKGROUND: Physical inactivity and obesity increase the risk of colorectal cancer but little is known about whether they influence prognosis after diagnosis. METHODS: Incident cases of colorectal cancer were identified among participants of the Melbourne Collaborative Cohort Study, a prospective cohort study of 41 528 Australians recruited from 1990 to 1994. Participants diagnosed with their first colorectal cancer between recruitment and 1 August 2002 were eligible. At the time of study entry, body measurements were taken and participants were interviewed about their physical activity. Information on tumour site and stage, treatments given, recurrences, and deaths were obtained from systematic review of the medical records. RESULTS: A total of 526 cases of colorectal cancer were identified. Median follow up among survivors was 5.5 years, and 208 deaths had occurred, including 181 from colorectal cancer. After adjusting for age, sex, and tumour stage, exercisers had an improved disease specific survival (hazard ratio 0.73 (95% confidence interval (CI) 0.54-1.00)). The benefit of exercise was largely confined to stage II-III tumours (hazard ratio 0.49 (95% CI 0.30-0.79)). Increasing per cent body fat resulted in an increase in disease specific deaths (hazard ratio 1.33 per 10 kg (95% CI 1.04-1.71)). Similarly, increasing
waist circumference reduced disease specific survival (hazard ratio 1.20 per 10 cm (95% CI 1.05-1.37)).

CONCLUSIONS: Increased central adiposity and a lack of regular physical activity prior to the diagnosis of colorectal cancer is associated with poorer overall and disease specific survival


Abstract: BACKGROUND: The KRAS mutation is not responsible for all cases of resistance to anti-epidermal growth factor receptors (EGFRs) in metastatic colorectal cancer (mCRC), and new predictive and prognostic factors are actively being sought. PATIENTS AND METHODS: We retrospectively evaluated the efficacy of a cetuximab-containing treatment in 73 patients with mCRC according to KRAS and BRAF mutational status as well as PTEN, c-MET, and insulin-like growth factor receptor (IGF1R) expression. RESULTS: Overall response rate (ORR), median progression-free survival (mPFS), and median overall survival (mOS) were significantly lower in patients with KRAS mutation than in patients with KRAS wild-type; among the population with KRAS wild-type, only 2 patients with BRAF mutations were found and neither of them achieved a response. No significant association was found between PTEN and clinical outcome. Compared with low/normal expression, c-MET overexpression significantly correlated with shorter mPFS and mOS: 3 vs. 5 months (P = .018) and 11 vs. 10 months (P = .037), respectively. In patients with high IGF1R expression, mOS was significantly longer than in those with low/normal expression (14 vs. 8 months; P = .015). CONCLUSION: KRAS mutation significantly correlates with a worse outcome in patients treated with cetuximab, whereas no definitive inference can be drawn about the role of BRAF mutation and PTEN loss of expression. Instead, c-MET overexpression might represent a negative prognostic factor in mCRC and may have a role in resistance to anti-EGFR therapy. Interestingly, IGF1R overexpression seems a favorable prognostic factor in mCRC


Abstract: Obesity has been associated with both the carcinogenesis and poor prognosis of colon cancer, one of the leading causes of cancer-related death. Increased blood levels of insulin in obese subjects have been demonstrated to play a key role in carcinogenesis. It is also possible that insulin affects treatment efficacy, leading to poor prognosis. In this study, we demonstrated that insulin can increase HT29 colon cancer cell line resistance to cycloheximide and 5-fluorouracil induced cytotoxicity. This effect can be inhibited by the PI3K/Akt inhibitor Ly294002, indicating the important role of this pathway in the insulin-induced inefficacy of chemotherapy. The insulin-induced resistance to cycloheximide and 5-fluorouracil can be used in drug screening to overcome the inefficacy of chemotherapy in obesity-associated colon cancer


Abstract: PURPOSE: To evaluate the safety and efficacy of IMC-A12, a human monoclonal antibody (mAb) that blocks insulin-like growth factor receptor-1 (IGF-1R), as monotherapy or in combination with cetuximab in patients with metastatic colorectal cancer. METHODS: A randomized, phase II study was performed in which patients in arm A received IMC-A12 10 mg/kg intravenously (IV) every 2 weeks, while patients in arm B received this same dose of IMC-A12 plus cetuximab 500 mg/m2 IV every 2 weeks. Subsequently, arm C (same combination treatment as arm B) was added to include patients who had disease control on a prior anti-EGFR mAb and wild-type KRAS tumors. Archived pretreatment tumor tissue was obtained when possible for KRAS, PIK3CA, and BRAF genotyping, and immunohistochemistry was obtained for pAKT
as well as IGF-1R. RESULTS: Overall, 64 patients were treated (median age, 61 years; range, 40 to 84 years): 23 patients in arm A, 21 in arm B, and 20 in arm C. No antitumor activity was seen in the 23 patients treated with IMC-A12 monotherapy. Of the 21 patients randomly assigned to IMC-A12 plus cetuximab, one patient (with KRAS wild type) achieved a partial response, with disease control lasting 6.5 months. Arm C (all patients with KRAS wild type), however, showed no additional antitumor activity. Serious adverse events thought possibly related to IMC-A12 included a grade 2 infusion-related reaction (2%; one of 64 patients), thrombocytopenia (2%; one of 64 patients), grade 3 hyperglycemia (2%; one of 64 patients), and grade 1 pyrexia (2%, one of 64 patients). CONCLUSION: IMC-A12 alone or in combination with cetuximab was insufficient to warrant additional study in patients with colorectal cancer refractory to EGFR inhibitors.


Abstract: Seventy to 40% of K-RAS wild type colorectal tumors does not seem to benefit from treatment with antiepidermal growth factor receptor (anti-EGFR) monoclonal antibodies. Recent data suggested that in presence of IGF-1 system, altered activation colorectal cancer cells may escape anti-EGFR mediated cell death. The interaction between IGF-1 expression and K-RAS mutational analysis was tested to verify the ability of IGF-1 to identify a subgroup of patients more likely to benefit from EGFR-targeted antibodies treatment. IGF-1 expression and K-RAS mutational status was assessed in advanced colorectal cancer patients receiving irinotecan/cetuximab. One hundred twelve patients were analyzed. IGF-1 was negative in 30 patients (27%) and overexpressed in the remaining 82 cases (73%). In IGF-1 negative and IGF-1 positive tumors, we observed progressive disease in 9 (30%) and 55 (67%) patients, respectively (p = 0.001). Median progression-free survival was 7.5 mo in patients showing IGF-1 negative tumors and 3 mo for IGF-1 expressing tumors (p = 0.002). Among K-RAS wild type patients, IGF-1 negative and positive tumors showed a partial response to cetuximab-irinotecan in 13 (65%) and 11 (22%) cases, respectively (p = 0.002). Median progression-free survival in IGF-1 negative tumors was 10 mo and 3.2 mo in IGF-1 positive colorectal cancers (p = 0.02). IGF-1 proved to be a possible predictive factor for resistance to anti-EGFR monoclonal antibodies in K-RAS wild type colorectal cancer. Combined IGF-1 and K-RAS analysis may represent an effective strategy for a better selection of responding colorectal cancer patients.


Abstract: BACKGROUND: Colorectal cancer is the second-leading cause of cancer death in the United States among men and women combined. Refinements in screening, staging, and treatment strategies have improved survival from this disease, with over 65% of patients diagnosed with colorectal cancer surviving over 5 years after diagnosis. In the prognosis of colorectal cancer, clinicopathological factors are important. However, modifiable prognostic factors are emerging as significant contributors to cancer outcomes, including obesity and obesity-related inflammation and metabolic conditions. METHODS: This report reviews the literature on obesity and obesity-related inflammation and metabolic disturbances and colorectal cancer outcomes (recurrence, disease-free survival, and/or mortality). A PubMed search was conducted of all English-language papers published between August 2003 and 2009 and cited in MEDLINE. RESULTS: Primary research papers were reviewed for colorectal cancer outcomes related to obesity, inflammation, or metabolic conditions. An association between body size and colorectal cancer recurrence and possibly survival was found; however, reports have been inconsistent. These inconsistent findings may be due to the complex interaction between adiposity, physical inactivity, and dietary intake. Circulating prognostic markers such as C-reactive protein, insulin-like growth factor, and insulin, alone or in combination, have been associated with prognosis in observational studies and should be evaluated in
randomized trials and considered for incorporation into surveillance. CONCLUSIONS: The literature suggests that obesity and obesity-related inflammation and metabolic conditions contribute to the prognosis of colorectal cancer; however, comprehensive large scale trials are needed. Interventions to reduce weight and control inflammation and metabolic conditions, such as diabetes, need to be evaluated and rapidly translated to behavior guidelines for patients


Abstract: PURPOSE: Obesity, sedentary lifestyle, and Western dietary pattern have been linked to increased risk of cancer recurrence and mortality among patients with surgically resected colorectal cancer. Excess energy balance leads to increased circulating insulin and depressed levels of circulating insulin-like growth factor binding protein (IGFBP) -1, which promote cancer cell growth in preclinical models. PATIENTS AND METHODS: Among 373 patients diagnosed with nonmetastatic colorectal cancer between 1991 and 2004, we performed a prospective observational study nested within two large US cohorts to evaluate the association between mortality and prediagnosis circulating C-peptide (a marker of insulin secretion), IGFBP-1, insulin-like growth factor-I (IGF-1), and IGFBP-3. RESULTS: Compared with patients in the bottom quartile, patients in the top quartile of plasma C-peptide had an age-adjusted hazard ratio (HR) for death of 1.87 (95% CI, 1.04 to 3.36; P = .03 for trend), whereas those in the top quartile of circulating IGFBP-1 had a significant reduction in mortality (HR = 0.48; 95% CI, 0.28 to 0.84; P = .02 for trend). Little change in these estimates was noted after adjusting for other covariates known or suspected to influence survival. No associations were noted between mortality and IGF-1 or IGFBP-3, which are two components of the IGF axis not closely correlated with lifestyle factors. CONCLUSION: Among patients with surgically resected colorectal cancer, higher levels of prediagnosis plasma C-peptide and lower levels of prediagnosis plasma IGFBP-1 were associated with increased mortality. Circulating insulin and IGFBP-1 are potential mediators of the association between lifestyle factors and mortality after colorectal cancer resection


Abstract: OBJECTIVES: The present study evaluated the prognostic implications of insulin-like growth factor-1 receptor (IGF-1R), epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor (HER)-2 in patients with colorectal cancer (CRC). METHODS: Our subjects were 91 patients who underwent surgery and subsequently received fluoropyrimidines. Expressions of IGF-1R, EGFR and HER-2 in primary lesions were analyzed immunohistochemically to determine the prognostic significance of these biomarkers. RESULTS: Overexpression was found for IGF-1R in 48 tumors (53%), EGFR in 57 (63%) and HER-2 in 2 (2%). Overexpression of IGF-1R was significantly correlated with shorter survival from the start of first-line chemotherapy (p = 0.033). Overexpression of EGFR was a significant predictor of clinical response to fluoropyrimidines (p = 0.032). Multivariate analysis of potential prognostic factors showed that IGF-1R expression and worsened performance status were independent predictors of poor outcomes. CONCLUSIONS: Our results suggest that anti-IGF-1R strategies may offer a useful approach in molecular therapy for CRC, which has the potential to improve outcomes


Abstract: BACKGROUND: Recent reports have shown that physical activity improves the outcome of patients with colorectal cancer as well as breast and prostate cancer. However, the mechanisms whereby
physical activity reduces cancer mortality are not well established. METHODS: Incident cases of colorectal cancer were identified among participants of the Melbourne Collaborative Cohort Study, a prospective cohort study of 41,528 Australians recruited from 1990 to 1994. Information on tumour site and stage, treatments given, recurrences, and deaths were obtained from systematic review of the medical records. Baseline assessments of physical activity and body size were made, and cases with available plasma had pre-diagnosis insulin-like growth factor 1 (IGF-1) and insulin-like growth factor binding protein 3 (IGFBP-3) levels measured. We assessed associations between these hormones and colorectal cancer specific deaths with respect to physical activity. RESULTS: A total of 526 cases of colorectal cancer were identified, of which 443 had IGF-1/IGFBP-3 levels measured. Median follow up among survivors was 5.6 years. For the physically active, increasing IGFBP-3 by 26.2 nmol/l was associated with a 48% reduction in colorectal cancer specific deaths (adjusted hazard ratio (HR) 0.52 (0.33-0.83); p = 0.006). No association was seen for IGF-1 (adjusted HR 0.90 (0.55-1.45); p = 0.65). For the physically inactive, neither IGF-1 nor IGFBP-3 was associated with disease specific survival. CONCLUSIONS: This study supports the hypothesis that the beneficial effects of physical activity in reducing colorectal cancer mortality may occur through interactions with the insulin-like growth factor axis and in particular IGFBP-3


Abstract: PURPOSE: The aim of this study was to evaluate the prognostic significance of insulin-like growth factor type 1 receptor (IGF-1R) expression in Dukes' C human colorectal cancers (CRCs). EXPERIMENTAL DESIGN: Immunohistochemical staining for IGF-1R was done on formalin-fixed, paraffin-embedded specimens from 161 patients with curatively resected Dukes' C CRC and at least 5-year follow-up periods. We investigated the association between the levels of IGF-1R expression and the clinicopathologic parameters. To evaluate the accurate prognostic value of IGF-1R expression, we investigated two patterns of recurrence-free survival (RFS) according to the mode of recurrence, the hepatic-RFS (H-RFS), and the nonhepatic-RFS (nH-RFS). The influence of the pattern of IGF-1R immunostaining (membranous or cytoplasmic) on RFS was also estimated. RESULTS: High (diffuse staining) and low (focal staining) levels of IGF-1R expression were found in 45 (28%) and 116 (72%) specimens, respectively. The recurrence rate was significantly higher in the latter group (49 of 116) than the former group (9 of 45; P = 0.01). H-RFS was significantly longer for the former group than the latter group (P = 0.021), whereas no difference was found in nH-RFS between the two groups (P = 0.121). In multivariate analysis, the level of IGF-1R expression was an independent factor for H-RFS (P = 0.015) as were the depth of invasion and lymph vessel invasion (P = 0.006 and 0.022, respectively). Using a combination of the level of IGF-1R expression and these two factors, the prognostic value was further increased. When IGF-1R staining patterns (membranous or cytoplasmic) were compared, membrane staining of IGF-1R possessed prognostic significance. CONCLUSIONS: In Dukes' C CRC, focal membrane expression of IGF-1R in the primary tumor can predict a high risk of recurrence, especially liver metastasis. Understanding the mechanisms involved could lead to new therapeutic approaches for advanced CRC

AMPK Activators - Metformin or Berberine

Abstract: BACKGROUND: Patients with type II diabetes mellitus (DM) have an increased risk of adenomatous colorectal (CRC) polyps and CRC cancer. The use of the anti-hyperglycemic agent metformin is associated with a reduced incidence of cancer-related deaths. METHODS: We retrospectively evaluated the medical records of 4758 patients seen at a single institution and determined that 424 patients were identified by their physicians as having type II DM and CRC cancer. Data were subsequently acquired determining the subject's age, body mass index (BMI), and disease date of diagnosis, stage, site of cancer, treatment, and survival. RESULTS: Patients with type II DM and CRC cancer treated with metformin as one of their diabetic medications had a survival of 76.9 months (95% CI=61.4-102.4) as compared with 56.9 months in those patients not treated with metformin (95% CI=44.8-68.8), P=0.048. By using a multivariable Cox regression model adjusted for age, sex, race, BMI, and initial stage of disease, we demonstrated that type II diabetic patients treated with metformin had a 30% improvement in overall survival (OS) when compared with diabetic patients treated with other diabetic agents. CONCLUSION: Colorectal cancer patients with DM treated with metformin as part of their diabetic therapy appear to have a superior OS


Abstract: Metformin use has been associated with decreased cancer risk and mortality. However, the effects of metformin on clinical outcomes of colorectal cancer (CRC) are not defined. This study aimed to evaluate the association between metformin use and mortality of CRC in diabetic patients. We identified 595 patients who were diagnosed both CRC and diabetes mellitus. Patients were compared by two groups; 258 diabetic patients taking metformin and 337 diabetic patients not taking metformin. Patient's demographics, clinical characteristics, overall mortality and CRC-specific mortality were analyzed. After a median follow-up of 41 months, there were 71 total deaths (27.5%) and 55 CRC-specific deaths (21.3%) among 258 patients who used metformin, compared with 136 total deaths (40.4%) and 104 CRC-specific deaths (30.9%) among 337 patients who did not use metformin. Metformin use was associated with decreased overall mortality (p = 0.018) and CRC-specific mortality (p = 0.042) by univariate analysis. After adjustment for clinically relevant factors, metformin use showed lower risk of overall mortality (HR, 0.66; 95% CI 0.476-0.923; p = 0.015) and CRC-specific mortality (HR, 0.66; 95% CI 0.45-0.975; p = 0.037) in CRC patients with diabetes. Metformin use in CRC patients with diabetes is associated with lower risk of CRC-specific and overall mortality


Abstract: The molecular mechanisms responsible for the association of obesity with adverse colon cancer outcomes are poorly understood. We investigated the effects of a high-energy diet on growth of an in vivo colon cancer model. Seventeen days following the injection of 5x10(5) MC38 colon carcinoma cells, tumors from mice on the high-energy diet were approximately twice the volume of those of mice on the control diet. These findings were correlated with the observation that the high-energy diet led to elevated insulin levels, phosphorylated AKT, and increased expression of fatty acid synthase (FASN) by the tumor cells. Metformin, an antidiabetic drug, leads to the activation of AMPK and is currently under investigation for its antineoplastic activity. We observed that metformin blocked the effect of the high-energy diet on tumor growth, reduced insulin levels, and attenuated the effect of diet on phosphorylation of AKT and expression of FASN. Furthermore, the administration of metformin led to the activation of AMPK, the inhibitory phosphorylation of acetyl-CoA carboxylase, the upregulation of BNIP3 and increased apoptosis as estimated by poly (ADP-ribose) polymerase (PARP) cleavage. Prior work showed that activating mutations of PI3K are associated with increased AKT activation and adverse outcome in
colon cancer; our results demonstrate that the aggressive tumor behavior associated with a high-energy
diet has similar effects on this signaling pathway. Furthermore, metformin is demonstrated to reverse the
effects of the high-energy diet, thus suggesting a potential role for this agent in the management of a
metabolically defined subset of colon cancers

Zakikhani M, Dowling RJ, Sonenberg N, Pollak MN. The effects of adiponectin and metformin on
prostate and colon neoplasia involve activation of AMP-activated protein kinase. Cancer Prev Res (Phila)
2008 October;1(5):369-75.

Abstract: Population studies provide evidence that obesity and insulin resistance are associated not only
with elevated serum insulin levels and reduced serum adiponectin levels but also with increased risk of
aggressive prostate and colon cancer. We show here that adiponectin activates AMP-activated protein
kinase (AMPK) in colon (HT-29) and prostate (PC-3) cancer cells. These results are consistent with prior
observations in myocytes, but we show that in epithelial cancer cells AMPK activation is associated with
reduction in mammalian target of rapamycin activation as estimated by Ser(2448) phosphorylation, with
reduction in p70S6 kinase activation as estimated by Thr(389) phosphorylation, with ribosomal protein
S6 activation as estimated by Ser(235/236) phosphorylation, with reduction in protein translation as
estimated by ([35]S)methionine incorporation, and with growth inhibition. Adiponectin-induced growth
inhibition is significantly attenuated when AMPK level is reduced using small interfering RNA,
indicating that AMPK is involved in mediating the antiproliferative action of this adipokine. Thus,
adiponectin has the characteristics of a AMPK-dependent growth inhibitor that is deficient in obesity, and
this may contribute to the adverse effects of obesity on neoplastic disease. Furthermore, metformin was
observed to activate AMPK and to have growth inhibitory actions on prostate and colon cancer cells,
suggesting that this compound may be of particular value in attenuating the adverse effects of obesity on
neoplasia


Abstract: Berberine, an isoquinoline alkaloid derived from plants, is a traditional medicine for treating
bacterial diarrhea and intestinal parasite infections. Although berberine has recently been shown to
suppress growth of several tumor cell lines, information regarding the effect of berberine on colon tumor
growth is limited. Here, we investigated the mechanisms underlying the effects of berberine on regulating
the fate of colon tumor cells, specifically the mouse immorto-Min colonic epithelial (IMCE) cells
carrying the Apc(min) mutation, and of normal colon epithelial cells, namely young adult mouse colonic
epithelium (YAMC) cells. Berberine decreased colon tumor colony formation in agar, and induced cell
death and LDH release in a time- and concentration-dependent manner in IMCE cells. In contrast, YAMC
cells were not sensitive to berberine-induced cell death. Berberine did not stimulate caspase activation,
and PARP cleavage and berberine-induced cell death were not affected by a caspase inhibitor in IMCE
cells. Rather, berberine stimulated a caspase-independent cell death mediator, apoptosis-inducing factor
(AIF) release from mitochondria and nuclear translocation in a ROS production-dependent manner.
Amelioration of berberine-stimulated ROS production or suppression of AIF expression blocked
berberine-induced cell death and LDH release in IMCE cells. Furthermore, two targets of ROS production
in cells, cathepsin B release from lysosomes and PARP activation were induced by berberine. Blockage
of either of these pathways decreased berberine-induced AIF activation and cell death in IMCE cells.
Thus, berberine-stimulated ROS production leads to cathepsin B release and PARP activation-dependent
AIF activation, resulting in caspase-independent cell death in colon tumor cells. Notably, normal colon
epithelial cells are less susceptible to berberine-induced cell death, which suggests the specific inhibitory
effects of berberine on colon tumor cell growth

11

Abstract: Colon cancer is one of the most common malignancies, mainly initiated by the abnormal activation of Wnt/beta-catenin signaling. In this study, we investigated the proliferation inhibitory effect of berberine on colon cancer cells and the molecular basis underlying this effect. With the viability, apoptosis and cell cycle assay, we demonstrated that berberine can inhibit proliferation, induce apoptosis and cell cycle arrest in colon cancer cells. In in vivo investigation, we demonstrated that berberine can prevent the colon cancer formation initiated by dimethylhydrazine (DMH) and dextran sodium sulfate (DSS) in rats. We employed western blotting, reverse transcription and polymerase chain reaction, special antagonist, overexpression and knockdown techniques to dissect the possible molecular mechanisms mediating the function of berberine. We found that the protein levels of beta-catenin in the nucleus and cytoplasm were all reduced after treating the colon cancer cells with berberine, and this may not result from accelerating the degradation of beta-catenin in the cytoplasm, but from inhibiting the mRNA expression of beta-catenin. Our results indicate that berberine can be a potential chemoprevention and chemotherapy agent for human colon cancer by targeting Wnt/beta-catenin signaling.


Abstract: We previously showed that the natural herb Coptidis rhizoma has an anticachectic effect in nude mice bearing human esophageal cancer cells. We further investigated this phenomenon by examining the anticachectic effect of C. rhizoma in syngeneic mice bearing colon 26/clone 20 carcinoma cells, which cause IL-6-related cachexia after cell injection. We evaluated nutritional parameters such as serum glucose level and wasting of adipose tissue and muscle in tumor-bearing and non-tumor-bearing mice treated with C. rhizoma (CR) supplement or a normal diet. IL-6 levels in those mice were quantified by ELISA and real-time RT-PCR. CR supplementation significantly attenuated weight loss in tumor-bearing mice without changing food intake or tumor growth. Furthermore, these mice maintained good nutritional status. IL-6 mRNA levels in tumors and spleens and IL-6 protein levels in tumors and sera were significantly lower in tumor-bearing mice treated with CR supplement than in those treated with a normal diet. CR supplementation did not affect food intake, body weight, nutritional parameters and IL-6 levels in non-tumor-bearing mice. An in vitro study showed that C. rhizoma and its major component, berberine, inhibited IL-1-induced IL-6 mRNA expression in a dose-dependent manner in colon 26/clone 20 cells. Our results showed that C. rhizoma exerts an anticachectic effect on colon 26/clone 20-transplanted mice and that its effect is associated with tumor IL-6 production. We also suggest that its effect might be due to berberine.


Abstract: The enzyme cyclooxygenase-2 (COX-2) is abundantly expressed in colon cancer cells and plays a key role in colon tumorigenesis. Compounds inhibiting COX-2 transcriptional activity have therefore potentially a chemopreventive property against colon tumor formation. An assay method for estimating COX-2 transcriptional activity in human colon cancer cells was established using a beta-galactosidase reporter gene system, and examination was made of various medicinal herbs and their ingredients for an inhibitory effect on COX-2 transcriptional activity. We found that berberine, an isoquinoline alkaloid present in plants of the genera Berberis and Coptis, effectively inhibits COX-2 transcriptional activity in colon cancer cells in a dose- and time-dependent manner at concentrations higher than 0.3 microM. The present findings may further explain the mechanism of anti-inflammatory and anti-tumor promoting effects of berberine.

Abstract: Widdrol, a natural sesquiterpene present in Juniperus sp., has been shown to exert anticancer and antifungal effects. Emerging evidence has suggested that AMP-activated protein kinase (AMPK), which functions as a cellular energy sensor, is a potential therapeutic target for human cancers. In this study, we found that AMPK mediates the anticancer effects of widdrol through induction of apoptosis in HT-29 colon cancer cells. We showed that widdrol induced the phosphorylation of AMPK in a dose- and time-dependent manner. The selective AMPK inhibitor compound C abrogated the inhibitory effect of widdrol on HT-29 cell growth. In addition, we demonstrated that widdrol induced apoptosis and this was associated with the activation of caspases, including caspase3/7 and caspase-9, in HT-29 cells. We also demonstrated that transfection of HT-29 cells with AMPK siRNAs significantly suppressed the widdrol-mediated apoptosis and the activation of caspases. However, cell cycle arrest induced by widdrol was not affected by transfection of HT-29 cells with AMPK siRNAs. Furthermore, widdrol inhibited HT-29 tumor growth in a human tumor xenograft model. Taken together, our results suggest that the anticancer effect of widdrol may be mediated, at least in part, by induction of apoptosis via AMPK activation.


Abstract: Adiponectin is a peptide hormone secreted by adipose tissue. It is a key hormone responsible for insulin sensitization, and its circulating level is inversely associated with abdominal obesity. Recent studies have shown that a reduced plasma adiponectin level is significantly correlated with the risk of various cancers. However, there are few studies regarding the association of adiponectin and colorectal cancer. To address this issue, we investigated the effect of adiponectin on colorectal cancer cells. Three colorectal cancer cell lines express both AdipoR1 and AdipoR2 receptors. MTT assay revealed that adiponectin inhibited human colorectal cancer cell growth. Furthermore, Western blot analysis revealed that adiponectin activated adenosine monophosphate-activated protein kinase (AMPK) and suppressed mammalian target of rapamycin (mTOR) pathways. Selective AMPK inhibitor compound C abrogated the inhibitory effect of adiponectin on cell growth. Our results clearly demonstrate the novel findings that adiponectin inhibits colorectal cancer cell growth via activation of AMPK, thereby down-regulating the mTOR pathway.


Abstract: Death receptor-mediated tumor cell death, either alone or in combination with other anticancer drugs, is considered as a new strategy for anticancer therapy. In this study, we have investigated the effects and molecular mechanisms of 5-aminoimidazole-4-carboxamide riboside [AICAR; a pharmacologic activator of AMP-activated protein kinase (AMPK)] in sensitizing tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL)- and TNFalpha-induced apoptosis of human colon cancer HCT116 cells. The cytotoxic action of AICAR requires AMPK activation and may occur at various stages of apoptotic pathways. AICAR cotreatment with either TRAIL or TNFalpha enhances activities of caspase-8, caspase-9, and caspase-3; down-regulates the antiapoptotic protein Bcl-2; increases the cleavage of Bid and results in the decrease of mitochondrial membrane potential; potentiates activation of p38 and c-Jun NH(2)-terminal kinase; and inhibits nuclear factor-kappaB activity. In addition, this sensitized cell apoptosis was neither observed in p53-null HCT116 cells nor affected by the cotreatment with mevalonate. In summary, we have developed a novel strategy of combining AICAR with TRAIL for the treatment of colon cancer cells. The sensitization effect of AICAR in cell apoptosis was mediated through AMPK pathway, requires p53 activity, and involves mitochondria-dependent apoptotic cascades, p38 and c-Jun NH(2)-terminal kinase.
Fish Omega-3s


Abstract: BACKGROUND: Polyunsaturated omega-3 fatty acids may beneficially influence healing processes and patient outcomes. The aim of this research was to study the clinical efficacy of fish oil enriched total parenteral nutrition in elderly patients after colorectal cancer surgery. METHODS: Fifty-seven elderly patients with colorectal cancer were enrolled in this prospective, randomized, double-blind, controlled clinical trial. All patients received isocaloric and isonitrogenous total parenteral nutrition by continuous infusion (20 - 24 hours per day) for seven days after surgery. The control group (n = 28) received 1.2 g/kg soybean oil per day, whereas the treatment group (n = 29) received 0.2 g/kg fish oil and 1.0 g/kg soybean oil per day. Blood samples were taken pre-operatively, and at days one and eight after the operation. The plasma levels of CD4, CD8, CD4/CD8, interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-alpha) were measured. Clinical outcomes were then analysed. RESULTS: Patient characteristics were comparable between the two groups. At day eight post-surgery, IL-6, TNF-alpha and CD8 titres were lower in the treatment group when compared to the control group; these results reached statistical significance. In the treatment group, there were fewer infectious complications and incidences of systemic inflammatory response syndrome (SIRS), and shorter lengths of hospital stay were observed. The total cost of medical care was comparable for the two groups. No serious adverse events occurred in either group. CONCLUSIONS: Fish oil 0.2 g/kg per day administrated to elderly patients after colorectal surgery was safe and may shorten the length of hospital stay and improve clinical outcomes.


Abstract: Inflammation is a common feature in cancer. The presence and magnitude of the chronic systemic inflammatory responses may produce progressive nutritional decline. This study aims at investigating whether there are changes in inflammation markers and/or in nutritional status of patients with colorectal cancer undergoing chemotherapy who were supplemented with fish oil. The clinical trial was conducted with 23 patients randomly distributed in 2 groups. The supplemented group (SG) consumed 2 g of fish oil containing 600 milligrams of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) for 9 wk. Nutritional and inflammatory markers status was available, both at a baseline (M0), and after 9 wk of chemotherapy (M9) in the SG and in the nonsupplemented group (NSG). Statistical analysis was conducted with STATA 11.0 software. SG and NSG presented the same baseline characteristics (P > 0.05). Nutritional status indicators such as body mass index and body weight were modified only in the NSG when comparing baseline and M9, P = 0.03 and P = 0.01 respectively, whereas in SG these indicators did not vary. Patients supplemented with fish oil (SG) showed a clinically relevant decrease in the C-reactive protein/albumin relation (P = 0.005). Low doses of fish oil supplement can positively modulate the nutritional status and the C-reactive protein/albumin ratio.


Abstract: We report a case of a Stage IV rectal cancer patient for whom EPA oral nutritional supplements promoted treatment compliance with cancer chemotherapy by resolving a refractory cachectic condition. A 76-year-old male who developed a local re-growth of residual disease and multiple lung metastases...
after abdomino-perineal resection for lower rectal cancer was referred to our clinic for chemotherapy. On admission, he suffered from a loss of appetite as well as a 30% loss of usual body weight, caused by a cachectic condition with systemic inflammatory response. On starting chemotherapy, his daily diet was supplemented with EPA containing oral nutritional supplements (EPA ONS). Within 2 weeks after initiating EPA ONS treatment, the systemic inflammatory response resolved, and at the same time, body weight and the serum level of albumin increased, which allowed treatment compliance with aggressive multidrug chemotherapy. The patient gained 10 kg in body weight even after 12 months of aggressive chemotherapy, and has attained a longstanding partial remission from the disease. Although cancer cachexia is generally regarded as an end-stage irreversible pathological condition, EPA ONS may promote patient compliance with cancer chemotherapy by resolving cachectic condition, and thus may improve survival.


Abstract: Omega (omega)-3 polyunsaturated fatty acids (PUFAs) are naturally occurring substances that are well tolerated and have been used extensively for the prevention of cardiovascular disease. More recently, omega-3 PUFAs have been recognised to have anticancer activity. There is also evidence suggesting improved efficacy and/or tolerability of conventional cancer chemotherapy when administered with omega-3 PUFAs. The purpose of this review is to (i) describe the mechanisms by which omega-3 PUFAs are thought to have antineoplastic activity, (ii) review published preclinical and clinical studies that support anti-colorectal cancer activity and (iii) summarise current clinical trials investigating the potential therapeutic role(s) of omega-3 PUFAs at different stages of colorectal carcinogenesis, from adenoma (polyp) prevention to treatment of established malignant disease and prevention of cancer recurrence.


Abstract: PURPOSE: The aim of this study was to evaluate whether the omega-3 polyunsaturated fatty acid cis-5,8,11,14,17-eicosapentanoic acid (EPA) can enhance the radiosensitivity of different human tumor cell lines. MATERIALS AND METHODS: Colon adenocarcinoma cells HT-29, and two glioblastoma multiforme tumor cells T98G and U251 were cultured under standard conditions. Cell growth was observed during administration with different concentrations of EPA, using it as the free fatty acid dissolved in ethanol or bound to bovine serum albumin. To investigate the influence of EPA (free and bound) on radiosensitivity, tumor cells were pretreated 30 minutes or 24 hours prior to irradiation with the fatty acid. Cell survival was measured by colony-forming assays. RESULTS: When combined with irradiation, incubation with EPA was found to result in enhanced radiosensitivity with substantial variation: while there was strong radiosensitization for HT-29 and U251 cells, almost no effect for T98G cells was observed. A marked radiosensitization was clearly dependent on the treatment schedule. CONCLUSION: The observations suggest that EPA is not only a nutritional adjuvant but also may be a potential candidate to enhance the efficacy of irradiation on human cancer cells.


Abstract: Diets rich in n-3 polyunsaturated fatty acids (PUFAs) have been associated with a reduced risk of several types of cancer. Recent reports have suggested that these PUFAs enhance the cytotoxic effect of cancer chemoradiotherapy. The effect of docosahexaenoic acid (DHA) on key cell cycle regulators and
target proteins of cancer therapy was investigated in the human malign colon cancer cell line SW620. Cell cycle check point proteins such as p21 and stratifin (14-3-3 sigma) increased at mRNA and protein level, whereas cell cycle progression proteins such as cell division cycle 25 homolog and cyclin-dependent kinase 1 decreased after DHA treatment. Protein levels of inhibitors of apoptosis family members associated with chemotherapy resistance and cancer malignancy, survivin and livin, decreased after the same treatment: likewise the expression of NF-kappaB. Levels of the proapoptotic proteins phosphorylated p38 MAPK and growth arrest-inducible and DNA damage-inducible gene 153/C/EBP-homologous protein (CHOP) increased. The results indicate that DHA treatment causes simultaneous cell cycle arrest in both the G1 and G2 phase. In conclusion, DHA affects several target proteins of chemotherapy in a favorable way. This may explain the observed enhanced chemosensitivity in cancer cells supplemented with n-3 PUFAs and encourage further studies investigating the role of n-3 PUFAs as adjuvant to chemotherapy and radiotherapy in vivo.


Abstract: Several studies have suggested that the n-3 fatty acids Docosahexaenoic (DHA) and Eicosapentaenoic (EPA) have an important protective effect on colorectal cancer, and this could be at least partly due to their proapoptotic activity. It is unclear, however, how this phenomenon is triggered and what mechanisms are implicated. Here, we show that both DHA and EPA have an important proapoptotic effect on colorectal cancer cells with different molecular phenotypes but not in noncancerous cells. Apoptosis is caspase dependent, and both intrinsic and extrinsic pathways are implicated. The dimerization of Bax and Bak, the depolarization of the mitochondrial membrane, and the subsequent release of cytochrome c and Smac/Diablo to the cytosol evidence the activation of the intrinsic pathway. The implication of the extrinsic pathway is shown by the activation of caspase-8, along with the down-regulation of FLIP. The timing of caspase-8 activation, and the oligomerization of Bid with Bax, suggest a cross-talk with the intrinsic pathway. None of the death receptors that commonly initiate the extrinsic pathway: FAS, TNF-R1, and TRAIL-R2 are found to be responsible for triggering the apoptosis cascade induced by DHA and EPA. Neither PPARgamma nor cyclooxygenase-2, two likely candidates to regulate this process, play a significant role. Our findings suggest that the down-regulation of two key regulatory elements of the extrinsic and intrinsic pathways, FLIP and XIAP, respectively, is determinant in the induction of apoptosis by DHA and EPA. These fatty acids could potentially be useful adjuvant anticancer agents in combination with other chemotherapeutic elements.


Abstract: The insulin-like growth factor (IGF) system plays a critical role in normal growth and development as well as in malignant states. Most of the biological activities of the IGFs are mediated by the IGF-IR, which is over-expressed in most tumours and cancer cell lines. Fatty acids have critical roles in both systemic physiological processes (e.g. metabolism) and cellular events (e.g. proliferation, apoptosis, signal transduction, and gene expression). Alpha-linolenic acid (ALA) and linoleic acid (LA) are essential fatty acids of the omega-3 and omega-6 families, respectively. The aim of this study was to investigate the potential interactions between fatty acids and the IGF signal transduction pathways, and to evaluate the impact of this interplay on colon cancer cells survival and proliferation. Results of Western blot analyses revealed that ALA and LA enhanced the ligand-induced IGF-IR phosphorylation and, in addition, increased receptor phosphorylation in an IGF-I independent manner. Furthermore, fatty acid treatment led to phosphorylation of downstream signalling molecules, including Akt and Erk. In addition, FACS analysis and apoptosis measurements indicated that ALA and LA have a potential mitogenic effect on HCT116 cells, as reflected by the number of cells in S phase and by a reduction of PARP cleavage.
implying a reduction in apoptotic activity. In summary, our results provide evidence that omega-3 and omega-6 fatty acids modulate IGF-I action in colon cancer cells


Abstract: AIM: To investigate the impact of arachidonic acid (AA) and docosahexaenoic acid (DHA) and their combination on colon cancer cell growth. METHODS: The LS-174T colon cancer cell line was used to study the role of the prostaglandin precursor AA and the omega-3 polyunsaturated fatty acid DHA on cell growth. Cell viability was assessed in XTT assays. For analysis of cell cycle and cell death, flow cytometry and DAPI staining were applied. Expression of cyclooxygenase-2 (COX-2), p21 and bcl-2 in cells incubated with AA or DHA was examined by real-time RT-PCR. Prostaglandin E(2) (PGE(2)) generation in the presence of AA and DHA was measured using a PGE(2)-ELISA. RESULTS: AA increased cell growth, whereas DHA reduced viability of LS 174T cells in a time- and dose-dependent manner. Furthermore, DHA down-regulated mRNA of bcl-2 and up-regulated p21. Interestingly, DHA was able to suppress AA-induced cell proliferation and significantly lowered AA-derived PGE(2) formation. DHA also down-regulated COX-2 expression. In addition to the effect on PGE(2) formation, DHA directly reduced PGE(2)-induced cell proliferation in a dose-dependent manner. CONCLUSION: These results suggest that DHA can inhibit the pro-proliferative effect of abundant AA or PGE(2)


Abstract: AIM: The purpose of this study was to examine the influence of fish oil on growth of colon cancer in nude mice. MATERIALS AND METHODS: Xenografts were initiated in mice receiving a standard diet or diets modified with corn or fish oil. After 3 weeks, mice were sacrificed, tumours were removed and processed for lipid analysis, histopathology and high resolution magic angle spinning magnetic resonance spectroscopy. RESULTS: Diet modified with fish oil suppressed tumour growth. Xenografts from mice receiving fish oil had higher levels of omega-3 polyunsaturated fatty acids (PUFAs) with concomitant reduced levels of omega-6 PUFAs. Furthermore, these xenografts had significantly lower levels of phosphocholine. Overall the results indicated less aggressive tumour growth in mice receiving a fish oil diet


Abstract: Polyunsaturated fatty acids (PUFAs) are normal constituents of the diet, but have properties different from other fatty acids (e.g., through generation of signaling molecules). N-3 PUFAs reduce cancer cell growth, but no unified mechanism has been identified. We show that docosahexaenoic acid (DHA; 22:6 n-3) causes extensive changes in gene expression patterns at mRNA level in the colon cancer cell line SW620. Early changes include unfolded protein response (UPR) and increased levels of phosphorylated eIF2alpha as verified at protein level. The latter is considered a hallmark of endoplasmic reticulum (ER) stress and is abundantly present already after 3 h. It may coordinate many of the downstream changes observed, including signaling pathways for cell cycle arrest/apoptosis, calcium homeostasis, cholesterol metabolism, ubiquitination, and proteasomal degradation. Also, eicosapentaenoic acid (EPA), but not oleic acid (OA), induced key mediators of ER stress and UPR at protein level. Accumulation of esterified cholesterol was not compensated for by increased total levels of cholesterol, and mRNAs for cholesterol biosynthesis as well as de novo synthesis of cholesterol were
reduced. These results suggest that cytotoxic effects of DHA are associated with signaling pathways involving lipid metabolism and ER stress.


Abstract: Human colon carcinoma COLO 205, carrying wild type p53, grown subcutaneously in athymic mice was inhibited 80% by a high fat menhaden oil diet containing a mixture of omega-3 fatty acids compared to the low fat corn oil diet containing omega-6 fatty acids. Feeding a high fat diet of golden algae oil containing docosahexaenoic acid (DHA) as the sole long chain omega-3 fatty acid resulted in 93% growth inhibition. Similar findings were previously reported for WiDr colon carcinoma containing mutated p53 (His237). In vitro, 125 μM DHA inhibited COLO 205 growth by 81%, WiDr by 42%, while eicosapentaenoic acid (EPA) marginally inhibited growth of both lines by approximately 30%. DHA inhibited cell proliferation by 41% in WiDr but did not significantly inhibit proliferation in COLO 205. Cell cycle analysis revealed that DHA arrested cell cycle at Resting/Gap 1 (G0/G1 phase) in WiDr and at Gap 2/Mitosis (G2/M) phase in COLO 205. DHA induced apoptosis in COLO 205 but not in WiDr, and EPA did not induce apoptosis in either line. Taken together, these findings suggest DHA is the primary tumor suppressive omega-3 fatty acid in vivo and in vitro and inhibits cancer growth by p53 dependent and independent pathways, while the marginal inhibition by EPA is p53 independent.


Abstract: BACKGROUND: Fish oil consisting of omega-3 polyunsaturated fatty acids (PUFA) seems to reduce the incidence of colon cancer. The effect of PUFAs on metastasis of colon carcinoma is still unclear. AIM: The study was designed to examine the effects of a diet rich in omega-3-PUFAs on a model of colorectal metastasis. METHODS: Thirty animals (WAG/Rij) were randomly assigned to receive an omega-3 diet or a control diet to evaluate their effect on tumor growth. The target male rats (WAG/Rij) were fed a diet containing 15% omega-3-fatty acids three days before and 28 days after intervention and the control rats received 15% coconut oil at the same time points. CC 531 cells, a moderately differentiated colon adenocarcinoma, were injected into the spleen of each rat. After 28 days of diet, animals were sacrificed. The tumor growth was evaluated macroscopically and microscopically in liver tissue. The tissue was examined after immunostaining and the use of monoclonal antibodies. RESULTS: PUFAs decreased the index of tumor load from 1.54 in the controls to 0.79 in the treatment group (P = 0.036). While 69.2% of the control animals were tumor positive, only 21.4% of the target animals showed tumor after omega-3-fatty acid (P < 0.05). CONCLUSION: We could show that omega-3-fatty acids may decrease malignant metastatic tumor growth in the liver.


Abstract: OBJECTIVE: This study evaluated whether omega-3 polyunsaturated fatty acids (PUFAs) could enhance the radiosensitivity of three different human colorectal adenocarcinoma cell lines. To understand the underlying mechanisms, the effects of omega-3 PUFAs on the cell growth, survival, and apoptosis were evaluated alone or in combination with an antioxidant (vitamin E) and compared with the effects of omega-6 PUFAs. METHODS: LS174T, CO112, and Caco-2 cell survival was assessed by clonogenic assay after a 3-d pretreatment with omega-3/omega-6 PUFAs and/or vitamin E before a single X-ray exposure to 4 Gy. Cell growth and viability were measured by double fluorescence-activated cell sorter analyses using propidium iodide and fluorescein isothiocyanate-conjugated annexin V. Student's t
test or multivariable linear regression analyses were used for comparison. RESULTS: Preincubation with 30 to 100 micromol/L of omega-3 PUFAs induced a dose-dependent additive decrease in cell survival after irradiation (P < 0.05). Evaluation of the underlying mechanisms indicated that omega-3 PUFAs mainly decreased the cell number via apoptosis induction. Moreover, formation of lipid peroxidation products and modulation of cyclooxygenase II activity seemed to be involved, because coincubation with 10 micromol/L vitamin E abolished the effect of 50 micromol/L of omega-3 PUFAs (P < 0.05), whereas omega-6 PUFAs could partly mimic omega-3 PUFA effects. CONCLUSION: These observations suggest that omega-3 PUFAs may be potential candidates as nutritional adjuvants to enhance the efficacy of human colorectal cancer radiotherapy

Vaculova A, Hofmanova J, Andera L, Kozubik A. TRAIL and docosahexaenoic acid cooperate to induce HT-29 colon cancer cell death. Cancer Lett 2005 November 8;229(1):43-8. Abstract: The resistance of some cancer cells to TRAIL-induced apoptosis is a major obstacle in successful clinical application of this cytokine. Combination treatment with agents capable of sensitising the cells to TRAIL effects is beneficial for new cancer treatment strategies. Docosahexaenoic acid (DHA) is under intense investigation for its ability to affect cancer cell growth and apoptosis. We demonstrated a modulation of TRAIL-induced apoptosis of HT-29 human colon cancer cells by DHA on the molecular (pro-caspase-3, -8, Bid, PARP cleavage) and cellular (cell viability and adhesion) level. To conclude, TRAIL and DHA were shown to cooperate in the induction of colon cancer cell apoptosis

Calviello G, Di NF, Serini S et al. Docosahexaenoic acid enhances the susceptibility of human colorectal cancer cells to 5-fluorouracil. Cancer Chemother Pharmacol 2005 January;55(1):12-20. Abstract: PURPOSE: Powerful growth-inhibitory action has been shown for n-3 polyunsaturated fatty acids against colon cancer cells. We have previously described their ability to inhibit proliferation of colon epithelial cells in patients at high risk of colon cancer. In the work reported here we investigated the ability of docosahexaenoic acid (DHA) to potentiate the antineoplastic activity of 5-fluorouracil (5-FU) in p53-wildtype (LS-174 and Colo 320) and p53-mutant (HT-29 and Colo 205) human colon cancer cells. METHODS: When in combination with DHA, 5-FU was used at concentrations ranging from 0.1 to 1.0 microM, much lower than those currently found in plasma patients after infusion of this drug. Similarly, the DHA concentrations (≤ or =10 microM) used in combination with 5-FU were lower than those widely used in vitro and known to cause peroxidative effects in vivo. RESULTS: Whereas the cells showed different sensitivity to the growth-inhibitory action of 5-FU, DHA reduced cell growth independently of p53 cellular status. DHA synergized with 5-FU in reducing colon cancer cell growth. The potentiating effect of DHA was attributable to the enhancement of the proapoptotic effect of 5-FU. DHA markedly increased the inhibitory effect of 5-FU on the expression of the antiapoptotic proteins BCL-2 and BCL-XL, and induced overexpression of c-MYC which has recently been shown to drive apoptosis and, when overexpressed, to sensitize cancer cells to the action of proapoptotic agents, including 5-FU. CONCLUSION: Our results indicate that DHA strongly increases the antineoplastic effects of low concentrations of 5-FU. Overall, the results suggest that combinations of low doses of the two compounds could represent a chemotherapeutic approach with low toxicity

Braga M, Gianotti L, Vignali A, Carlo VD. Preoperative oral arginine and n-3 fatty acid supplementation improves the immunometabolic host response and outcome after colorectal resection for cancer. Surgery 2002 November;132(5):805-14. Abstract: BACKGROUND: Previous trials showed that perioperative immunonutrition improved outcome in patients with gastrointestinal cancer. This study was designed to appraise the impact of the simple preoperative oral arginine and n-3 fatty acids supplementation on immune response, gut oxygenation, and postoperative infections. METHODS: Two hundred patients with colorectal neoplasm
were randomized to: (a) oral intake for 5 days before surgery of a formula enriched with arginine and n-3 fatty acids (pre-op group; n = 50); (b) same preoperative treatment prolonged after surgery by jejunal infusion (peri-op group; n = 50); (c) oral intake for 5 days before surgery of a standard isoenergetic, isonitrogenous formula (control group; n = 50); and (d) no supplementation before and after operation (conventional group; n = 50). The immune response was measured by phagocytosis ability of polymorphonuclear cells and delayed hypersensitivity response to skin tests. Gut oxygenation and microperfusion were assessed by polarographic probes and laser Doppler flowmetry, respectively.

RESULTS: The 4 groups were comparable for demographics, comorbidity, and surgical variables. The 2 groups receiving immunoutrients (pre-op and peri-op) had a significantly better immune response, gut oxygenation, and microperfusion than the other 2 groups. Intent-to-treat analysis showed an overall infection rate of 12% in pre-op, 10% in peri-op, 32% in control, and 30% in conventional groups (P <.04 pre-op and peri-op vs control and conventional). CONCLUSION: Preoperative oral arginine and n-fatty acids improves the immunometabolic response and decreases the infection rate. Postoperative prolongation with such supplemented formula has no additional benefit.


Abstract: The present study investigated the influence of dietary omega-3 fatty acid supplementation on the growth of human colon carcinoma xenograft in athymic nude mice. Four diets were fed to evaluate the effect of levels and types of fat on colon tumor growth. Animals were maintained on a standard diet modified by addition of fats containing omega-3 and omega-6 fatty acids to represent high and low fat intakes for 53 days. The final mean estimated tumor weight for the high fat corn oil (24%) fed group was 2,302 mg, whereas the low fat (8% corn oil) group was 1,681 mg. The final mean tumor weight of the high fat menhaden oil fed group was 782 mg representing a 66% decrease in growth compared to the high fat corn oil group and a decrease of 54% compared to the low corn oil fed group. The high fat golden algae oil fed group resulted in a mean final tumor weight of 223 mg representing a 90% inhibition of tumor growth relative to the high fat corn oil fed group and 87% inhibition of growth compared to the low fat corn oil fed group. These findings indicate that dietary omega-3 fatty acids possess significant tumor suppressing properties and that the primary tumor suppressing fatty acid is docosahexaenoic acid. Histopathologic examination of control and treated tumors and expression array analyses (human cytokine and apoptosis arrays) support the tumor growth inhibition data and provide evidence for discussion of possible mechanisms for the observed growth inhibition.


Abstract: Epidemiological and preclinical studies demonstrate that consumption of diets high in omega-3 fatty acids (n-3 PUFAs) reduce the risk of colon cancer. Docosahexaenoic acid (DHA), a long chain polyunsaturated fatty acid (PUFAs) is a major constituent of nutrients rich in n-3 PUFAs. There are studies to indicate that colon tumor inhibition by n-3 PUFA-rich diets is, in part, mediated through modulation of signaling pathways that alter gene expression which are involved in colon tumor growth. In the present study using CaCo-2 colon cancer cell lines we examined the effects of DHA on the genetic precursors of human colon cancer at the transcription level using DNA oligonucleotide arrays. Our results indicated that DHA inhibits the growth of CaCo-2 cells and induces apoptosis. For gene expression analysis using DNA microarrays, total RNA extracted from DHA treated CaCo-2 cells was converted to cDNA, labeled with Cy5-dCTP (DHA-treated) and Cy3-dCTP (untreated cells) and used as probes for hybridization in human chip spotted with 3,800 oligonucleotides consisting of 156 functional categories. The expression profiles of genes indicated a reprogramming pattern of previously known and unknown genes and transcription factors that provided clues to the possible functional mechanism of DHA. An average of (ratios from triplicate experiments) 504 out of 3,800 genes expressed after 48 h of DHA
treatment. Altered expression on the transcription factors includes down regulation of nine members of
the RNA II polymerases, transcription co-repressor associated protein and enhancer binding proteins such
as AP2, in addition to changes in the expression of zinc finger group of transcription factors. Activation
of cytochrome c which triggers caspases was associated with the elevated expression of pro-apoptotic
caspases 10, 13, 8, 5 and 9 in DHA treated cells. Activation of cyclin-dependent kinase inhibitors such as
p21 (waf1/cip1), p27, p57, p19 and growth arrest specific proteins by more than 2-fold is consistent with
the induction of apoptosis and inactivation of antiapoptotic Bcl-2 family of genes. Inactivation of
prostaglandin family of genes, lipoxygenases and altered expression of peroxisome proliferators
(PPARalpha and gamma) by DHA seem to indicate a lipid peroxidation-induced apoptosis in addition to
effect reflected on the modification of cell cycle regulatory genes. These findings support the conclusion
that a genomewide expression profiling of human colon cancer precursor genes and transcription factors
provides a set of novel regulatory mechanism(s) to determine the chemopreventive efficacy of DHA and
thus to prevent the inflammation and neoplasia

Klieveri L, Fehres O, Griffini P, Van Noorden CJ, Frederiks WM. Promotion of colon cancer metastases

Abstract: Recently, it was demonstrated that dietary omega-3 polyunsaturated fatty acids (PUFAs) induce
10-fold more metastases in number and 1000-fold in volume in an animal model of colon cancer
metastasis in rat liver. It was observed that tumors of rats on a fish oil diet lacked peritumoral stroma
unlike tumors in livers of rats on a low fat diet or a diet containing omega-6 PUFAs. In the present study,
only one-third of the tumors in livers of rats on omega-3 PUFA diet contained peritumoral stroma,
whereas peritumoral stroma was present in 87% of the tumors in livers of rats on low fat diet. To explain
these findings, we tested the hypothesis that fish oil exerts a direct inhibiting effect on the formation of
extracellular matrix in tumor stroma as a consequence of blocking transformation of fat storing cells into
myofibroblasts. It was found with immunohistochemical analysis of desmin as marker for fat storing cells
and alpha-smooth muscle actin as marker for myofibroblasts that numbers of myofibroblasts were higher
in tumors containing intratumoral stroma only than in tumors containing both peritumoral and
intratumoral stroma. As most of the tumors in fish oil-treated rats contained intratumoral stroma only, this
suggests that transformation of fat storing cells into myofibroblasts was highest in tumor stroma of fish
oil-treated rats. Therefore, it is unlikely that the lack of stroma around tumors in fish oil-treated rats is
due to inhibition of transformation of fat storing cells into myofibroblasts, but lack of peritumoral stroma is
rather a consequence of rapid development of tumors in livers of fish oil-treated rats

Kontogiannnea M, Gupta A, Ntanios F, Graham T, Jones P, Meterissian S. omega-3 fatty acids decrease

Abstract: BACKGROUND: Diets rich in omega-3 fatty acids have been shown to decrease both the
initiation and promotion of colon carcinogenesis although their effect on hepatic metastasis formation is
less well understood. Since adhesion of human colorectal carcinoma (HCRC) cells to hepatic endothelial
cells is an important step in the metastatic cascade, the effect of membrane omega-3 fatty acid alterations
on endothelial cell adhesion was studied. MATERIALS AND METHODS: CX-1 cells, a moderately
derifferentiated HCRC cell line known to produce hepatic metastases in an athymic mouse intrasplenic
injection model, were used. Cells were grown in omega-3 fatty acid-enriched medium and membrane-free
fatty acid modifications confirmed with gas chromatography. Both human umbilical vein and hepatic
sinusoidal endothelial cells were used in the binding assays. Adhesion assays were performed in a
standard fashion using (51)Cr-labeled cells to tumor necrosis factor (TNF)-stimulated endothelial cell
monolayers. Immunohistochemical analysis was performed for sialyl-Lewis(x), the receptor involved in
endothelial adhesion on the surface of control and fatty acid-modified cells. Adhesion assays were performed in a
standard fashion using (51)Cr-labeled cells to tumor necrosis factor (TNF)-stimulated endothelial cell
monolayers. Immunohistochemical analysis was performed for sialyl-Lewis(x), the receptor involved in
endothelial adhesion on the surface of control and fatty acid-modified cells. Adhesion assays were performed in a
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endothelial adhesion on the surface of control and fatty acid-modified cells. Adhesion assays were performed in a
standard fashion using (51)Cr-labeled cells to tumor necrosis factor (TNF)-stimulated endothelial cell
monolayers. Immunohistochemical analysis was performed for sialyl-Lewis(x), the receptor involved in
endothelial adhesion on the surface of control and fatty acid-modified cells.
of CX-1 to both human umbilical vein and hepatic sinusoidal endothelial cells decreased from 38.4 +/- 0.44 to 11.58 +/- 0.87% (P < 0.01). Immunocytochemical analysis showed a decrease in sialyl-Lewis(x) expression with omega-3 treatment. CONCLUSIONS: These data indicate that omega-3 fatty acids may also be protective against the formation of hepatic metastases. The mechanism for this may be decreased endothelial cell adhesion which in turn may be due to decreased expression of the endothelial receptor sialyl-Lewis(x).


Abstract: The effects of omega-3 polyunsaturated fatty acids (PUFAs) and omega-6 PUFAs on the development of experimentally induced colon carcinoma metastasis in rat liver were investigated quantitatively in vivo. Rats were kept on either a low-fat diet or on a fish oil (omega-3 PUFAs) or safflower oil (omega-6 PUFAs) diet for 3 weeks before the administration of colon cancer cells to the portal vein, until they were sacrificed at 1 or 3 weeks after tumor transplantation. At 1 week after transplantation, the fish oil diet had induced 7-fold more metastases (in terms of number and size) than had the low-fat diet, whereas the safflower oil diet had not affected the number and total volume of metastases. At 3 weeks after tumor transplantation, the fish oil diet and the safflower oil diet had induced, respectively, 10- and 4-fold more metastases (number) and over

Green Tea Polyphenols


Abstract: AIM: Epigallocatechin-3-gallate (EGCG) is the major polyphenolic constituent in green tea. The aim of this study is to investigate the effects of EGCG on proliferation and migration of the human colon cancer SW620 cells. METHODS: Proliferation and migration of SW620 cells were induced by the protease-activated receptor 2-agonist peptide (PAR2-AP, 100 mumol/L) or factor VIIa (10 nmol/L), and analyzed using MTT and Transwell assays, respectively. The cellular cytoskeleton was stained with rhodamine-conjugated phalloidin and examined with a laser scanning confocal fluorescence microscope. The expression of caspase-7, tissue factor (TF) and matrix metalloproteinase (MMP)-9 in the cells was examined using QT-PCR, ELISA and Western blot assays. The activation of extracellular signal-regulated kinase 1 and 2 (ERK1/2) and nuclear factor-kappa B (NF-kappaB) signaling pathways was analyzed with Western blot. RESULTS: Both PAR2-AP and factor VIIa promoted SW620 cell proliferation and migration, and caused cytoskeleton reorganization (increased filopodia and pseudopodia). Pretreatment with EGCG (25, 50, 75, and 100 mug/mL) dose-dependently blocked the cell proliferation and migration induced by PAR2-AP or factor VIIa. EGCG (100 mug/mL) prevented the cytoskeleton changes induced by PAR2-AP or factor VIIa. EGCG (100 mug/mL) counteracted the down-regulation of caspase-7 expression and up-regulation of TF and MMP-9 expression in the cells treated with PAR2-AP or factor VIIa. Furthermore, it blocked the activation of ERK1/2 and NF-kappaB (p65/RelA) induced by PAR2-AP or factor VIIa. CONCLUSION: EGCG blocks the proliferation and migration of SW620 cells induced by PAR2-AP and factor VIIa via inhibition of the ERK1/2 and NF-kappaB pathways. The compound may serve as a preventive and therapeutic agent for colon cancers.

Abstract: BACKGROUND: TROP-2 is a tumor-promoting molecule that has been found to be overexpressed in many cancer cells, making it a plausible biomarker of carcinogenesis. The main aim of this study was to examine the effect of green tea catechins (namely, (-)-epigallocatechin-3-gallate; EGCG) on TROP-2 expression. MATERIALS AND METHODS: Western blot and RT-PCR were applied to assess TROP2 expression in colorectal cancer cells and tissues. RESULTS: Two different mechanisms were found to operate in diverse cell lines. In SW480 cells, EGCG affected the post-transcriptional processing of the TROP-2 mRNA, as this was quickly and specifically degraded in the presence of EGCG. In HCT-116 cells, EGCG affected TROP-2 expression at the post-translational level. TROP-2 was found to be highly expressed in colorectal tumors compared to adjacent normal tissues.

CONCLUSION: This study provided a novel beneficial activity of green tea as an anti-tumorigen agent causing the suppression of TROP-2 in colorectal cancer.


Abstract: (-)-Epigallocatechin gallate (EGCG), the major constituent of green tea, inhibits the growth of colorectal cancer cells by inhibiting the activation of various types of receptor tyrosine kinases (RTKs). The RTK vascular endothelial growth factor (VEGF)/VEGF receptor (VEGFR) axis induces tumor angiogenesis in colorectal cancer. This study examined the effects of EGCG on the activity of the VEGF/VEGFR axis and the expression of hypoxia-inducible factor (HIF)-1alpha, which promotes angiogenesis by elevating VEGF levels, in human colorectal cancer cells. Total and phosphorylated (i.e., activated) form (p-VEGFR-2) of VEGFR-2 proteins were overexpressed in a series of human colorectal cancer cell lines. Within 3h, EGCG caused a decrease in the expression of HIF-1alpha protein and VEGF, HIF-1alpha, insulin-like growth factor (IGF)-1, IGF-2, epidermal growth factor (EGF), and heregulin mRNAs in SW837 colorectal cancer cells, which express a constitutively activated VEGF/VEGFR axis. A decrease was also observed in the expression of VEGFR-2, p-VEGFR-2, p-IGF-1 receptor, p-ERK, and p-Akt proteins within 6h after EGCG treatment. Drinking EGCG significantly inhibited the growth of SW837 xenografts in nude mice, and this was associated with the inhibition of the expression and activation of VEGFR-2. The consumption of EGCG also inhibited activation of ERK and Akt, both of which are downstream signaling molecules of the VEGF/VEGFR axis, and reduced the expression of VEGF mRNA in xenografts. These findings suggest that EGCG may exert, at least in part, growth-inhibitory effects on colorectal cancer cells by inhibiting the activation of the VEGF/VEGFR axis through suppressing the expression of HIF-1alpha and several major growth factors. EGCG may therefore be useful in the chemoprevention and/or treatment of colorectal cancer.


Abstract: BACKGROUND: Epigallocatechin-3-gallate (EGCG), one of the major catechins in green tea, is a potential chemopreventive agent for various cancers. The aim of this study was to examine the effect of EGCG on the expression of heat shock proteins (HSPs) and tumor suppression. METHODS: Cell colony formation was evaluated by a soft agar assay. Transcriptional activity of HSP70 and HSP90 was determined by luciferase reporter assay. An EGCG-HSPs complex was prepared using EGCG attached to the cyanogen bromide (CNBr)-activated Sepharose 4B. In vivo effect of EGCG on tumor growth was examined in a xenograft model. RESULTS: Treatment with EGCG decreased cell proliferation and colony formation of MCF-7 human breast cancer cells. EGCG specifically inhibited the expression of HSP70 and HSP90 by inhibiting the promoter activity of HSP70 and HSP90. Pretreatment with EGCG increased the stress sensitivity of MCF-7 cells upon heat shock (44 degrees C for 1 h) or oxidative stress (H2O2, 500 microM for 24 h). Moreover, treatment with EGCG (10 mg/kg) in a xenograft model resulted in delayed tumor incidence and reduced tumor size, as well as the inhibition of HSP70 and HSP90.
expression. CONCLUSIONS: Overall, these findings demonstrate that HSP70 and HSP90 are potent molecular targets of EGCG and suggest EGCG as a drug candidate for the treatment of human cancer


Abstract: This study investigated the apoptotic regulation by green tea catechin epigallcatechin-3-gallate (EGCG) on colon cancer cells in the presence of low-dose H(2)O(2) known to exert the activation of signal pathways leading to cell proliferation. In the presence of low-dose H(2)O(2), EGCG induced apoptosis and abolished the cell-proliferative effect exhibited by low-dose H(2)O(2). This reduction of growth was accompanied by an activation of AMP-activated kinase (AMPK), a decrease in cyclooxygenase-2 (COX-2) expression and prostaglandin E(2) (PGE(2)) levels, and the induction of apoptotic markers such as p53 and poly(ADP-ribose) polymerase (PARP) cleavage. The low-dose H(2)O(2) stimulated COX-2 expression, and treating cells with synthetic AMPK activator AICAR (5-aminoimiazole-4-carboxamide-1-beta-d-ribofuranoside) resulted in greater suppression of COX-2 expression and PGE(2). By treating cells with high concentrations of the reactive oxygen species (ROS) scavenger NAC (N-acetyl-1-cysteine), the apoptotic effect of EGCG was abolished and led to suppression of AMPK and COX-2, indicating that the liberation of excessive ROS might be the upstream signal of the AMPK-COX-2 signaling pathway even in the presence of low-dose H(2)O(2)


Abstract: We previously reported that (-)-epigallocatechin gallate (EGCG) in green tea alters plasma membrane organization and causes internalization of epidermal growth factor receptor (EGFR), resulting in the suppression of colon cancer cell growth. In the present study, we investigated the detailed mechanism underlying EGCG-induced downregulation of EGFR in SW480 colon cancer cells. Prolonged exposure to EGCG caused EGFR degradation. However, EGCG required neither an ubiquitin ligase (c-Cbl) binding to EGFR nor a phosphorylation of EGFR at tyrosine residues, both of which are reportedly necessary for EGFR degradation induced by epidermal growth factor. In addition, EGCG induced phosphorylation of p38 mitogen-activated protein kinase (MAPK), a stress-inducible kinase believed to negatively regulate tumorigenesis, and the inhibition of p38 MAPK by SB203580, a specific p38 MAPK inhibitor, or the gene silencing using p38 MAPK-small interfering RNA (siRNA) suppressed the internalization and subsequent degradation of EGFR induced by EGCG. EGFR underwent a gel mobility shift upon treatment with EGCG and this was canceled by SB203580, indicating that EGCG causes EGFR phosphorylation via p38 MAPK. Moreover, EGCG caused phosphorylation of EGFR at Ser1046/1047, a site that is critical for its downregulation and this was also suppressed by SB203580 or siRNA of p38 MAPK. Taken together, our results strongly suggest that phosphorylation of EGFR at serine 1046/1047 via activation of p38 MAPK plays a pivotal role in EGCG-induced downregulation of EGFR in colon cancer cells


Abstract: We investigated the possible mechanisms of inhibition of colorectal carcinogenesis by green tea (GT) in azoxymethane-treated (AOM) Apc(Min+)/+ mice. Mice received water or a 0.6% (w/v) solution of GT as the only source of beverage. GT treatment commenced at the 8th week of age and lasted for 8 wk. The treatment caused a statistically significant reduction in the number of newly formed tumors (28%, P
< 0.05). Immunohistochemical analysis showed that GT decreased the levels of beta-catenin and its downstream target cyclin D1. To probe a mechanism, we further investigated the expression of retinoic X receptor alpha (RXR alpha) in AOM/Apc(Min/+) tumors. Our results show that RXR alpha is selectively downregulated in AOM/Apc(Min/+) mouse intestinal tumors. In contrast, other retinoic receptors including retinoic acid receptor alpha (RAR alpha), RAR beta, RXR beta, and RXR gamma were all expressed in Apc(Min/+) adenomas. Furthermore, our results show that RXR alpha downregulation is an early event in colorectal carcinogenesis and is independent of beta-catenin expression. GT significantly increased the protein levels of RXR alpha. In addition, RT-PCR analysis showed that GT induced a similar increase in the levels of RXR alpha mRNA. Genomic bisulfite treatment of colonic DNA followed by pyrosequencing of 24 CpG sites in the promoter region of RXR alpha gene showed a significant decrease in CpG methylation with GT treatment. The results suggest that a low concentration of GT is sufficient to desilence RXR alpha and inhibit intestinal tumorigenesis in the Apc(Min/+) mouse.


Abstract: BACKGROUND & AIMS: Green tea catechins are known to have anticarcinogenic effects. Epigallocatechin-3-gallate (EGCG) accounts for almost 50% of the total catechin content in green tea extract and has very potent antioxidant effects. EGCG also inhibits angiogenesis, possibly through the inhibition of proangiogenic factors including vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), which in turn, inhibits tumor growth and metastasis. However, the exact molecular mechanism by which EGCG suppresses bFGF expression is not known. Our objective was to elucidate the molecular mechanisms by which EGCG inhibits bFGF expression in colorectal cancer.

METHODS: We examined posttranslational regulation of bFGF by EGCG in human colorectal cancer cells. We also examined bFGF in intestinal tumor formation of APC(Min/+) mice with and without catechin treatment. RESULTS: The bFGF protein was quickly degraded in the presence of EGCG, but a proteasome inhibitor suppressed this degradation. EGCG was also found to increase ubiquitination of bFGF and trypsin-like activity of the 20S proteasome, thereby resulting in the degradation of bFGF protein. Furthermore, EGCG suppressed tumor formation in APC(Min/+) mice, compared with vehicle-treated mice, in association with reduced bFGF expression. CONCLUSIONS: The ubiquitin-proteasome degradation pathway contributes significantly to down-regulation of bFGF expression by EGCG. Catechin compounds have fewer adverse effects than chemotherapeutic agents and hence can be used as proof-of-concept in cancer therapeutics to suppress growth and metastasis by targeting proteins such as bFGF.


Abstract: The ability of (-)-epicatechin (EC), (-)-epigallocatechin (EGC) and (-)-epigallocatechin gallate (EGCG) to inhibit the growth of HCT 116 colorectal and Hep G2 hepatocellular carcinoma cells was examined by MTT and clonogenic assays (CA). The respective catechins inhibited the growth of HCT 116 more strongly than Hep G2. In MTT assay, IC(50) values of EGC and EGCG against HCT 116 were smaller on prolongation of the exposure times of the cells to the catechins. In CA, however, these two catechins had IC(50) values ranging between 7.6+/-0.4 and 11.2+/-0.5 microM against the same cells regardless of the exposure times. EC showed much weaker growth inhibitions relative to the two aforementioned catechins.

Abstract: Catechins are key components of teas that have antiproliferative properties. We investigated the effects of green tea catechins on intracellular signalling and VEGF induction in vitro in serum-deprived HT29 human colon cancer cells and in vivo on the growth of HT29 cells in nude mice. In the in vitro studies, (-)-epigallocatechin gallate (EGCG), the most abundant catechin in green tea extract, inhibited Erk-1 and Erk-2 activation in a dose-dependent manner. However, other tea catechins such as (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG), and (-)-epicatechin (EC) did not affect Erk-1 or 2 activation at a concentration of 30 microM. EGCG also inhibited the increase of VEGF expression and promoter activity induced by serum starvation. In the in vivo studies, athymic BALB/c nude mice were inoculated subcutaneously with HT29 cells and treated with daily intraperitoneal injections of EC (negative control) or EGCG at 1.5 mg day(-1)mouse(-1) starting 2 days after tumour cell inoculation. Treatment with EGCG inhibited tumour growth (58%), microvessel density (30%), and tumour cell proliferation (27%) and increased tumour cell apoptosis (1.9-fold) and endothelial cell apoptosis (3-fold) relative to the control condition (P< 0.05 for all comparisons). EGCG may exert at least part of its anticancer effect by inhibiting angiogenesis.

**Grape Seed Extract**


Abstract: BACKGROUND: Grape seed procyanidins (GSP) can inhibit cell proliferation and tumorigenesis, and induce apoptosis in human breast, prostate, skin and colorectal carcinoma cell lines. MATERIALS AND METHODS: In order to study the mechanism of apoptosis, four colorectal cell lines, HT-29, SW-480, LoVo and Colo 320DM, were used. GSP-treated cells were assessed for viability by trypan blue exclusion, for loss of mitochondrial membrane potential by rhodamine 123 staining, for increased apoptosis by annexin V labeling, and for changes in the levels of proteins involved in apoptosis by immunoblotting. RESULTS: GSP had no significant pro-apoptotic effect on the Colo 320DM cell line. In HT-29, SW-480 and LoVo cells, GSP (12.5-50 mg/l) inhibited proliferation in a dose-dependent manner. In these three lines, GSP treatment increased the proportion of rhodamine 123-negative cells and annexin V-positive cells, while immunoblotting revealed increased levels of apoptosis activation protein, caspase-3 and the cleavage fragment of PARP (a caspase-3 substrate), but the level of Bcl-2 did not change. CONCLUSION: GSP inhibited the proliferation of some colorectal carcinoma cell lines and was associated with an apoptotic mechanism involving a loss of mitochondrial membrane potential and caspase-3 activation in these cells.


Abstract: One approach to control colorectal cancer (CRC) is its preventive intervention by dietary agents or those consumed as supplements. However, because most of these products are often consumed by patients as a complementary and alternative medicine practice, a scientific base such as efficacy, mechanism, and standardized preparation needs to be developed. Grape seed extract (GSE) is one such supplement widely consumed by humans for its several health benefits. We reported recently that GSE inhibits CRC cell HT29 growth in culture and nude mouse xenograft. Because GSE is available commercially through different vendors, here we assessed whether GSE from 2 different manufacturers produces comparable biological effects in a panel of human CRC cell lines. Our results show that irrespective of source, GSE strongly inhibits LoVo, HT29, and SW480 cell growth, with a G1 arrest in
LoVo and HT29 cells but an S and/or G2/M arrest in SW480 cell cycle progression. GSE also induced Cip/p21 levels in all 3 cell lines. Furthermore, an induction of apoptosis was observed in all 3 cell lines by GSE. Taken together, our findings suggest that GSE could be an effective CAM agent against CRC possibly due to its strong growth inhibitory and apoptosis-inducing effects.


Abstract: PURPOSE: Accumulating evidences suggest the beneficial effects of fruit-and-vegetable consumption in lowering the risk of various cancers, including colorectal cancer. Herein, we investigated the in vitro and in vivo anticancer effects and associated mechanisms of grape seed extract (GSE), a rich source of proanthocyanidins, against colorectal cancer. EXPERIMENTAL DESIGN: Effects of GSE were examined on human colorectal cancer HT29 and LoVo cells in culture for proliferation, cell cycle progression, and apoptosis. The in vivo effect of oral GSE was examined on HT29 tumor xenograft growth in athymic nude mice. Xenografts were analyzed by immunohistochemistry for proliferation and apoptosis. The molecular changes associated with the biological effects of GSE were analyzed by Western blot analysis. RESULTS: GSE (25-100 microg/mL) causes a significant dose- and time-dependent inhibition of cell growth with concomitant increase in cell death. GSE induced G1 phase cell cycle arrest along with a marked increase in Cip1/p21 protein level and a decrease in G1 phase-associated cyclins and cyclin-dependent kinases. GSE-induced cell death was apoptotic and accompanied by caspase-3 activation. GSE feeding to mice at 200 mg/kg dose showed time-dependent inhibition of tumor growth without any toxicity and accounted for 44% decrease in tumor volume per mouse after 8 weeks of treatment. GSE inhibited cell proliferation but increased apoptotic cell death in tumors. GSE-treated tumors also showed enhanced Cip1/p21 protein levels and poly(ADP-ribose) polymerase cleavage. CONCLUSIONS: GSE may be an effective chemopreventive agent against colorectal cancer, and that growth inhibitory and apoptotic effects of GSE against colorectal cancer could be mediated via an up-regulation of Cip1/p21.


Abstract: Abnormalities in cell cycle progression provide unlimited replicative potential to cancer cells, and therefore targeting of key cell cycle regulators could be a sound cancer chemopreventive strategy. Earlier, we found that grape seed extract (GSE) increases Cip/p21 protein level and inhibits growth and induces apoptosis in human colon carcinoma HT29 cells both in vitro and in vivo. However, the mechanism of GSE-induced p21 upregulation and its role in biological efficacy of GSE are not known, which were investigated here. GSE treatment of HT29 cells resulted in a strong dose- and time-dependent phosphorylation of extracellular signal regulated kinase 1/2 (ERK1/2), consistent with p21 induction. The inhibition of sustained ERK1/2 activation by GSE using pharmacological inhibitors abrogated GSE-induced p21 upregulation. Furthermore, pretreatment of cells with N-acetylcysteine inhibited GSE-induced ERK1/2 phosphorylation as well as p21 upregulation, suggesting the involvement of GSE-induced oxidative stress as an upstream event. Consistent with this, GSE also decreased intracellular level of reduced glutathione. Next, we determined whether GSE-induced signaling regulates p21 expression at transcriptional and/or translational levels. GSE was found to increase the stability of p21 message with resultant increase in p21 protein level, but it did not alter the protein stability to a great extent. Importantly, knock-down of p21 abrogated GSE-induced G(1) arrest suggesting that p21 induction by GSE is essential for its G(1) arrest effect. Together, our results for the first time identify a central role of p21 induction and associated mechanism in GSE-induced cell cycle arrest in HT29 cells.
Soy Isoflavones


Abstract: To understand the relationship between the role of soy isoflavones and estrogen receptor (ER)-beta in colon tumorigenesis, we investigated the cellular effects of soy isoflavones (composed of genistein, daidzein, and glycitein) in DLD-1 human colon adenocarcinoma cells with or without ER-beta gene silencing by RNA interference (RNAi). Soy isoflavones decreased the expression of proliferating cell nuclear antigen (PCNA), extracellular signal-regulated kinase (ERK)-1/2, AKT, and nuclear factor (NF)-kappaB. Soy isoflavones dose-dependently caused G2/M cell cycle arrest and downregulated the expression of cyclin A. This was associated with inhibition of cyclin dependent kinase (CDK)-4 and up-regulation of its inhibitor p21(cip1) expressions. ER-beta gene silencing lowered soy isoflavone-mediated suppression of cell viability and proliferation. ERK-1/2 and AKT expressions were unaltered and NF-kappaB was modestly upregulated by soy isoflavones after transient knockdown of ER-beta expression. Soy isoflavone-mediated arrest of cells at G2/M phase and upregulation of p21(cip1) expression were not observed when ER-beta gene was silenced. These findings suggest that maintaining the expression of ER-beta is crucial in mediating the growth-suppressive effects of soy isoflavones against colon tumors. Thus upregulation of ER-beta status by specific food-borne ER-ligands such as soy isoflavones could potentially be a dietary prevention or therapeutic strategy for colon cancer.


Abstract: Epidemiologic studies suggest that nutritional phytoestrogens contained in soy are causally related to protection against hormone-dependent cancers. The incidence of colorectal cancer is at least 30% lower in women than in men in the United States. This suggests that estrogen and, conceivably, nutritional phytoestrogens are protective compounds against colorectal cancer for both sexes. Prevention of colorectal, mammary, and prostate cancer may also depend on optimal synthesis of the antimitotic prodifferentiating vitamin D hormonal metabolite 1,25-(OH)(2)-cholecalciferol (1,25-D3). Cytochrome-P450-hydroxylases responsible for synthesis (CYP27B1; 25-D3-1 alpha-hydroxylase) and catabolism (CYP24; 1,25-D3-24-hydroxylase) of 1,25-D3 are not only present in the kidney but are also expressed in human colonocytes, prostate cells, and mammary cells. In addition, levels of CYP27B1, vitamin D receptor, and estrogen receptor-beta (the high-affinity receptor for phytoestrogens) are enhanced early during human colorectal cancer, which suggests an interactive physiological defense against tumor progression. We demonstrate in human mammary and prostate cells concentration-dependent regulation of CYP27B1 and of CYP24 by genistein at 0.05-50 micromol/L. The high concentration of 50 micromol/L is very effective in eliminating CYP24 expression in prostate cancer cells. This high concentration can be achieved in vivo in the prostate by an as-yet-unknown concentative mechanism. Soy feeding, or more effectively genistein feeding, elevates CYP27B1 and reduces CYP24 expression in the mouse colon. In mice fed low nutritional calcium, CYP24 rises in parallel to enhanced colonic proliferation, and genistein counteracts both. We suggest that nutritional soy or genistein can optimize extrarenal 1,25-D3 synthesis, which could result in growth control and, conceivably, in inhibition of tumor progression.


Abstract: BACKGROUND: Previous studies suggest that sex steroids influence colorectal cancer (CRC) carcinogenesis. The oestrogen receptor beta (ERbeta) is the predominantly expressed ER in the colon and
loss of ERbeta in CRC has been associated with advanced cancer stages. METHODS: Information on vital status by the end of 2009 was obtained for 1262 CRC patients recruited between 2003 and 2007. The ERbeta expression was immunohistochemically measured and associations of ERbeta scores with overall survival (OS), disease-specific survival (DSS) and disease-free survival (DFS) were evaluated using Cox proportional hazard models adjusted for prognostic factors, such as tumour stage and second primary tumours. RESULTS: Of the 1101 tumour samples with successful measurement, 535 were ERbeta negative (48.6%), 381 (34.6%) showed moderate and 185 (16.8%) showed high ERbeta expression. Compared with high ERbeta expression, lack of ERbeta was associated with higher cancer stages as well as greater tumour extent. In multivariate analyses, ERbeta negativity was associated with an increased hazard ratio for death (HR=1.61, 95% CI 1.09-2.40, P=0.02), death attributed to CRC (HR=1.54, 95% CI 0.99-2.39, P=0.06) as well as a poorer DFS (DFS HR=1.64, 95% CI 1.23-3.36, P=0.04). The associations were stronger in stage I-III patients (OS HR=2.20, 95% CI 1.28-4.06, P=0.007, DSS HR=2.38, 95% CI 1.20-5.39, P=0.02, respectively). CONCLUSIONS: Lack of ERbeta expression is associated with advanced cancer stages and independently associated with poor survival.


Abstract: BACKGROUND: Estrogen receptor beta (ERbeta) is the predominant ER in the colorectal epithelium, whose expression is greatly reduced in colorectal cancer compared with normal colon tissue. Recent in vitro studies suggested that ERbeta may suppress tumor growth. No research was reported whether ERbeta can be used as therapeutic agent for colon cancer. METHODS: In this study, ERbeta gene constructed into adenoviral (Ad) vectors was used to treat colon cancer HCT-116 cells alone or in combination with raloxifene. In vitro and in vivo studies were conducted to investigate the therapeutic effects of ERbeta and raloxifene in HCT-116 cells. RESULTS: Our results indicated that, although Ad-ERbeta alone had no effect on the proliferation of HCT-116 cells, the combination of Ad-ERbeta with raloxifene significantly inhibited the proliferation of HCT-116 cells. The apparently apoptotic induction effects may partly explain the cytotoxicity of the two agents. The results of the study of ERbeta on migration and invasion of HCT-116 cells demonstrated that overexpression of ERbeta significantly decreased cell migration and increased invasion of cells. The antitumor efficacies of ERbeta as well as raloxifene were further investigated on HCT-116 tumor bearing mice. Results demonstrated that both Ad-ERbeta and raloxifene individually inhibited tumor growth. The combination group showed the highest inhibitory efficiency compared with other three groups. CONCLUSION: These findings demonstrated that combined administration of Ad-ERbeta with raloxifene represents a promising colon cancer therapeutic strategy.


Abstract: The present study assesses the effects of two isoflavones, genistein and glycine, and equol - a product of intestinal bacterial metabolism of dietary isoflavones, on vitamin D receptor (VDR) expression in an intestinal HT29 cell line. Genistein and glycine significantly upregulated the VDR transcription and translation in HT29 cells. The effect of equol was less pronounced. Treating HT29 cells transfected with a vector containing the VDR promoter next to a luciferase reporter with genistein or glycine resulted in significant upregulation of VDR promoter activity, in a manner similar to that induced by 17beta-estradiol (E2). Again, the effect of equol was less pronounced. VDR luciferase promoter activity was upregulated most by genistein, then by glycine and least by equol when the VDR promoter was cotransfected with estrogen receptor beta. Reporter gene and chromatin immunoprecipitation (ChIP) assays demonstrated that E2 upregulates AP-1 and Sp-1 sites present on the VDR gene. In contrast, the same assays demonstrated that the Sp-1, but not AP-1, site is induced by the phytoestrogens. Similar to
E2, genistein, glycine and the isoflavonoid metabolite equol induced higher concentrations of intracellular free calcium, an event that could provide the upstream mechanism(s) induced by E2 and phytoestrogens that initiates the signaling cascade which results in the activation of extracellular signal-regulated kinase (ERK) signaling pathways and modulation of Sp-1 sites of the VDR gene, and culminates in enhanced VDR expression.


Abstract: Several strands of evidence indicate that oestrogens exert a protective role against the development of colon cancer through indirect and direct effects on colonic epithelium. Oestrogen receptor beta (ERbeta), the predominant ER subtype in human colon, is significantly decreased in colonic tumours compared with normal mucosa suggesting a potential role in the regulation of colon tumour growth. To investigate this hypothesis we engineered human colon cancer ERLalpha-negative HCT8 cells in order to obtain ERbeta protein over-expression. Stably transfected cells were cloned and ERbeta expression and functionality were monitored by RT-PCR, Western blotting and transactivation in an assay using oestrogen-responsive reporter constructs. Over-expression of ERbeta inhibited cell proliferation and increased cell adhesion in a ligand-independent manner. Its constitutive activation is possibly due to cross-talk with intracellular signalling pathways, as epidermal growth factor and IGF-I were able to induce ERbeta transactivation. A possible mechanism by which ERbeta over-expression inhibits proliferation in HCT8 cells is by modulation of some key regulators of the cell cycle; there is a decrease in cyclin E and an increase in the cdk inhibitor p21CIP1. In fact, flow cytometry analysis provided evidence for blocking of the G1-S phase progression induced by ERbeta over-expression. The magnitude of this effect was affected by the level of ERbeta expression. These results provide the first direct evidence that ERbeta plays an important role in colon cancer as a regulator of cell proliferation through the control of key cell cycle modulators and arrest in G1-S phase transition. These findings are compatible with the hypothesis that the loss of ERbeta expression could be one of the events involved in the development or progression of colon cancer.

**Lycopene**


Abstract: A previous study indicated that lycopene could significantly inhibit the proliferation of human colon cancer cells in vitro. However, the in vivo anticancer effects of lycopene against colon cancer have not been demonstrated yet. Therefore, this study investigated whether consumption of lycopene could prevent the growth and progression of colorectal tumor in a mouse xenograft model. Bioluminescence imaging, histopathological, immunofluorescence (IFC), and immunohistochemical (IHC) staining results indicated that lycopene could effectively suppress the growth and progression of colon cancer in tumor-bearing mice. The results demonstrated that lycopene significantly suppressed the nuclear expression of PCNA and beta-catenin proteins in tumor tissues. Consumption of lycopene could also augment the E-cadherin adherent molecule and nuclear levels of cell cycle inhibitor p21(CIP1/WAF1) protein. The chemopreventive effects of lycopene were associated with suppression of COX-2, PGE(2), and phosphorylated ERK1/2 proteins. Furthermore, the inhibitory effects of lycopene were inversely correlated with the plasma levels of matrix metalloproteinase 9 (MMP-9) in tumor-bearing mice. These results suggested that lycopene could act as a chemopreventive agent against the growth and progression of colorectal cancer in a mouse xenograft model.

Abstract: Several studies indicated that people who live in the Mediterranean region have very low rates of chronic diseases such as cardiovascular disease and cancer. It is well known that Mediterranean-style diet is rich in vegetables, tomato, fruit, fish and olive oil. These important dietary components may contribute to lower risk of cancer. Lycopene, a major component in tomato, exhibited potential anticarcinogenic activity. Previous studies showed that consumption of fish containing eicosapentaenoic acid (EPA) correlated with reduced risk of cancer. However, the combined effects of lycopene and EPA on the proliferation of human colon cancer have not been studied well yet. Thus, we investigated the anticancer properties and therapeutic potential of lycopene and EPA in human colon cancer HT-29 cells. In this study, we determined the combined effects of lycopene and EPA on the proliferation of human colon cancer cells. We demonstrated that low concentration of lycopene and EPA could synergistically inhibit the proliferation of colon cancer cells. The inhibitory mechanism was associated with suppression of phosphatidylinositol 3-kinase/Akt signaling pathway. Furthermore, treatment of lycopene and EPA also synergistically blocked the activation of downstream mTOR molecule. Immunocytochemical staining results revealed that lycopene and EPA could also up-regulate the expression of apoptotic proteins such as Bax and Fas ligand to suppress cell survival. In conclusion, our novel findings suggest that lycopene and EPA synergistically inhibited the growth of human colon cancer cells at low concentration. The inhibitory effects of lycopene and EPA on cell proliferation of human colon cancer HT-29 cells were, in part, associated with the down-regulation of the PI-3K/Akt/mTOR signaling pathway.


Abstract: The aberrant regulation of the phosphoinositide 3-kinase/Akt survival signaling pathway in cancer has prompted significant interest in suppression of this pathway to treat cancer. Previous studies identified an important role for phosphoinositide 3-kinase/Akt in colon cancer progression. Lycopene, a major component in tomato, exhibited potential anti-carcinogenic activity. Consumption of tomato has been associated with reduced risk of several types of human cancer. However, the inhibitory mechanisms of lycopene on the proliferation of human colon cancer have not been studied well yet. Thus we investigated the inhibitory effects of lycopene on the Akt signaling pathway in human colon cancer HT-29 cells. Lycopene inhibited cell proliferation in human colon cancer HT-29 cells with a IC(50) value of 10 microM. Lycopene treatment suppressed Akt activation and non-phosphorylated beta-catenin protein level in human colon cancer cells. Immunocytochemical results indicated that lycopene increased the phosphorylated form of beta-catenin proteins. These effects were also associated with reduced promoter activity and protein expression of cyclin D1. Furthermore, lycopene significantly increased nuclear cyclin-dependent kinase inhibitor p27(kip)abundance and inhibited phosphorylation of the retinoblastoma tumor suppressor protein in human colon cancer cells. In conclusion, lycopene inhibited cell proliferation of human colon cancer cells via suppression of the Akt signaling pathway and downstream targeted molecules.


Abstract: BACKGROUND: Higher circulating insulin-like growth factor I (IGF-I) concentrations have been related to a greater risk of cancer. Lycopene intake is inversely associated with cancer risk, and experimental studies have shown that it may affect the IGF system, possibly through an effect on IGF-binding proteins (IGFBPs). OBJECTIVE: The objective of our study was to investigate the effect of an 8-
wk supplementation with tomato-derived lycopene (30 mg/d) on serum concentrations of total IGF-I, IGF-II, IGFBP-1, IGFBP-2, and IGFBP-3. DESIGN: We conducted a randomized, placebo-controlled, double-blinded crossover study in 40 men and 31 postmenopausal women with a family history of colorectal cancer, a personal history of colorectal adenoma, or both. RESULTS: Lycopene supplementation significantly (P = 0.01) increased serum IGFBP-1 concentrations in women (median relative difference between serum IGFBP-1 concentrations after lycopene supplementation and after placebo, 21.7%). Serum IGFBP-2 concentrations were higher in both men and women after lycopene supplementation than after placebo, but to a lesser extent (mean relative difference 8.2%; 95% CI: 0.7%, 15.6% in men and 7.8%; 95% CI: -5.0%, 20.6% in women). Total IGF-I, IGF-II, and IGFBP-3 concentrations were not significantly altered by lycopene supplementation. CONCLUSIONS: This is the first study known to show that lycopene supplementation may increase circulating IGFBP-1 and IGFBP-2 concentrations. Because of high interindividual variations in IGFBP-1 and IGFBP-2 effects, these results should be confirmed in larger randomized intervention studies.


**Abstract:** Epidemiological studies have shown that high serum levels of insulin-like growth factor-I are associated with an increased risk of colon and other types of cancer. The aim of this study was to determine whether short intervention with dietary tomato lycopene extract will affect serum levels of the insulin-like growth factor system components in colon cancer patients. The study had a double-blind, randomized, placebo-controlled design. Colon cancer patients (n=56), candidates for colectomy, were recruited from the local community a few days to a few weeks before surgery. Personal and medical data were recorded. Plasma concentrations of insulin-like growth factor-I and II and insulin-like growth factor-I-binding protein-3 were assayed by routine laboratory methods. Lycopene was assayed by high-performance liquid chromatography. Plasma lycopene levels increased by twofold after supplementation with tomato lycopene extract. In the placebo-treated group, there was a small nonsignificant increase in lycopene plasma levels. The plasma concentration of insulin-like growth factor-I decreased significantly by about 25% after tomato lycopene extract supplementation as compared with the placebo-treated group (P<0.05). No significant change was observed in insulin-like growth factor-I-binding protein-3 or insulin-like growth factor-II, whereas the insulin-like growth factor-I/insulin-like growth factor-I-binding protein-3 molar ratio decreased significantly (P<0.05). Given that high plasma levels of insulin-like growth factor-I have been suggested as a risk factor for various types of cancer including colon cancer, the results support our suggestion that tomato lycopene extract has a role in the prevention of colon and possibly other types of cancer.

**Melatonin**


**Abstract:** Drug-target interactions can be modified by adding modulatory agents to increase treatment efficacy and clinical outcome. Combination chemotherapy has become increasingly important because drugs acting synergistically can achieve therapeutic effects at substantially lower doses and with a limited spectrum of side effects. Irinotecan, known as one of the camptothecin analogs, has shown a broad spectrum of antitumor activity against various malignancies. In this study, we evaluated the effect of melatonin on the genotoxic activity of irinotecan in healthy human lymphocytes and a lung cancer cell line (A549) and a colorectal adenocarcinoma cell line (HT29) in vitro. Irinotecan, as a single agent, was shown to induce DNA damage in all types of analyzed cells. The combination of melatonin at
concentrations of 50 μM with increasing doses of irinotecan (7.5, 15, 30, and 60 μM) resulted in an increase in the amount of DNA damage in A549 and HT29 cancer cells, but was not effective in inducing DNA damage in healthy human lymphocytes. Analysis of the efficacy of DNA repair, performed after 60 and 120 minutes of postincubation, showed the gradual decrease of DNA percentage in comet tails during repair postincubation in all experimental samples. Our results indicate that melatonin can modulate the genotoxic activity of irinotecan and DNA repair efficacy in human cancer cells in vitro. These findings may be supportive for the optimization of therapeutic efficacy in irinotecan treatment.


Abstract: Angiogenesis is an important mediator of tumor progression. As tumors expand, diffusion distances from the existing vascular supply increases, resulting in hypoxia in the cancer cells. Sustained expansion of a tumor mass requires new blood vessel formation to provide rapidly proliferating tumor cells with an adequate supply of oxygen and nutrients. The key regulator of hypoxia-induced angiogenesis is the transcription factor known as hypoxia-inducible factor (HIF)-1. HIF-1alpha is stabilized by hypoxia-induced reactive oxygen species (ROS) and enhances the expression of several types of hypoxic genes, including that of the angiogenic activator known as vascular endothelial cell growth factor (VEGF). In this study, we found that melatonin, a small lipophilic molecule secreted primarily by the pineal gland, destabilizes hypoxia-induced HIF-1alpha protein levels in the HCT116 human colon cancer cell line. This destabilization of HIF-1alpha resulted from the antioxidant activity of melatonin against ROS induced by hypoxia. Moreover, under hypoxia, melatonin suppressed HIF-1 transcriptional activity, leading to a decrease in VEGF expression. Melatonin also blocked in vitro tube formation and invasion and migration of human umbilical vein endothelial cells induced by hypoxia-stimulated conditioned media of HCT116 cells. These findings suggest that melatonin could play a pivotal role in tumor suppression via inhibition of HIF-1-mediated angiogenesis.


Abstract: The antiproliferative and proapoptotic properties of melatonin in human colon cancer cells in culture were recently reported. To address the mechanisms involved in these actions, HT-29 human colon cancer cells were cultured in RPMI 1640 medium supplemented with fetal bovine serum at 37 degrees C. Cell proliferation was assessed by the incorporation of [3H]-thymidine into DNA. Cyclic nucleotide levels, nitrite concentration, glutathione peroxidase and reductase activities, and glutathione levels were assessed after the incubation of these cells with the following drugs: melatonin membrane receptor agonists 2-iodo-melatonin, 2-iodo-N-butanoyl-5-methoxytriptamine, 5-methoxycarbonylamino-N-acetyltryptamine (GR-135,531), and the antagonists luzindole, 4-phenyl-2-propionamidotetralin, and prazosin; the melatonin nuclear receptor agonist CGP 52608, and four synthetic kynurenines analogs to melatonin 2-acetamide-4-(3-methoxyphenyl)-4-oxobutyric acid, 2-acetamide-4-(2-amino-5-methoxyphenyl)-4-oxobutyric acid, 2-butyramide-4-(3-methoxyphenyl)-4-oxobutyric acid and 2-butyramide-4-(2-amino-5-methoxyphenyl)-4-oxobutyric acid. The results show that the membrane receptors are not necessary for the antiproliferative effect of melatonin and the participation of the nuclear receptor in this effect is suggested. Moreover, the antioxidative and anti-inflammatory actions of melatonin, counteracting the oxidative status and reducing the production of nitric oxide by cultured HT-29 cells seem to be directly involved in the oncostatic properties of melatonin. Some of the synthetic kynurenines exert higher antiproliferative effects than melatonin. The results reinforce the clinical interest of melatonin due to the different mechanisms involved in its oncostatic role, and suggest a new synthetic pathway to obtain melatonin agonists with clinical applications to oncology.

Abstract: It is known since many years that the pineal hormone melatonin (MLT) may play anticancer activity through several mechanisms, including antiproliferative and immunostimulating effects. Moreover, it exerts an important antioxidant action. Therefore, MLT could be useful in the treatment of human neoplasms, either alone or in association with chemotherapy. The present study was performed to evaluate the influence of a concomitant MLT administration on efficacy and toxicity of several chemotherapeutic combinations in metastatic solid tumor patients, suffering from non-small cell lung cancer (NSCLC) or gastrointestinal tumors. The study included 370 patients who were randomized to receive chemotherapy alone or chemotherapy plus MLT (20 mg/day orally in the evening every day). NSCLC patients received cisplatin (CDDP) plus etoposide or CDDP plus gemcitabine. Colorectal cancer patients were treated with oxaliplatin plus 5-fluorouracil (5-FU), or weekly CPT-11 or 5-FU and folates (FA). Finally, gastric cancer patients received CDDP, epirubicin, 5-FU and FA or weekly 5-FU plus FA. The overall tumor regression rate achieved in patients concomitantly treated with MLT was significantly higher than that found in those treated with chemotherapy alone. Moreover, the 2-year survival rate was significantly higher in patients concomitantly treated with MLT. These results confirm in human the anticancer therapeutic properties of the pineal hormone MLT, which may enhance the efficacy of the standard anticancer chemotherapies.


Abstract: Recent advances in immunobiological knowledge have suggested the possibility of enhancing the therapeutic activity of various chemotherapeutic agents by a concomitant administration of antioxidant drugs and/or immunomodulating neurohormones. In particular, the pineal neurohormone melatonin (MLT), which is able to exert both antioxidant and immunomodulating effects, has been proven to enhance the efficacy of various chemotherapeutic drugs, namely cisplatin, anthracyclines and 5-fluorouracil, whereas at present there are no data about its possible influence on cytotoxic drugs effective in the treatment of colon cancer other than 5-fluorouracil, such as irinotecan (CPT-11). The present study was performed to evaluate the influence of a concomitant administration of MLT on CPT-11 therapeutic activity in metastatic colorectal cancer. The study included 30 metastatic colorectal cancer patients progressing after at least one previous chemotherapeutic line containing 5-fluorouracil, who were randomized to be treated with CPT-11 alone or CPT-11 plus MLT. According to a weekly low-dose schedule, CPT-11 was given i.v. at 125 mg/m2/week for 9 consecutive weeks. MLT was administered orally at 20 mg/day during the dark period of the day. No complete response was observed. A partial response (PR) was achieved in 2 out of 16 patients treated with CPT-11 alone and in 5 out of 14 patients concomitantly treated with MLT. Moreover, a stable disease (SD) was obtained in 5 out of 16 patients treated with CPT-11 alone and in 7 out of 14 patients treated with CPT-11 plus MLT. Therefore, the percent of disease-control achieved in patients concomitantly treated with MLT was significantly higher than that observed in those treated with chemotherapy alone (12 out of 14 vs 7 out of 16, p < 0.05). The only important toxicity was diarrhoea grade 3-4, which occurred in 6 out of 16 patients treated with CPT-11 alone and in 4 out of 14 patients treated with CPT-11 plus MLT, which required a 50% dose reduction. However, taken together, patients treated with CPT-11 at 50% of the planned dose showed a percent of disease control comparable to that achieved in patients who had no dose reduction (6 out of 10 vs 13 out of 20). This preliminary study shows that the efficacy of weekly low-dose CPT-11 in pretreated metastatic colorectal cancer patients may be enhanced by a concomitant daily administration of the pineal hormone MLT, according to the results previously reported for other chemotherapeutic agents. Moreover,
since the dose reduction of CPT-11 does not influence its efficacy, the dose of CPT-11 for successive studies might be not greater than 70 mg/m2


Abstract: OBJECTIVES: The anticancer activity of the indole melatonin has been explained to be due to its immunomodulatory, anti-proliferative and anti-oxidant effects, whereas at present no data are available about its possible influence on the angiogenesis, which has been shown to be one of the main biological mechanisms responsible for tumor dissemination. Vascular endothelial growth factor (VEGF) is the most active angiogenic factor, and the evidence of abnormally high blood levels or VEGF has been proven to be associated with poor prognosis in cancer patients. To investigate the influence of melatonin on angiogenesis, in this preliminary study we have evaluated the effects of melatonin therapy on VEGF blood levels in advanced cancer patients. MATERIAL & METHODS: The study included 20 metastatic patients, who progressed on previous conventional antitumor therapies and for whom no other effective treatment was available. Melatonin was given orally at 20 mg/day in the evening for at least 2 months. Serum levels of VEGF were measured by an enzyme immunoassay on venous blood samples collected at 15-day intervals. RESULTS: The clinical response consisted of minor response (MR) in 2, stable disease (SD) in 6 and progressive disease (PD) in the remaining 12 patients. VEGF mean levels decreased on therapy, without, however, statistical differences with respect to the pre-treatment values. In contrast, by evaluating changes in VEGF levels in relation to the clinical response, non-progressing patients (MR + SD) showed a significant decline in VEGF mean concentrations, whereas no effect was achieved in progressing patients. CONCLUSIONS: This study, by showing that melatonin-induced control or the neoplastic growth is associated with a decline in VEGF secretion, would suggest that the pineal hormone may control tumor growth at least in part by acting as a natural anti-angiogenic molecule, with a following opposition or angiogenesis-dependent cancer proliferation


Abstract: The effect of melatonin on inhibition of cell growth was studied in CT-26, a murine colon carcinoma-derived cell line. Cells growing in exponential phase were exposed to low (10(-7)-10(-10) M) and high doses (1, 2 and 3 x 10(-3) M) of melatonin during 24 h. Synthesis of DNA was measured by 5-bromo-2'-deoxyuridine incorporation. There was no effect at low doses, but a statistically significant correlation was found between the decrease in DNA synthesis and the dose of melatonin used (r = -0.52, P < 0.001). This implied the following percentages of inhibition: 1 mM, 22%; 2 mM, 25%; 3 mM, 47%. Potential cell membrane damage by high doses of melatonin was investigated by lactate dehydrogenase measurement and no significant levels were observed. Analysis with a single saturation technique showed no detectable oestradiol receptors in this cell type; therefore, we can assume that the effects occurring with the addition of melatonin were not mediated by modulation of this hormone on oestrogen receptors. The decreases in cell growth were attributed to a moderate, but significant antiproliferative action of melatonin on this non-hormone-dependent cell line


Abstract: Chemotherapy with 5-fluorouracil (5-FU) and folates represents the first-line standard therapy for metastatic colorectal cancer, whereas at present there is no conventional second-time treatment. Because of its importance in generating an effective anticancer immune response, interleukin-2 (IL-2) could constitute a new promising therapy of advanced colon cancer. Generally, IL-2 may determine tumor
regressions in colon cancer only when it is given at high toxic doses. Our preliminary studies have shown that the pineal hormone melatonin may amplify IL-2 activity, which becomes active also at low doses in several tumor histotypes. On the basis, we have performed a clinical trial to evaluate the impact of low-dose IL-2 plus melatonin on the survival time in metastatic colon cancer, which progressed in response to 5-FU plus folates. The study included 50 metastatic colorectal cancer patients, who did not respond or progressed after initial response to first-line chemotherapy with 5-FU and folates. Patients were randomized to receive supportive care alone or low-dose subcutaneous IL-2 (3 million IU/day for 6 days/week for 4 weeks) plus melatonin (40 mg/day orally). No spontaneous tumor regression occurred in patients receiving supportive care alone. A partial response was achieved in 3/25 patients treated with immunotherapy. Percent survival at 1 year was significantly higher in patients treated with immunotherapy than in those treated with supportive care alone (9/25 vs. 3/25, p < 0.05). This study suggests that low-dose subcutaneous IL-2 plus melatonin may be effective as a second-line therapy to induce tumor regression and to prolong percent survival at 1 year in metastatic colorectal cancer patients progressing under 5-FU and folates.


Abstract: Since there is no effective second line chemotherapy in colorectal cancer resistant to fluorouracil, this study was carried out to evaluate the therapeutic activity of the pineal hormone melatonin, which has appeared to have antineoplastic activity in some experimental conditions, in patients with metastatic colorectal carcinoma who did not respond to fluorouracil. The study included 14 patients (8 men, 6 women; mean age 58 years). Melatonin was given intramuscularly at a daily dose of 20 mg at 3.00 p.m. for 2 months; after that, melatonin therapy was continued at 10 mg/day orally in responder patients, in those with stable disease and/or an evident improvement in PS. One patient had a minor response; 3 other patients had a stable disease, whereas the other 10 cases progressed. An evident improvement in PS was seen in 5/14 (36%) patients. These preliminary results show that melatonin does not have important antitumor activity in metastatic colorectal cancer patients resistant to fluorouracil. However, the pineal hormone could be usefully employed as supportive care to improve the quality of life in these patients for whom no standard treatment is yet available.


Abstract: Plasma melatonin was determined in samples of patients with colorectal carcinoma and in controls using an iodinated radioimmunoassay. Both groups showed large individual variability in absolute melatonin levels. However, during the night, melatonin concentration in cancer patients was significantly lower than in controls.

**Low-Dose Aspirin**


Abstract: BACKGROUND: Daily aspirin reduces the long-term incidence of some adenocarcinomas, but effects on mortality due to some cancers appear after only a few years, suggesting that it might also reduce growth or metastasis. We established the frequency of distant metastasis in patients who
developed cancer during trials of daily aspirin versus control. METHODS: Our analysis included all five large randomised trials of daily aspirin (\(\geq 75\) mg daily) versus control for the prevention of vascular events in the UK. Electronic and paper records were reviewed for all patients with incident cancer. The effect of aspirin on risk of metastases at presentation or on subsequent follow-up (including post-trial follow-up of in-trial cancers) was stratified by tumour histology (adenocarcinoma vs other) and clinical characteristics. FINDINGS: Of 17,285 trial participants, 987 had a new solid cancer diagnosed during mean in-trial follow-up of 6.5 years (SD 2.0). Allocation to aspirin reduced risk of cancer with distant metastasis (all cancers, hazard ratio [HR] 0.64, 95% CI 0.48-0.84, p=0.001; adenocarcinoma, HR 0.54, 95% CI 0.38-0.77, p=0.0007; other solid cancers, HR 0.82, 95% CI 0.53-1.28, p=0.39), due mainly to a reduction in proportion of adenocarcinomas that had metastatic versus local disease (odds ratio 0.52, 95% CI 0.35-0.75, p=0.0006). Aspirin reduced risk of adenocarcinoma with metastasis at initial diagnosis (HR 0.69, 95% CI 0.50-0.95, p=0.02) and risk of metastasis on subsequent follow-up in patients without metastasis initially (HR 0.45, 95% CI 0.28-0.72, p=0.0009), particularly in patients with colorectal cancer (HR 0.26, 95% CI 0.11-0.57, p=0.0008) and in patients who remained on trial treatment up to or after diagnosis (HR 0.31, 95% CI 0.15-0.62, p=0.0009). Allocation to aspirin reduced death due to cancer in patients who developed adenocarcinoma, particularly in those without metastasis at diagnosis (HR 0.50, 95% CI 0.34-0.74, p=0.0006). Consequently, aspirin reduced the overall risk of fatal adenocarcinoma in the trial populations (HR 0.65, 95% CI 0.53-0.82, p=0.0002), but not the risk of other fatal cancers (HR 1.06, 95% CI 0.84-1.32, p=0.64; difference, p=0.003). Effects were independent of age and sex, but absolute benefit was greatest in smokers. A low-dose, slow-release formulation of aspirin designed to inhibit platelets but to have little systemic bioavailability was as effective as higher doses. INTERPRETATION: That aspirin prevents distant metastasis could account for the early reduction in cancer deaths in trials of daily aspirin versus control. This finding suggests that aspirin might help in treatment of some cancers and provides proof of principle for pharmacological intervention specifically to prevent distant metastasis. FUNDING: None


Abstract: BACKGROUND: High-dose aspirin (\(\geq 500\) mg daily) reduces long-term incidence of colorectal cancer, but adverse effects might limit its potential for long-term prevention. The long-term effectiveness of lower doses (75-300 mg daily) is unknown. We assessed the effects of aspirin on incidence and mortality due to colorectal cancer in relation to dose, duration of treatment, and site of tumour. METHODS: We followed up four randomised trials of aspirin versus control in primary (Thrombosis Prevention Trial, British Doctors Aspirin Trial) and secondary (Swedish Aspirin Low Dose Trial, UK-TIA Aspirin Trial) prevention of vascular events and one trial of different doses of aspirin (Dutch TIA Aspirin Trial) and established the effect of aspirin on risk of colorectal cancer over 20 years during and after the trials by analysis of pooled individual patient data. RESULTS: In the four trials of aspirin versus control (mean duration of scheduled treatment 6.0 years), 391 (2.8%) of 14 033 patients had colorectal cancer during a median follow-up of 18.3 years. Allocation to aspirin reduced the 20-year risk of colon cancer (incidence hazard ratio [HR] 0.76, 0.60-0.96, p=0.02; mortality HR 0.65, 0.48-0.88, p=0.005), but not rectal cancer (0.90, 0.63-1.30, p=0.58; 0.80, 0.50-1.28, p=0.35). Where subsite data were available, aspirin reduced risk of cancer of the proximal colon (0.45, 0.28-0.74, p=0.001; 0.34, 0.18-0.66, p=0.001), but not the distal colon (1.10, 0.73-1.64, p=0.66; 1.21, 0.66-2.24, p=0.54; for incidence difference p=0.04, for mortality difference p=0.01). However, benefit increased with scheduled duration of treatment, such that allocation to aspirin of 5 years or longer reduced risk of proximal colon cancer by about 70% (0.35, 0.20-0.63; 0.24, 0.11-0.52; both p<0.0001) and also reduced risk of rectal cancer (0.58, 0.36-0.92, p=0.02; 0.47, 0.26-0.87, p=0.01). There was no increase in benefit at doses of aspirin greater than 75 mg daily, with an absolute reduction of 1.76% (0.61-2.91; p=0.001) in 20-year risk of any fatal colorectal cancer after 5-years scheduled treatment with 75-300 mg daily. However, risk of fatal colorectal cancer was higher on 30 mg versus 283 mg daily on long-term follow-up of the Dutch TIA trial.
(odds ratio 2.02, 0.70-6.05, p=0.15). INTERPRETATION: Aspirin taken for several years at doses of at least 75 mg daily reduced long-term incidence and mortality due to colorectal cancer. Benefit was greatest for cancers of the proximal colon, which are not otherwise prevented effectively by screening with sigmoidoscopy or colonoscopy. FUNDING: None

Vitamin D


Abstract: BACKGROUND: Individuals with higher blood 25-hydroxyvitamin D [25(OH)D] levels have a lower risk of developing colorectal cancer (CRC), but the influence of 25(OH)D on mortality after CRC diagnosis is unknown. METHODS: The association between prediagnostic 25(OH)D levels and CRC-specific (N = 444) and overall mortality (N = 541) was prospectively examined among 1,202 participants diagnosed with CRC between 1992 and 2003 in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. Multivariable Cox proportional hazards models were used to calculate HRs and corresponding 95% CIs according to 25(OH)D quintiles and genetic variation within the VDR and CASR genes. Potential dietary, lifestyle, and metabolic effect modifiers were also investigated. RESULTS: There were 541 deaths, 444 (82%) due to CRC. Mean follow-up was 73 months. In multivariable analysis, higher 25(OH)D levels were associated with a statistically significant reduction in CRC-specific (P(trend) = 0.04) and overall mortality (P(trend) = 0.01). Participants with 25(OH)D levels in the highest quintile had an adjusted HR of 0.69 (95% CI: 0.50-0.93) for CRC-specific mortality and 0.67 (95% CI: 0.50-0.88) for overall mortality, compared with the lowest quintile. Except for a possible interaction by prediagnostic dietary calcium intake (P(interaction) = 0.01), no other potential modifying factors related to CRC survival were noted. The VDR (FokI and BsmI) and CASR (rs1801725) genotypes were not associated with survival. CONCLUSIONS: High prediagnostic 25(OH)D levels are associated with improved survival of patients with CRC. IMPACT: Our findings may stimulate further research directed at investigating the effects of blood vitamin D levels before, at, and after CRC diagnosis on outcomes in CRC patients.


Abstract: PURPOSE: Previous studies have suggested that higher plasma 25-hydroxyvitamin D(3) [25(OH)D] levels are associated with decreased colorectal cancer risk and improved survival, but the prevalence of vitamin D deficiency in advanced colorectal cancer and its influence on outcomes are unknown. PATIENTS AND METHODS: We prospectively measured plasma 25(OH)D levels in 515 patients with stage IV colorectal cancer participating in a randomized trial of chemotherapy. Vitamin D deficiency was defined as 25(OH)D lower than 20 ng/mL, insufficiency as 20 to 29 ng/mL, and sufficiency as >/= 30 ng/mL. We examined the association between baseline 25(OH)D level and selected patient characteristics. Cox proportional hazards models were used to calculate hazard ratios (HR) for death, disease progression, and tumor response, adjusted for prognostic factors. RESULTS: Among 515 eligible patients, 50% of the study population was vitamin D deficient, and 82% were vitamin D insufficient. Plasma 25(OH)D levels were lower in black patients compared to white patients and patients of other race (median, 10.7 v 21.1 v 19.3 ng/mL, respectively; P < .001), and females compared to males (median, 18.3 v 21.7 ng/mL, respectively; P = .0005). Baseline plasma 25(OH)D levels were not associated with patient outcome, although given the distribution of plasma levels in this cohort, statistical power for survival analyses were limited. CONCLUSION: Vitamin D deficiency is highly prevalent.
among patients with stage IV colorectal cancer receiving first-line chemotherapy, particularly in black and female patients.


Abstract: BACKGROUND: Recently, serum 25-hydroxyvitamin D (25OHD) levels were shown to be associated with the survival of patients with colorectal cancer. However, 25OHD levels were measured a median of 6 years before diagnosis or were predicted levels. In this study, we directly measured serum 25OHD levels at surgery and examined the association with survival among patients with colorectal cancer. METHODS: We started a prospective cohort study to find prognostic factors in patients with colorectal cancer from 2003 to 2008 and stored serum samples and clinical data. As part of a post-hoc analysis, serum 25OHD levels were measured by radioimmunoassay. Association between overall survival and serum 25OHD levels were computed using the Cox proportional hazard model adjusted for month of serum sampling as well as age at diagnosis, gender, cancer stage, residual tumor after surgery, time period of surgery, location of tumor, adjuvant chemotherapy and number of lymph nodes with metastasis at surgery. Unadjusted and adjusted hazard ratios (HR) and 95% confidence intervals (95% CI) were determined. RESULTS: Serum 25OHD levels were measured in 257 patients. Only 3% had sufficient levels (30 ng/ml and greater). Based on month of blood sampling, an annual oscillation of 25OHD levels was seen, with levels being lower in spring and higher in late summer. Higher 25OHD levels were associated with better overall survival under multi-variate analysis (HR, 0.91: 95% CI, 0.84 to 0.99, P = 0.027). CONCLUSIONS: These results suggest that higher 25OHD levels at surgery may be associated with a better survival rate of patients with colorectal cancer.


Abstract: Several non-hypercalcemic analogs of 1alpha,25-dihydroxyvitamin D3 (1,25(OH)(2)D(3)) show antitumor activity in a subset of cancer patients. High vitamin D receptor (VDR) expression, which is associated with good prognosis but is lost during tumor progression. We show that the SNAIL transcription factor represses VDR gene expression in human colon cancer cells and blocks the antitumor action of EB1089, a 1,25(OH)(2)D(3) analog, in xenografted mice. In human colon cancers, elevated SNAIL expression correlates with downregulation of VDR.


Abstract: OBJECTIVE: To investigate whether prognosis of breast-, colon- and prostate cancer may be related to vitamin D(3), induced from solar ultra-violet (UV) radiation, through studies on geographical and seasonal variations in UV radiation. METHODS: This study includes 115,096 cases of breast-, colon- or prostate cancer, diagnosed between 1964 and 1992. Among these, 45,667 deaths due to the cancer were registered. On the basis of a north-south gradient in solar UV radiation and geographical climatic differences, Norway was divided into eight residential regions. According to seasonal variations in UV radiation, four periods of diagnosis during the year were used. Case fatality according to residential region and to season of diagnosis was estimated using Cox regression. The effects of occupational sun exposure, childbirth pattern and educational level were also evaluated. RESULTS: No geographic variation in case fatality was observed for the three cancer types studied. A significant variation in prognosis by season of diagnosis was observed. Diagnoses during summer and fall, the seasons with the highest level of vitamin D(3), revealed the lowest risk of cancer death. CONCLUSION: The results suggest that a high level of vitamin D(3) at the time of diagnosis, and thus, during cancer treatment, may improve prognosis of the three cancer types studied.

Abstract: BACKGROUND: Epidemiologic studies have demonstrated an inverse correlation between dietary calcium and vitamin D intake and the incidence of colorectal carcinoma. Elevated serum levels of 25-hydroxyvitamin D3 (25-OH-D3) are associated with a major reduction in the incidence of this neoplasm. The reduction in tumor size and number induced by calcium supplements in an experimental carcinogenesis model was neutralized by vitamin D3 deficiency. To the authors’ knowledge, vitamin D serum levels have never been determined previously in colorectal carcinoma patients. They compared serum 1,25-dihydroxyvitamin D3 (1,25(OH)2D3), 25-OH-D3, and parathyroid hormone (PTH) levels of colorectal carcinoma patients with those of healthy controls. METHODS: Serum 1,25(OH)2D3, 25-OH-D3, and PTH levels were determined in 84 colorectal carcinoma patients (10 with Stage I, 29 with Stage II, 25 with Stage III, and 20 with Stage IV) and 30 healthy controls, all of whom were normocalcemic and not taking calcium or vitamin D supplements. RESULTS: 25-OH-D3 serum levels were higher in cancer patients than controls, irrespective of stage. Serum 1,25(OH)2D3 decreased with advancing stage: 73 +/- 18, 48 +/- 16, 39 +/- 12, 34 +/- 13, and 75 +/- 20 pg/mL in Stages I, II, III, IV, and controls, respectively. There was a corresponding increase in serum PTH levels: 58.0 +/- 9.4, 73.7 +/- 14.4, 79.0 +/- 21.3, 100.4 +/- 30.9, and 51.2 +/- 3.9 pg/mL in Stages I, II, III, IV, and controls, respectively. Serum vitamin D metabolite levels did not correlate with gender, age, tumor localization, or histologic grade.

CONCLUSIONS: An inverse correlation between serum levels of the active metabolite of vitamin D and colorectal carcinoma stage has been demonstrated for the first time, to the authors’ knowledge, in colorectal carcinoma patients. Because 1,25(OH)2D3 has been shown to inhibit proliferation of colonic epithelial cells, decreased serum levels may facilitate the growth of colorectal carcinoma and influence its biologic behavior.


Abstract: PURPOSE: We investigated the association between serum levels of 25-hydroxyvitamin D (25-OHD) and risk of death in Norwegian cancer patients. METHODS: The study population was 658 patients with cancers of the breast (n = 251), colon (n = 52), lung (n = 210), and lymphoma (n = 145), obtained from JANUS, a population-based serum bank in Norway. Serum samples were collected within 90 days of cancer diagnosis and were analyzed for 25-OHD. Patients were diagnosed during 1984-2004 and were followed for death throughout 2008. We used Cox regression models to assess the relationship between serum 25-OHD and risk of death. RESULTS: Three hundred and ninety-nine patients died during follow-up, of whom 343 (86%) died from cancer. Adjusted for sex, age at diagnosis, and season of blood sampling, patients with 25-OH levels below 46 nmol/L at diagnosis experienced shorter survival. Compared to patients in the lowest quartile of serum 25-OHD, the risk of cancer death among patients in the highest quartile was significantly reduced (HR 0.36 95% CI 0.27, 0.51). The estimated change in risk of cancer death was most pronounced between the first and the second quartile. The associations between 25-OH levels and survival were observed for all four cancers. CONCLUSIONS: Higher circulating serum levels of 25-OHD were positively associated with the survival for cancers of the breast, colon, lung, and lymphoma.


Abstract: BACKGROUND: Preclinical and clinical evidence support an association between vitamin D deficiency and an increased risk of colorectal cancer. Normal vitamin D status has been linked to favorable health outcomes ranging from decreased risk of osteoporosis to improved cancer mortality. We
performed a retrospective study to assess the impact of metastatic disease and chemotherapy treatment on vitamin D status in patients with colorectal cancer residing in Western New York. MATERIALS AND METHODS: Patients, 315, with colorectal cancer treated in a single institute were assayed for 25-OH vitamin D. The association of age, gender, primary disease site and stage, body mass index, and chemotherapy with vitamin D status was investigated. RESULTS: Vitamin D deficiency was common among participants with a median 25-OH vitamin D level of 21.3 ng/ml (optimal range 32-100 ng/ml). Primary site of disease and chemotherapy status were associated with very low 25-OH vitamin D levels (< or =15 ng/ml) on multivariate analysis. Patients receiving chemotherapy and patients with a rectal primary were 3.7 and 2.6-fold more likely to have severe vitamin D deficiency on multivariate analysis than nonchemotherapy patients and colon cancer primary patients, respectively. CONCLUSIONS: Chemotherapy is associated with a significant increase in the risk of severe vitamin D deficiency. Patients with colorectal cancer, especially those receiving chemotherapy, should be considered for aggressive vitamin D replacement strategies.


Abstract: PURPOSE: Higher plasma 25-hydroxyvitamin D(3) (25(OH)D) levels are associated with a decreased incidence of colorectal cancer, but the influence of plasma 25(OH)D on the outcome of patients with established colorectal cancer is unknown. PATIENTS AND METHODS: We prospectively examined the association between prediagnosis 25(OH)D levels and mortality among 304 participants in the Nurses' Health Study (NHS) and the Health Professionals Follow-Up Study (HPFS) who were diagnosed with colorectal cancer from 1991 to 2002. Participants diagnosed within 2 years of blood collection were excluded. Participants were observed until death, June 2005 (NHS), or January 2005 (HPFS), whichever came first. The primary end point was overall mortality. Cox proportional hazards models were used to calculate hazard ratios (HR) adjusted for other risk factors for cancer survival. RESULTS: Higher plasma 25(OH)D levels were associated with a significant reduction in overall mortality (P for trend = .02). Compared with the lowest quartile, participants in the highest quartile had an adjusted HR of 0.52 (95% CI, 0.29 to 0.94) for overall mortality. A trend toward improved colorectal cancer-specific mortality was also seen (HR = 0.61; 95% CI, 0.31 to 1.19). The results remained unchanged after excluding patients diagnosed within 5 years of blood collection (P for trend = .04); the multivariate HR for overall mortality comparing extreme quartiles was 0.45 (95% CI, 0.19 to 1.09). CONCLUSION: Among patients with colorectal cancer, higher prediagnosis plasma 25(OH)D levels were associated with a significant improvement in overall survival. Further study of the vitamin D pathway and its influence on colorectal carcinogenesis and cancer progression is warranted.


Abstract: Solar radiation contributes significantly to the status of serum calcidiol (25-hydroxyvitamin D3, 25-(OH)D3) in humans, even at the high latitudes of northern Norway. Thus, in late summer the serum concentration of calcidiol is roughly 50% larger than that in late winter, when the solar radiation in Norway contains too little ultraviolet radiation to induce any synthesis of vitamin D3 in human skin. This seems to influence the prognosis of colon cancer. We here report that the survival rate of colon cancer in men and women, assessed 18 months after diagnosis, is dependent on the season of diagnosis. A high serum concentration of calcidiol at the time of diagnosis, i.e. at the start of conventional therapy, seems to give an increased survival rate. This agrees with cell and animal experiments reported in the literature, as well as with epidemiological data from some countries relating colon cancer survival with latitude and vitamin D3 synthesis in skin. One possible interpretation of the present data is that, the level of calcidiol, or its derivative calcitriol (1alpha,25-dihydroxyvitamin D3, 1alpha,25-(OH)2D3), may act positively in concert with conventional therapies of colon cancer.


Abstract: BACKGROUND: 1,25-Dihydroxyvitamin D(3) inhibits growth of several types of human cancer cells in vitro, but its therapeutic use is hampered because it causes hypercalcemia. 19-nor-1,25-Dihydroxyvitamin D(2) (paricalcitol) is a noncalcemic vitamin D analog that is approved by the Food and Drug Administration for the treatment of secondary hyperparathyroidism. We investigated the antitumor activity and mechanism of action of paricalcitol in vitro and in vivo. METHODS: Effects of paricalcitol on proliferation, the cell cycle, differentiation, and apoptosis were examined in cancer cell lines. Effects on tumor growth were examined with colon cancer cell xenografts in nude mice (five in the experimental group and five in the control group). The interaction of paricalcitol with the vitamin D receptor (VDR) in mononuclear spleen cells and myeloid stem cells from wild-type and VDR knockout mice was examined. All statistical tests were two-sided. RESULTS: Paricalcitol inhibited the proliferation of myeloid leukemia cell lines HL-60, NB-4, and THP-1 cells at an effective dose that inhibited growth 50% (ED(50)) of 2.4-5.8 x 10(-9) M by inducing cell cycle arrest and differentiation. Paricalcitol inhibited the proliferation of NCI-H929 myeloma cells at an ED(50) of 2.0 x 10(-10) M by inducing cell cycle arrest and apoptosis. Paricalcitol also inhibited the proliferation of colon cancer cell lines HT-29 (ED(50) = 1.7 x 10(-8) M) and SW837 (ED(50) = 3.2 x 10(-8) M). HT-29 colon cancer xenografts in paricalcitol-treated nude mice were smaller (1044 mm(3) and 1752 mm(3), difference = 708 mm(3), 95% confidence interval = 311 to 1104 mm(3); P =.03) and weighed less (1487 mg and 4162 mg, difference = 2675 mg, 95% confidence interval = 2103 to 3248 mg; P<.001) than those in vehicle-treated mice. Paricalcitol induced committed myeloid hematopoietic stem cells from wild-type but not from VDR knockout mice to differentiate as macrophages. CONCLUSION: Paricalcitol has anticancer activity against myeloid leukemia, myeloma, and colon cancer cells that may be mediated through the VDR. Because it has been approved by the Food and Drug Administration, clinical trials of this agent in certain cancers are reasonable.


Abstract: Vitamin D affects calcium metabolism and prevents proliferation of colon cells in vitro. In human beings the main circulating form of vitamin D is 25-hydroxyvitamin D; to regulate calcium homoeostasis, this form must be converted to 1alpha, 25-dihydroxyvitamin D by 1alpha-hydroxylation in the kidney with 25-hydroxyvitamin D-1alpha-hydroxylase. Cultured transformed colon cancer cells can convert 25-hydroxyvitamin D(3) to 1alpha,25-dihydroxyvitamin D(3). We identified messenger RNA (mRNA) for 25-hydroxyvitamin D-1alpha-hydroxylase in normal colon tissue and in malignant and adjacent normal colon tissue. These findings support the notion that vitamin D might have a role in cell growth regulation and cancer protection, and might be the explanation for why the risk of dying from colorectal cancer is highest in areas with the least amount of sunlight.


Abstract: Vitamin D and its analogs are potent inhibitors of colorectal cancer growth and metastasis. A number of recent studies have defined the intersections between the beta-catenin-TCF pathway (a known contributor to colorectal cancer progression) and the vitamin D receptor (VDR) pathway, shedding light on the underlying mechanisms. Vitamin D also regulates the innate immune response, and as such influences susceptibility to inflammatory bowel disease, a predisposing factor in colorectal cancer. Understanding the role of vitamin D in these different contexts will enable development of next generation vitamin D analogs that will serve as both chemopreventatives and cancer therapeutics, without
the accompanying side effects of hypercalcemia usually associated with high vitamin D intake. This review summarizes the mechanisms of action of vitamin D and the VDR in the context of the gastrointestinal tract and colorectal carcinogenesis


Abstract: Aberrant activation of the Wnt/beta-catenin pathway is critical for the initiation and progression of most colon cancers. This activation provokes the accumulation of nuclear beta-catenin and the induction of its target genes. Apc(min/+) mice are the most commonly used model for colon cancer. They harbor a mutated Apc allele and develop intestinal adenomas and carcinomas during the first months of life. This phenotype is caused by the mutation of the second Apc allele and the consequent accumulation of nuclear beta-catenin in the affected cells. Here we describe that vitamin D receptor (VDR) is a crucial modulator of nuclear beta-catenin levels in colon cancer in vivo. By appropriate breeding of Apc(min/+) and Vdr(+/-) mice we have generated animals expressing a mutated Apc allele and two, one, or none Vdr wild type alleles. Lack of Vdr increased the number of colonic Aberrant Crypt Foci (ACF) but not that of adenomas or carcinomas in either small intestine or colon. Importantly, colon ACF and tumors of Apc(min/+)Vdr(-/-) mice had increased nuclear beta-catenin and the tumors reached a larger size than those of Apc(min/+)Vdr(+/-). Both ACF and carcinomas in Apc(min/+)Vdr(-/-) mice showed higher expression of beta-catenin/TCF target genes. In line with this, VDR knock-down in cultured human colon cancer cells enhanced beta-catenin nuclear content and target gene expression. Consistently, VDR depletion abrogated the capacity of 1,25(OH)(2)D(3) to promote the relocation of beta-catenin from the nucleus to the plasma membrane and to inhibit beta-catenin/TCF target genes. In conclusion, VDR controls the level of nuclear beta-catenin in colon cancer cells and can therefore attenuate the impact of oncogenic mutations that activate the Wnt/beta-catenin pathway.


Abstract: BACKGROUND: The vitamin D receptor (VDR) pathway is important in the prevention and potentially in the treatment of many cancers. One important mechanism of VDR action is related to its interaction with the Wnt/beta-catenin pathway. Agonist-bound VDR inhibits the oncogenic Wnt/beta-catenin/TCF pathway by interacting directly with beta-catenin and in some cells by increasing cadherin expression which, in turn, recruits beta-catenin to the membrane. Here we identify TCF-4, a transcriptional regulator and beta-catenin binding partner as an indirect target of the VDR pathway. METHODOLOGY/PRINCIPAL FINDINGS: In this work, we show that TCF-4 (gene name TCF7L2) is decreased in the mammary gland of the VDR knockout mouse as compared to the wild-type mouse. Furthermore, we show 1,25(OH)2D3 increases TCF-4 at the RNA and protein levels in several human colorectal cancer cell lines, the effect of which is completely dependent on the VDR. In silico analysis of the human and mouse TCF7L2 promoters identified several putative VDR binding elements. Although TCF7L2 promoter reporters responded to exogenous VDR, and 1,25(OH)2D3, mutation analysis and chromatin immunoprecipitation assays, showed that the increase in TCF7L2 did not require recruitment of the VDR to the identified elements and indicates that the regulation by VDR is indirect. This is further confirmed by the requirement of de novo protein synthesis for this up-regulation. CONCLUSIONS/SIGNIFICANCE: Although it is generally assumed that binding of beta-catenin to members of the TCF/LEF family is cancer-promoting, recent studies have indicated that TCF-4 functions instead as a transcriptional repressor that restricts breast and colorectal cancer cell growth. Consequently, we conclude that the 1,25(OH)2D3/VDR-mediated increase in TCF-4 may have a protective role in colon cancer as well as diabetes and Crohn's disease.

Abstract: The active vitamin D metabolite 1alpha,25-dihydroxyvitamin D3 [1alpha,25(OH)2D3] has wide but not fully understood antitumor activity. A previous transcriptomic analysis of 1alpha,25(OH)2D3 action on human colon cancer cells revealed cystatin D (CST5), which encodes an inhibitor of several cysteine proteases of the cathepsin family, as a candidate target gene. Here we report that 1alpha,25(OH)2D3 induced vitamin D receptor (VDR) binding to, and activation of, the CST5 promoter and increased CST5 RNA and protein levels in human colon cancer cells. In cells lacking endogenous cystatin D, ectopic cystatin D expression inhibited both proliferation in vitro and xenograft tumor growth in vivo. Furthermore, cystatin D inhibited migration and anchorage-independent growth, antagonized the Wnt/beta-catenin signaling pathway, and repressed c-MYC expression. Cystatin D repressed expression of the epithelial-mesenchymal transition inducers SNAI1, SNAI2, ZEB1, and ZEB2 and, conversely, induced E-cadherin and other adhesion proteins. CST5 knockdown using shRNA abrogated the antiproliferative effect of 1alpha,25(OH)2D3, attenuated E-cadherin expression, and increased c-MYC expression. In human colorectal tumors, expression of cystatin D correlated with expression of VDR and E-cadherin, and loss of cystatin D correlated with poor tumor differentiation. Based on these data, we propose that CST5 has tumor suppressor activity that may contribute to the antitumoral action of 1alpha,25(OH)2D3 in colon cancer.


Abstract: Colorectal cancer is a major health problem worldwide. Aberrant activation of the Wingless-type mouse mammary tumour virus integration site family (Wnt)/beta-catenin signalling pathway due to mutation of adenomatous polyposis coli (APC), beta-catenin (CTNNB1) or AXIN genes is the most common and initial alteration in sporadic colorectal tumours. Numerous epidemiological and experimental studies have indicated a protective action of vitamin D against colorectal cancer. Previous work has demonstrated that the most active vitamin D metabolite, 1alpha,25-dihydroxyvitamin D3 (1,25(OH)2D3) inhibits beta-catenin transcriptional activity by promoting vitamin D receptor (VDR) binding to beta-catenin and the induction of E-cadherin expression. Recently, 1,25(OH)2D3 has been shown to distinctly regulate two genes encoding the extracellular Wnt inhibitors DICKKOPF-1 and DICKKOPF-4 (DKK-1, DKK-4). By an indirect transcriptional mechanism, 1,25(OH)2D3 increases the expression of DKK-1 RNA and protein, which acts as a tumour suppressor in human colon cancer cells harbouring endogenous mutations in the Wnt/beta-catenin pathway. In contrast, 1,25(OH)2D3 represses DKK-4 transcription by inducing direct VDR binding to its promoter. Unexpectedly, DKK-4 is a target of the Wnt/beta-catenin pathway and is up-regulated in colorectal tumours, and it has been shown to increase cell migration and invasion and to promote a proangiogenic phenotype. Together, these results show that 1,25(OH)2D3 exerts a complex set of regulatory actions leading to the inhibition of the Wnt/beta-catenin pathway in colon cancer cells that is in line with its protective effect against this neoplasia.


Abstract: The active vitamin D metabolite 1,25-dihydroxyvitamin D(3) (1,25(OH)(2)D(3)) inhibits proliferation and promotes differentiation of colon cancer cells through the activation of vitamin D receptor (VDR), a transcription factor of the nuclear receptor superfamily. Additionally, 1,25(OH)(2)D(3) has several nongenomic effects of uncertain relevance. We show that 1,25(OH)(2)D(3) induces a transcription-independent Ca(2+) influx and activation of RhoA-Rho-associated coiled kinase (ROCK).
This requires VDR and is followed by activation of the p38 mitogen-activated protein kinase (p38MAPK) and mitogen- and stress-activated kinase 1 (MSK1). As shown by the use of chemical inhibitors, dominant-negative mutants and small interfering RNA, RhoA-ROCK, and p38MAPK-MSK1 activation is necessary for the induction of CDH1/E-cadherin, CYP24, and other genes and of an adhesive phenotype by 1,25(OH)(2)D(3). RhoA-ROCK and MSK1 are also required for the inhibition of Wnt-beta-catenin pathway and cell proliferation. Thus, the action of 1,25(OH)(2)D(3) on colon carcinoma cells depends on the dual action of VDR as a transcription factor and a nongenomic activator of RhoA-ROCK and p38MAPK-MSK1.


Abstract: The beta-catenin signaling pathway is deregulated in nearly all colon cancers. Nonhypercalcemic vitamin D3 (1alpha,25-dehydroxyvitamin D(3)) analogues are candidate drugs to treat this neoplasia. We show that these compounds promote the differentiation of human colon carcinoma SW480 cells expressing vitamin D receptors (VDRs) (SW480-ADH) but not that of a malignant subline (SW480-R) or metastasic derivative (SW620) cells lacking VDR. 1alpha,25(OH)2D(3) induced the expression of E-cadherin and other adhesion proteins (occludin, Zonula occludens [ZO]-1, ZO-2, vinculin) and promoted the translocation of beta-catenin, plakoglobin, and ZO-1 from the nucleus to the plasma membrane. Ligand-activated VDR competed with T cell transcription factor (TCF)-4 for beta-catenin binding. Accordingly, 1alpha,25(OH)2D(3) repressed beta-catenin-TCF-4 transcriptional activity. Moreover, VDR activity was enhanced by ectopic beta-catenin and reduced by TCF-4. Also, 1alpha,25(OH)2D(3) inhibited expression of beta-catenin-TCF-4-responsive genes, c-myc, peroxisome proliferator-activated receptor delta, Tcf-1, and CD44, whereas it induced expression of ZO-1. Our results show that 1alpha,25(OH)2D(3) induces E-cadherin and modulates beta-catenin-TCF-4 target genes in a manner opposite to that of beta-catenin, promoting the differentiation of colon carcinoma cells.


Abstract: The Wnt/beta-catenin signalling pathway is activated in 90% of human colon cancers by nuclear accumulation of beta-catenin protein due to its own mutation or to that of adenomatous polyposis coli. In the nucleus, beta-catenin regulates gene expression promoting cell proliferation, migration and invasiveness. 1alpha,25-dihydroxyvitamin D(3) (1,25(OH)(2)D(3)) inhibits beta-catenin signalling by inducing its binding to vitamin D receptor (VDR) and by promoting beta-catenin nuclear export. The transcription factor Snail1 represses VDR expression and we demonstrate here that Snail1 also abolishes the nuclear export of beta-catenin induced by 1,25(OH)(2)D(3) in SW480-ADH cells. Accordingly, Snail1 relieves the inhibition exerted by 1,25(OH)(2)D(3) on genes whose expression is driven by beta-catenin, such as c-MYC, ectodermal-neural cortex-1 (ENC-1) or ephrin receptor B2 (EPHB2). In addition, Snail1 abrogates the inhibitory effect of 1,25(OH)(2)D(3) on cell proliferation and migration. In xenografted mice, Snail1 impedes the nuclear export of beta-catenin and the inhibition of ENC-1 expression induced by EB1089, a 1,25(OH)(2)D(3) analogue. The elevation of endogenous SNAIL1 protein levels reproduces the effect of an ectopic Snail1 gene. Remarkably, the expression of exogenous VDR in cells with high levels of Snail1 normalizes the transcriptional responses to 1,25(OH)(2)D(3). However, this exogenous VDR failed to fully restore the blockage of the Wnt/beta-catenin pathway by 1,25(OH)(2)D(3). This suggests that the effects of Snail1 on this pathway are not merely due to the repression of VDR gene. We conclude that Snail1 is a positive regulator of the Wnt/beta-catenin signalling pathway in part through the abrogation of the inhibitory action of 1,25(OH)(2)D(3).

**Spirulina (inhibitor of NADPH oxidase)**


Abstract: The NADPH oxidase (Nox) proteins catalyze the regulated formation of reactive oxygen species (ROS), which play key roles as signaling molecules in several physiological and pathophysiological processes. ROS generation by the Nox1 member of the Nox family is necessary for the formation of extracellular matrix (ECM)-degrading, actin-rich cellular structures known as invadopodia. Selective inhibition of Nox isoforms can provide reversible, mechanistic insights into these cellular processes in contrast to scavenging or inhibition of ROS production. Currently no specific Nox inhibitors have been described. Here, by high-throughput screening, we identify a subset of phenothiazines, 2-acetylphenothiazine (here referred to as ML171) (and its related 2-(trifluoromethyl)-phenothiazine) as nanomolar, cell-active, and specific Nox1 inhibitors that potently block Nox1-dependent ROS generation, with only marginal activity on other cellular ROS-producing enzymes and receptors including the other Nox isoforms. ML171 also blocks the ROS-dependent formation of ECM-degrading invadopodia in colon cancer cells. Such effects can be reversed by overexpression of Nox1 protein, which is suggestive of a selective mechanism of inhibition of Nox1 by this compound. These results elucidate the relevance of Nox1-dependent ROS generation in mechanisms of cancer invasion and define ML171 as a useful Nox1 chemical probe and potential therapeutic agent for inhibition of cancer cell invasion.


Abstract: NADPH oxidase/dual-oxidase (Nox/Duox) family members have been implicated in nuclear factor kappa-B (NFkappaB)-mediated inflammation and inflammation-associated pathologies. We sought to examine, for the first time, the role of Nox/Duox and NFkappaB in rats treated with the cooked meat heterocyclic amine carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP). In the PhIP-induced colon tumors obtained after 1 year, Nox1, Nox4, NFkappaB-p50 and NFkappaB-p65 were all highly overexpressed compared with their levels in adjacent normal-looking colonic mucosa. Nox1 and Nox4 mRNA and protein levels also were markedly elevated in a panel of primary human colon cancers, compared with their matched controls. In HT29 human colon cancer cells, Nox1 knockdown induced G1 cell cycle arrest, whereas in Caco-2 cells there was a strong apoptotic response, with increased levels of cleaved caspase-3, -6, -7 and poly(ADP-ribose)polymerase. Nox1 knockdown blocked lipopolysaccharide-induced phosphorylation of IkappaB kinase, inhibited the nuclear translocation of NFkappaB (p50 and p65) proteins, and attenuated NFkappaB DNA binding activity. There was a corresponding reduction in the expression of downstream NFkappaB targets, such as MYC, CCND1 and IL1beta. The results provide the first evidence for a role of Nox1, Nox4 and NFkappaB in PhIP-induced colon carcinogenesis, including during the early stages before tumor onset. Collectively, the findings from this investigation and others suggest that further work is warranted on the role of Nox/Duox family members and NFkappaB in colon cancer development.

Abstract: BACKGROUND AND PURPOSE: Oxaliplatin is the first platinum-based compound effective in the treatment of colorectal cancer. Oxaliplatin combined with cetuximab for metastatic colorectal cancer is under evaluation. The preliminary results seem controversial, particularly for the use of cetuximab in K-Ras mutated patients. K-Ras mutation is known to affect redox homeostasis. Here we evaluated how the efficacy of oxaliplatin alone or combined with cetuximab varied according to the Ras mutation and redox status in a panel of colorectal tumour cell lines. EXPERIMENTAL APPROACH: Viability was evaluated by methylthiazol tetrazolium assay, reactive oxygen species production by DCFDA and lucigenin on HT29-D4, Caco-2, SW480 and SW620 cell lines. KEY RESULTS: Combination of oxaliplatin and cetuximab was less cytotoxic than oxaliplatin alone in colorectal cells harbouring wild-type Ras and membrane expression of receptors for epidermal growth factor receptor (EGFR), such as HT29-D4 and Caco-2 cells. In contrast, cetuximab did not affect oxaliplatin efficiency in cells harbouring K-Ras(V12) mutation, irrespective of membrane EGFR expression (SW620 and SW480 cells). Transfection of HT29-D4 with K-Ras(V12) decreased oxaliplatin IC50 and impaired cetuximab sensitivity, without affecting expression of membrane EGFR compared with HT29-D4 control. Oxaliplatin efficacy relies on endogenous production of H2O2. Cetuximab inhibits H2O2 production inhibiting the EGFR/Nox1 NADPH oxidase pathway. Oxaliplatin efficacy was impaired by short hairpin RNA for Nox1 and by catalase (H2O2 scavenger). CONCLUSIONS AND IMPLICATIONS: Cetuximab limited oxaliplatin efficiency by affecting the redox status of cancer cells through Nox1. Such combined therapy might be improved by controlling H2O2 elimination.


Abstract: The NADPH-oxidase 1 (Nox1) is a homolog of gp91phox, the catalytic subunit of the phagocyte superoxide-generating NADPH-oxidase. Nox1 is expressed in normal colon epithelial cells and in colon tumor cell lines, and overexpression in model cells has been implicated in stimulation of mitogenesis and angiogenesis and inhibition of apoptosis. This suggests that aberrant expression of Nox1 could contribute to the development of colorectal cancer. Herein, we examine the expression of Nox1 mRNA in 24 colon tumors of various stages compared with paired adjacent normal tissue from the same patient, and correlate expression with some common mutations associated with colon cancer. Nox1 was overexpressed compared with paired normal tissue in 57% of tumors as early as the adenoma stage, with no correlation of expression level with tumor stage. Overexpression of Nox1 mRNA correlated with Nox1 protein levels assessed by immunofluorescence and immunohistochemistry with an antibody specific for Nox1. There was a strong correlation between Nox1 mRNA level and activating mutations in codons 12 and 13 of K-Ras. Eighty percent (8/10) of tumors with codons 12 and 13 mutations had a 2-fold or more increase in Nox1 mRNA, and 70% (7/10) had a 5-fold or greater increase. Transgenic mice expressing K-Ras(G12V) in the intestinal epithelium also expressed markedly elevated Nox1 in both small and large intestine. There was no correlation between inactivating mutations in the tumor suppressor p53 and Nox1 expression. We conclude that Nox1 mRNA and protein are overexpressed in colon cancer and are strongly correlated with activating mutations in K-Ras.

de Carvalho DD, Sadok A, Bourgarel-Rey V et al. Nox1 downstream of 12-lipoxygenase controls cell proliferation but not cell spreading of colon cancer cells. *Int J Cancer* 2008 April 15;122(8):1757-64.

Abstract: The catalytic subunit of the NADPH oxidase complex, Nox1 (homologue of gp91phox/Nox2), expressed mainly in intestinal epithelial and vascular smooth muscle cells, functions in innate immune defense and cell proliferation. The molecular mechanisms underlying these functions, however, are not
We measured Nox1-dependent O2- production during cell spreading on Collagen IV (Coll IV) in colon carcinoma cell lines. Knocking down Nox1 by shRNA, we showed that Nox1-dependent O2- production is activated during cell spreading after 4 hr of adhesion on Collagen IV. Nox1 activation during cell spreading relies on Rac1 activation and arachidonic metabolism. Our results showed that mannoalide (a secreted phospholipase A2 inhibitor) and cinnamyl-3,4-dihydroxy-alpha-cynocinnamate (a 12-lipoxygenase inhibitor) inhibit O2- production, cell spreading and cell proliferation in these colonic epithelial cells. 12-Lipoxygenase inhibition of ROS production and cell spreading can be reversed by adding 12-HETE, a 12-lipoxygenase product, supporting the specific effect observed with cinnamyl-3,4-dihydroxy-alpha-cynocinnamate. In contrast, Nox1 shRNA and DPI (NADPH oxidase inhibitor) weakly affect cell spreading while inhibiting O2- production and cell proliferation. These results suggest that the 12-lipoxygenase pathway is upstream of Nox1 activation and controls cell spreading and proliferation, while Nox1 specifically affects cell proliferation.


Abstract: Reactive oxygen species are well-known mediators of various biological responses. Recently, new homologues of the catalytic subunit of NADPH oxidase have been discovered in non-phagocytic cells. These new homologues (Nox1-Nox5) produce low levels of superoxides compared to the phagocytic homologue Nox2/gp91phox. Using Nox1 siRNA, we show that Nox1-dependent superoxide production affects the migration of HT29-D4 colonic adenocarcinoma cells on collagen-I. Nox1 inhibition or down-regulation led to a decrease of superoxide production and alpha 2 beta 1 integrin membrane availability. An addition of arachidonic acid stimulated Nox1-dependent superoxide production and HT29-D4 cell migration. Pharmacological evidences using phospholipase A2, lipoxygenases and protein kinase C inhibitors show that upstream regulation of Nox1 relies on arachidonic acid metabolism. Inhibition of 12-lipoxygenase decreased basal and arachidonic acid induced Nox1-dependent superoxide production and cell migration. Migration and ROS production inhibited by a 12-lipoxygenase inhibitor were restored by the addition of 12(S)-HETE, a downstream product of 12-lipoxygenase. Protein kinase C delta inhibition by rottlerin (and also GO6983) prevented Nox1-dependent superoxide production and inhibited cell migration, while other protein kinase C inhibitors were ineffective. We conclude that Nox1 activation by arachidonic acid metabolism occurs through 12-lipoxygenase and protein kinase C delta, and controls cell migration by affecting integrin alpha 2 subunit turn-over.


Abstract: Recent research reveals that free bilirubin functions physiologically as a potent inhibitor of NADPH oxidase activity. The chromophore phycoerythobilin (PCB), found in blue-green algae and cyanobacteria such as Spirulina, also has been found to be a potent inhibitor of this enzyme complex, likely because in mammalian cells it is rapidly reduced to phycoerythorubin, a close homolog of bilirubin. In light of the protean roles of NADPH oxidase activation in pathology, it thus appears likely that PCB supplementation may have versatile potential in prevention and therapy -- particularly in light of rodent studies demonstrating that orally administered Spirulina or phycoerythrin (the Spirulina holoprotein that contains PCB) can exert a wide range of anti-inflammatory effects. Until PCB-enriched Spirulina extracts or synthetically produced PCB are commercially available, the most feasible and least expensive way to administer PCB is by ingestion of whole Spirulina. A heaping tablespoon (about 15 g) of Spirulina can be expected to provide about 100 mg of PCB. By extrapolating from rodent studies, it can be concluded that an intake of 2 heaping tablespoons daily would be likely to have important antioxidant activity in humans -- assuming that humans and rodents digest and absorb Spirulina-bound PCB in a comparable manner. An intake of this magnitude can be clinically feasible if Spirulina is
incorporated into "smoothies" featuring such ingredients as soy milk, fruit juices, and whole fruits. Such a regimen should be evaluated in clinical syndromes characterized and in part mediated by NADPH oxidase overactivity in affected tissues.


Abstract: In many cancers, a chronic increase in oxidant stress - associated with elevated levels of hydrogen peroxide - contributes to the increased proliferative rate, diminished apoptosis, increased angiogenic and metastatic capacity, and chemoresistance that often characterize advanced malignancies. This oxidant stress often reflects up-regulation of expression and activity of NADPH oxidase, and/or decreased activity of catalase, which functions as suppressor gene in oxidant-dependent cancers. These characteristics of oxidant-dependent cancers suggest a dual strategy for treatment of these cancers. Since ascorbate can react spontaneously with molecular oxygen to generate hydrogen peroxide, high-dose intravenous ascorbate should be selectively toxic to tumors that are low in catalase activity - as suggested by numerous cell culture studies. Measures which concurrently improve the oxygenation of hypoxic tumor regions would be expected to boost the efficacy of such therapy; calcitriol and high-dose selenium might also be useful in this regard. Secondly, during the intervals between sessions of ascorbate therapy, administration of agents which can safely inhibit NADPH oxidase would be expected to slow the proliferation and spread of surviving tumor cells - while providing selection pressure for a further decline in catalase activity. In effect, cancers treated in this way would be whipsawed between lethally excessive and inadequately low oxidant stress. An additional possibility is that ascorbate-induced oxidant stress in tumors might potentiate the cell kill achieved with concurrently administered cytotoxic drugs, inasmuch as oxidant mechanisms appear to play a mediating role in the apoptosis induced by many such drugs, largely via activation of c-Jun NH(2)-terminal kinase; cell culture studies would be useful for evaluating this possibility.

**High-Dose Biotin and or Tadalafil – Boosters of cGMP**


Abstract: Nonsteroidal anti-inflammatory drugs (NSAIDs) have been widely reported to inhibit tumor growth by a COX-independent mechanism, although alternative targets have not been well defined or used to develop improved drugs for cancer chemoprevention. Here, we characterize a novel sulindac derivative referred to as sulindac benzylamine (SBA) that does not inhibit COX-1 or COX-2, yet potently inhibits the growth and induces the apoptosis of human colon tumor cells. The basis for this activity appears to involve cyclic guanosine 3',5'-monophosphate phosphodiesterase (cGMP PDE) inhibition as evident by its ability to inhibit cGMP hydrolysis in colon tumor cell lysates and purified cGMP-specific PDE5, increase intracellular cGMP levels, and activate cGMP-dependent protein kinase G at concentrations that suppress tumor cell growth. PDE5 was found to be essential for colon tumor cell growth as determined by siRNA knockdown studies, elevated in colon tumor cells as compared with normal colonocytes, and associated with the tumor selectivity of SBA. SBA activation of PKG may suppress the oncogenic activity of beta-catenin as evident by its ability to reduce beta-catenin nuclear levels, Tcf (T-cell factor) transcriptional activity, and survivin levels. These events preceded apoptosis induction and appear to result from a rapid elevation of intracellular cGMP levels following cGMP PDE inhibition. We conclude that PDE5 and possibly other cGMP degrading isozymes can be targeted to develop safer and more efficacious NSAID derivatives for colorectal cancer chemoprevention.

Abstract: Nitric oxide (NO), an uncharged free radical is implicated in various physiological and pathological processes. The present study is an investigation on the effect of NO on proliferation, apoptosis and migration of colon cancer cells. Colon adenocarcinoma cells, WiDr, were used for the in vitro experiments. Tissues from colon adenocarcinoma, adjacent normal and inflammatory tissue and lymph node with metastasis were evaluated for iNOS, MMP-2/9 and Fra-1/Fra-2. NO increases the proliferation of cancer cells and simultaneously prevents apoptosis. Expression of MMP-2/9, RhoB and Rac-1 was enhanced by NO in a time dependent manner. Further, NO increased phosphorylation of ERK1/2 and induced nuclear translocation of Fra-1 and Fra-2. Electrophoretic mobility shift analysis and use of deletion mutant promoter constructs identified role of AP-1 in NO-mediated regulation of MMP-2/9. iNOS, MMP-2/9, Fra-1 and Fra-2 in normal and colon adenocarcinoma tissues were analyzed and it was found that increased expression of these proteins in cancer when compared to normal provides support to our in vitro findings. The study showed that the NO-cGMP-PKG promotes MMP-2/9 expression by activating ERK-1/2 and AP-1. This study reveals the insidious role of NO in imparting tumor aggressiveness


Abstract: In colorectal cancer, the antitumorigenic guanylyl cyclase C (GCC) signalome is defective reflecting ligand deprivation from downregulation of endogenous hormone expression. Although the proximal intracellular mediators of that signal transduction system, including cyclic guanosine monophosphate (cGMP) and cGMP-dependent protein kinase (PKG), are well characterized, the functional significance of its distal effectors remain vague. Dysregulation of ligand-dependent GCC signaling through vasodilator-stimulated phosphoprotein (VASP), an actin-binding protein implicated in membrane protrusion dynamics, drastically reduced cGMP-dependent VASP phosphorylation levels in colorectal tumors from patients. Restoration of cGMP-dependent VASP phosphorylation by GCC agonists suppressed the number and length of locomotory (filopodia) and invasive (invadopodia) actin-based organelles in human colon cancer cells. Membrane organelle disassembly reflected specific phosphorylation of VASP Ser239, the cGMP/PKG preferred site, and rapid VASP removal from tumor cell protrusions. Importantly, VASP Ser239 phosphorylation inhibited the proteolytic function of invadopodia, reflected by suppression of the cancer cell ability to digest DQ-collagen IV embedded in Matrigel. These results demonstrate a previously unrecognized role for VASP Ser239 phosphorylation, a single intracellular biochemical reaction, as an effective mechanism which opposes tumor cell shape promoting colon cancer invasion and metastasis. Reconstitution of physiological cGMP circuitry through VASP, in turn, represents an attractive targeted approach for patients with colorectal cancer


Abstract: Nonsteroidal anti-inflammatory drugs (NSAID) display promising antineoplastic activity, but toxicity resulting from cyclooxygenase (COX) inhibition limits their clinical use for chemoprevention. Studies suggest that the mechanism may be COX independent, although alternative targets have not been well defined. Here, we show that the NSAID sulindac sulfide (SS) inhibits cyclic guanosine 3',5'-monophosphate (cGMP) phosphodiesterase (PDE) activity in colon tumor cell lysates at concentrations that inhibit colon tumor cell growth in vitro and in vivo. A series of chemically diverse NSAIDs also
inhibited cGMP hydrolysis at concentrations that correlate with their potency to inhibit colon tumor cell growth, whereas no correlation was observed with COX-2 inhibition. Consistent with its selectivity for inhibiting cGMP hydrolysis compared with cyclic AMP hydrolysis, SS inhibited the cGMP-specific PDE5 isozyme and increased cGMP levels in colon tumor cells. Of numerous PDE isozyme-specific inhibitors evaluated, only the PDE5-selective inhibitor MY5445 inhibited colon tumor cell growth. The effects of SS and MY5445 on cell growth were associated with inhibition of beta-catenin-mediated transcriptional activity to suppress the synthesis of cyclin D and survivin, which regulate tumor cell proliferation and apoptosis, respectively. SS had minimal effects on cGMP PDE activity in normal colonocytes, which displayed reduced sensitivity to SS and did not express PDE5. PDE5 was found to be overexpressed in colon tumor cell lines as well as in colon adenomas and adenocarcinomas compared with normal colonic mucosa. These results suggest that PDE5 inhibition, cGMP elevation, and inhibition of beta-catenin transcriptional activity may contribute to the chemopreventive properties of certain NSAIDs.


Abstract: Activation of cGMP-dependent protein kinase (PKG) has anti-tumor effects in colon cancer cells but the mechanisms are not fully understood. This study has examined the regulation of beta-catenin/TCF signaling, as this pathway has been highlighted as central to the anti-tumor effects of PKG. We show that PKG activation in SW620 cells results in reduced beta-catenin expression and a dramatic inhibition of TCF-dependent transcription. PKG did not affect protein stability, nor did it increase phosphorylation of the amino-terminal Ser33/37/Thr41 residues that are known to target beta-catenin for degradation. However, we found that PKG potently inhibited transcription from a luciferase reporter driven by the human CTNNB1 promoter, and this corresponded to reduced beta-catenin mRNA levels. Although PKG was able to inhibit transcription from both the CTNNB1 and TCF reporters, the effect on protein levels was less consistent. Ectopic PKG had a marginal effect on beta-catenin protein levels in SW480 and HCT116 but was able to inhibit TCF-reporter activity by over 80%. Investigation of alternative mechanisms revealed that cJun-N-terminal kinase (JNK) activation was required for the PKG-dependent regulation of TCF activity. PKG activation caused beta-catenin to bind to FOXO4 in colon cancer cells, and this required JNK. Activation of PKG was also found to increase the nuclear content of FOXO4 and increase the expression of the FOXO target genes MnSOD and catalase. FOXO4 activation was required for the inhibition of TCF activity as FOXO4-specific short-interfering RNA completely blocked the inhibitory effect of PKG. These data illustrate a dual-inhibitory effect of PKG on TCF activity in colon cancer cells that involves reduced expression of beta-catenin at the transcriptional level, and also beta-catenin sequestration by FOXO4 activation.


Abstract: The synthesis of novel tadalafil analogues in which the benzodioxole moiety is replaced by 2-bromophenyl; the chiral carbons swing from R,R to R,S, S,R and S,S; the piprazinedione ring is maintained or reduced to the 5-membered imidazolidinedione or thioxoimidazolinone is described. The prepared analogues were evaluated for their capacity to inhibit the cyclic guanosine monophosphate (cGMP) selective phosphodiesterase 5 (PDE5) isozyme and the growth of human HT-29 colon adenocarcinoma cells. The R absolute configuration of C-5 in the beta-carboline-hydantoin and C-6 in the beta-carboline-piprazinedione derivatives was found to be essential for the PDE5 inhibition. In addition, tadalafil analogues that were synthesized from l-tryptophan were more active than those derived from d-tryptophan, which is of economic value and expands the horizon for the discovery of new carbolines as PDE5 inhibitors. While some analogues displayed potent tumor cell growth inhibitory activity, there was
no apparent correlation with their PDE5 inhibitory activity, which leads us to conclude that other PDE isozymes or PDE5 splice variants may be involved


Abstract: In recent years, several antitumor signaling pathways mediated by the cGMP-dependent protein kinases have been identified in colon cancer cells. This review aims to present the mounting evidence in favor of cGMP/protein kinase G (PKG) signaling as a therapeutic strategy in colon cancer. The homeostatic and tumor suppressive effects of cGMP in the intestine are uncontested, but the signaling details are not understood. PKG is the central cGMP effector, and can block proliferation and tumor angiogenesis by inhibiting beta-catenin/TCF and SOX9 signaling. Therapeutic activation of cGMP/PKG offers a promising avenue for the prevention and treatment of colon cancer, but additional preclinical studies are needed to fully understand the potential of this system


Abstract: Matrix metalloproteinase-9 (MMP-9) produced by colorectal cancer cells is a critical determinant of metastatic disease progression and an attractive target for antimetastatic strategies to reduce colon cancer mortality. Cellular signaling by cyclic GMP (cGMP) regulates MMP-9 dynamics in various cell systems, and the bacterial enterotoxin receptor guanylyl cyclase C (GCC), the principle source of cGMP in colonocytes, which is overexpressed in colorectal cancers, inhibits tumor initiation and progression in the intestine. Here, we show that ligand-dependent GCC signaling through cGMP induces functional remodeling of cancer cell MMP-9 reflected by a compartmental redistribution of this gelatinase, in which intracellular retention resulted in reciprocal extracellular depletion. Functional remodeling of MMP-9 by GCC signaling reduced the ability of colon cancer cells to degrade matrix components, organize the actin cytoskeleton to form locomotory organelles and spread, and hematogenously seed distant organs. Of significance, GCC effects on cancer cell MMP-9 prevented establishment of metastatic colonies by colorectal cancer cells in the mouse peritoneum in vivo. Because endogenous hormones for GCC are uniformly deficient in intestinal tumors, reactivation of dormant GCC signaling with exogenous administration of GCC agonists may represent a specific intervention to target MMP-9 functions in colon cancer cells. The notion that GCC-mediated regulation of cancer cell MMP-9 disrupts metastasis, in turn, underscores the unexplored utility of GCC hormone replacement therapy in the chemoprevention of colorectal cancer progression


Abstract: Colorectal cancer is a leading cause of cancer-related death in the world and there is an urgent need for new strategies to combat this disease. Findings from several independent laboratories have converged on cGMP signaling as an exciting new therapeutic target, but the mechanisms remain controversial. A key intracellular effector of cGMP is protein kinase G (PKG). This article reviews the scientific literature concerning PKG effects on tumor development and progression, and discusses possible strategies for its exploitation in future cancer therapies. Studies from several independent laboratories have described novel anti-tumor effects of PKG in colon cancer cells that include inhibition of tumor growth and angiogenesis. While more preclinical research is warranted to better understand signaling mechanisms, these properties support the notion that PKG is a novel cancer target

Kwon IK, Schoenlein PV, Delk J et al. Expression of cyclic guanosine monophosphate-dependent protein kinase in metastatic colon carcinoma cells blocks tumor angiogenesis. *Cancer* 2008 April 1;112(7):1462-
Abstract: BACKGROUND: Type 1 cyclic guanosine monophosphate (cGMP)-dependent protein kinase (PKG) reportedly has exhibited antitumor properties, and its expression is down-regulated in many tumors. METHODS: The authors recently demonstrated that PKG re-expression in metastatic colon carcinoma cells results in decreased tumorigenesis: In the current study, they addressed that mechanism. RESULTS: Over-expression of PKG in SW620 cells produced smaller, more apoptotic subcutaneous tumors in athymic mice, but the observed effect of PKG expression on growth and apoptosis in vitro was minimal. Closer examination of the subcutaneous xenografts revealed highly vascular tumors produced by the parental SW620 cells, which contrasted greatly with the PKG-expressing tumors, in which cell growth was limited to "islands" surrounding CD31-positive cells. The idea that PKG expression was associated with reduced tumor angiogenesis was supported by decreased levels of vascular endothelial growth factor in these tumors compared with tumors that were derived from parental SW620 cells. Investigation of potential mechanisms revealed that PKG expression was associated with reduced levels of beta-catenin compared with parental cells. Moreover, this effect of exogenous PKG on beta-catenin expression in SW620 cells also occurred in vitro, where the decrease was associated with reduced T-cell factor-dependent transcription. CONCLUSIONS: Together the findings indicated that PKG down-regulation in colon cancer cells is important for optimal tumor angiogenesis and that regulation of beta-catenin expression may be important to this process.


Abstract: Although it is often assumed that the antitumor effects of nonsteroidal anti-inflammatory drugs (NSAIDs) are due to inhibition of cyclooxygenase (COX) activity, specifically COX-2, there is accumulating evidence that COX-2 independent mechanisms can also play an important role. Studies with sulindac sulfone (Aptosyn) and related derivatives have revealed a novel pathway of tumor growth inhibition and apoptosis mediated by activation of the guanosine 3',5' monophosphate (cGMP)-dependent enzyme protein kinase G (PKG). The present study indicates that concentrations of the NSAIDs celecoxib, indomethacin, and meclofenamic acid that inhibit growth of SW480 human colon cancer cells inhibit subcellular cGMP-phosphodiesterase (PDE) enzymatic activity and in intact cells induce a two- to threefold increase in intracellular levels of cGMP. This is associated with phosphorylation of the protein VASP, a marker of PKG activation, activation of JNK1 and a decrease in cellular levels of cyclin D1; effects seen with other agents that cause activation of PKG in these cells. On the other hand even a high concentration of the COX-2 specific inhibitor rofecoxib (500 microM) did not inhibit growth of SW480 cells. Nor did rofecoxib inhibit cGMP-PDE activity or cause other changes related to PKG activation in these cells. Since activation of the PKG pathways by celecoxib, indomethacin, and meclofenamic acid in this cell culture system required high concentrations of these compounds, it remains to be determined whether activation of this pathway contributes to the in vivo antitumor effects of specific NSAIDs.


Abstract: The nonsteroidal anti-inflammatory drug sulindac is metabolized to sulindac sulfone (exisulind), an antineoplastic agent that inhibits growth and induces apoptosis in solid tumors. In colon cancer cells, the antineoplastic effects of exisulind have been attributed, in part, to induction of cyclic guanosine 3',5'-monophosphate (cGMP) signaling through inhibition of cGMP-specific phosphodiesterases, which elevates intracellular cGMP, and novel expression of cGMP-dependent protein kinase (PKG) Ibeta, the presumed downstream effector mediating apoptosis. Here, inhibition of proliferation and induction of cell death by exisulind was dissociated from cGMP signaling in human colon cancer cells. Accumulation of
intracellular cGMP produced by an exogenous cell-permeant analogue of cGMP or a potent agonist of guanylyl cyclase C yielded cytostasis without cell death. Surprisingly, the antiproliferative effects of induced cGMP accumulation were paradoxically less than additive, rather than synergistic, when combined with exisulind. Further, although exisulind induced expression of PKG Ibeta, it did not elevate intracellular cGMP and its efficacy was not altered by inhibition or activation of PKG I. Rather, PKG I induced by exisulind may mediate desensitization of cytostasis induced by cGMP. Thus, cytotoxic effects of exisulind are independent of cGMP signaling in human colon cancer cells. Moreover, combination therapies, including exisulind and agents that induce cGMP signaling, may require careful evaluation in patients with colon cancer


Abstract: This study compared Type-1 cGMP-dependent protein kinase (PKG) expression in normal and tumor tissues and examined PKG function in tumor growth. Studies with a cDNA array revealed that PKG expression was reduced in many tumors compared to respective normal tissue. This decrease in PKG expression was confirmed using quantitative RT-PCR and western blotting of matched colon specimens from normal epithelium and tumor tissue, and also in colon derived cell lines where luciferase reporter analysis revealed that the decreased expression occurred at the transcriptional level. Using SW620 colon carcinoma cells engineered for inducible expression of PKG1beta, it was found that exogenous PKG1beta lead to decreased tumor growth and invasiveness in nude mouse xenografts


Abstract: Recent studies indicate that the induction of apoptosis in human colon cancer cells by certain nonsteroidal antiinflammatory drugs involves increased expression of 15-LOX-1 and synthesis of its major product 13-S-hydroxyoctadecadienoic acid (13-S-HODE). Evidence was obtained that this occurs via a cyclooxygenase-2 (COX-2)-independent mechanism, but the actual mechanism of induction of 15-LOX-1 by these compounds is not known. There is extensive evidence that treatment of SW480 human colon cancer cells with sulindac sulfone (Exisulind, Aposyn) or the related derivative OSI-461, both of which inhibit cyclic GMP (cGMP)-phosphodiesterases but lack COX-2 inhibitory activity, causes an increase in intracellular levels of cGMP, thus activating protein kinase G (PKG), which then activates pathways that lead to apoptosis. Therefore, in the present study, we examined the effects of various agents that cause increased cellular levels of cGMP on the expression of 15-LOX-1 in SW480 human colon cancer cells. Treatment of the cells with Exisulind, sulindac sulfide, OSI-461, the guanylyl cyclase activator YC-1, or the cell-permeable cGMP compound 8-para-chlorophenylthio-cGMP (8-pCPT-cGMP) caused an increase in cellular levels of 15-LOX-1. Exisulind, OSI-461, and 8-pCPT-cGMP also increased mRNA levels of 15-LOX-1, suggesting that the effects were at the level of transcription. The cGMP-phosphodiesterase inhibitors and YC-1 increased the production of 13-S-HODE, which is the linoleic acid metabolite of 15-LOX-1. Treatment of SW480 cells with the PKG inhibitor Rp-8-pCPT-cGMP blocked Exisulind-induced 15-LOX-1 expression. Furthermore, derivatives of SW480 cells that were engineered to stably overexpress wild-type PKG Ibeta displayed increased cellular levels of 15-LOX-1 when compared with vector control cells. Taken together, these results provide evidence that the cGMP/PKG pathway can play an important role in the induction of 15-LOX-1 expression by nonsteroidal antiinflammatory drugs and related agents


Abstract: Phosphodiesterase 5 (PDE5) is a major isoform of cGMP phosphodiesterase in a variety of
human tumor cell lines and plays a key role in regulating intracellular cGMP concentrations ([cGMP]i). Here, we demonstrate that suppression of PDE5 gene expression by antisense pZeoSV2/ASP5 plasmid transfection results in a sustained increase in [cGMP]i, growth inhibition, and apoptosis in human colon tumor HT29 cells. With stable transfection, antisense transcripts exhibited a specific suppression in PDE5 activity, mRNA levels, and a 93 kDa hPDE5A1 protein. In cloned antisense cells, prolongation of the cell growth doubling times correlate positively with suppressed PDE5 activity and increased [cGMP]i. The growth inhibition in PDE5 antisense clones is due to an increased apoptotic rate and delayed cell-cycle progression. These results corroborate previous findings with the PDE5 inhibitor exisulind and its derivatives showing that sustained [cGMP]i induces apoptosis and growth inhibition in tumor cells. Furthermore, an inducible mitotic inhibitor p21WAF1/CIP1 has been found to account for the delay of cell-cycle progression in PDE5 antisense clones at G2/M phase. A proteolytic cleavage of p21WAF1/CIP1 in the antisense clones is also increased at the later stage of serum stimulation. The protein kinase G (PKG) inhibitor, KT5823, can prevent the cleavage of p21(WAF1/CIP). These data substantiate a pivot role for PDE5 as a modulator of apoptosis and cell-cycle progression for human carcinoma via a mechanism involving the activation of [cGMP]i/PKG signaling pathways.


Abstract: The activation of protein kinase G (PKG) by cGMP has become of considerable interest as a novel molecular mechanism for the induction of apoptosis in cancer cells, because sulindac sulfone (exisulind, Aptosyn) and certain derivatives that inhibit cGMP-phosphodiesterases and thereby increase cellular levels of cGMP appear to induce apoptosis via this mechanism. However, other effects of these compounds have not been excluded, and the precise mechanism by which PKG activation induces apoptosis has not been elucidated in detail. To directly examine the effects of PKG on cell growth and apoptosis, we generated a series of mutants of PKG Ialpha: PKG IalphaS65D, a constitutively activated point mutant; PKG IalphaDelta, a constitutively activated N-terminal truncated mutant; and PKG IalphaK390R, a dominant-negative point mutant. A similar series of mutants of PKG Ibeta were also constructed (Deguchi et al., Mol. Cancer Ther., 1: 803-809, 2002). The present study demonstrates that when transiently expressed in SW480 colon cancer, the constitutively activated mutants of PKG Ibeta, and to a lesser extent PKG Ialpha, inhibit colony formation and induce apoptosis. We were not able to obtain derivatives of SW480 cells that stably expressed these constitutively activated mutants, presumably because of toxicity. However, derivatives that stably overexpressed wild-type PKG Ibeta displayed growth inhibition, whereas derivatives that stably expressed the dominant-negative mutant (KR) of PKG Ibeta grew more rapidly and were more resistant to Aptosyn-induced growth inhibition than vector control cells. Stable overexpression of PKG Ibeta was associated with decreased cellular levels of beta-catenin and cyclin D1 and increased levels of p21(CIP1). Reporter assays indicated that activation of PKG Ibeta inhibits the transcriptional activity of the cyclin D1 promoter. We also found that transient expression of the constitutively activated mutants of PKG Ibeta inhibited cell migration. Taken together, these results indicate that activation of PKG Ibeta is sufficient to inhibit growth and cell migration and induce apoptosis in human colon cancer cells and that these effects are associated with inhibition of the transcription of cyclin D1 and an increase in the expression of p21(CIP1).


Abstract: The effects of Escherichia coli heat-stable enterotoxin (ST) and uroguanylin were examined on the proliferation of T84 and Caco2 human colon carcinoma cells that express guanylyl cyclase C (GC-C) and SW480 human colon carcinoma cells that do not express this receptor. ST or uroguanylin inhibited proliferation of T84 and Caco2 cells, but not SW480 cells, in a concentration-dependent fashion, assessed
by quantifying cell number, cell protein, and [(3)H]thymidine incorporation into DNA. These agonists did not inhibit proliferation by induction of apoptosis, assessed by TUNEL (terminal deoxynucleotidyl transferase-mediated dNTP-biotin nick end labeling of DNA fragments) assay and DNA laddering, or necrosis, assessed by trypan blue exclusion and lactate dehydrogenase release. Rather, ST prolonged the cell cycle, assessed by flow cytometry and [(3)H]thymidine incorporation into DNA. The cytostatic effects of GC-C agonists were associated with accumulation of intracellular cGMP, mimicked by the cell-permeant analog 8-Br-cGMP, and reproduced and potentiated by the cGMP-specific phosphodiesterase inhibitor zaprinast but not the inactive ST analog TJU 1-103. Thus, GC-C agonists regulate the proliferation of intestinal cells through cGMP-dependent mechanisms by delaying progression of the cell cycle. These data suggest that endogenous agonists of GC-C, such as uroguanylin, may play a role in regulating the balance between epithelial proliferation and differentiation in normal intestinal physiology. Therefore, GC-C ligands may be novel therapeutic agents for the treatment of patients with colorectal cancer.


Abstract: These studies report on the activation and induction of cGMP-dependent protein kinase (PKG) by exisulind and analogs and test the hypothesis that PKG is involved in the induction of apoptosis in colon tumor cells. Exisulind and analogs are proapoptotic drugs developed as inhibitors of cGMP phosphodiesterase gene families 5 and 2 that have been shown to sustain increased cGMP in SW480 and HT29 cells. At concentrations that induced apoptosis, both exisulind and CP461 increased PKG activity in SW480 cell supernatants. PKG activation was dose-dependent and sustained. Activation of PKG by exisulind and analogs was also seen in the colon tumor cell lines HT29, T84, and HCT116. The guanylyl cyclase activators YC-1 and guanylin increased PKG activity secondary to increased cellular cGMP and induced apoptosis in colon tumor cells. Exisulind and CP461 had no direct effect on purified PKG activity or on basal and stimulated PKG activity from cell supernatants. An additional effect of exisulind after 8 h of drug treatment was a dose-dependent increase of PKG Ibeta protein expression. beta-Catenin, a potential new substrate for PKG, whose regulation influences apoptosis, was phosphorylated by PKG in vitro. 32P-labeled cells treated with exisulind showed increased phosphorylation of beta-catenin. These data indicate that exisulind and analogs activate and induce PKG, resulting in increased phosphorylation of beta-catenin and enhanced apoptosis to promote colon tumor cell death.


Abstract: Sulindac sulfone (Exisulind) induces apoptosis and exhibits cancer chemopreventive activity, but in contrast to sulindac, it does not inhibit cyclooxygenases 1 or 2. We found that sulindac sulfone and two potent derivatives, CP248 and CP461, inhibited the cyclic GMP (cGMP) phosphodiesterases (PDE) 2 and 5 in human colon cells, and these compounds caused rapid and sustained activation of the e-Jun NH2-terminal kinase 1 (JNK1). Rapid activation of stress-activated protein/ERK kinase 1 (SEK1) and mitogen-activated protein kinase kinase kinase (MEKK1), which are upstream of JNK1, was also observed. Other compounds that increase cellular levels of cGMP also activated JNK1, and an inhibitor of protein kinase G (PKG), Rp-8-pCPT-cGMPS, inhibited JNK1 activation by the sulindac sulfone derivatives. Expression of a dominant-negative JNK1 protein inhibited CP248-induced cleavage of poly(ADP-ribose) polymerase, a marker of apoptosis. Thus, it appears that sulindac sulfone and related compounds induce apoptosis, at least in part, through activation of PKG, which then activates the MEKK1-SEK1-JNK1 cascade. These studies also indicate a role for cGMP and PKG in the JNK pathway.

Abstract: Biotin is a member of the vitamin B-complex family. Biotin deficiency has been associated with hyperglycaemia and insulin resistance in animals and humans. In the present study, we investigated the pharmacological effects of biotin on hypertension in the stroke-prone spontaneously hypertensive rat (SHRSP) strain. We observed that long-term administration of biotin decreased systolic blood pressure in the SHRSP strain; also, a single dose of biotin immediately decreased systolic blood pressure in this strain. Pretreatment with the guanylate cyclase inhibitor 1H-[1,2,4]oxadiazole [4,3-alpha]quinoxalin-1-one abolished the hypotensive action of biotin in the SHRSP strain, while pretreatment with the NO synthase inhibitor NG-nitro-l-arginine methyl ester had no effect on the action of biotin. Biotin reduced coronary arterial thickening and the incidence of stroke in the SHRSP strain. These results suggest that the pharmacological dose of biotin decreased the blood pressure of the SHRSP via an NO-independent direct activation of soluble guanylate cyclase. Our findings reveal the beneficial effects of biotin on hypertension and the incidence of stroke


Abstract: HeLa cells cultured in a biotin-deficient medium showed reduced rates of protein synthesis, DNA synthesis and growth. Continuous synthesis is required for the increase in DNA synthesis observed upon addition of biotin to cells cultured in biotin-deficient medium. The addition of biotin to the biotin-deficient culture medium increased the activity of guanylate cyclase in both HeLa cells and fibroblasts. Both cell types cultured in biotin deficient medium showed reduced activity of RNA Polymerase II. The exogenous addition of biotin to the biotin-deficient cell cultures also resulted in increased activity of RNA Polymerase II in HeLa cells and fibroblasts. The maximal response was observed in 4 hours. Significant increase in enzyme activity was observed at 10(-8) M biotin in the culture medium. The growth promoting effect of biotin seems to involve stimulations of cellular guanylate cyclase and RNA Polymerase II activity


Abstract: Biotin and its analog, (+)-biotin-p-nitrophenyl ester enhanced guanylate cyclase activity two- to threefold in rat liver, kidney, colon, cerebellum, and heart. Dose-response relationships revealed that at concentrations as low as 1 micromolar, both biotin and its analog caused maximal augmentation of guanylate cyclase activity. These data suggest a role for the activation of guanylate cyclase in the mechanism of action of this vitamin


Abstract: The treatment for erectile dysfunction (ED) was revolutionized with the development of phosphodiesterase type 5 (PDE5) inhibitors. Tadalafil (Cialis((R)); Eli Lilly and Company, Indianapolis, IN, USA) is the newest and most versatile PDE5 inhibitor in the clinical armamentarium for the treatment of ED. Its most unique characteristic is its long half-life of 17.5 hours, which lends itself to a longer therapeutic window with on-demand dosing and effective steady-state plasma concentrations with once-daily dosing. Clinical trials have proven its safety and efficacy with both dosing strategies for all severities and etiologies of ED, including difficult-to-treat ED. This thorough review will discuss ED, the physiology of penile erection and the role of PDE5, and all aspects of tadalafil, from its development, through its pharmacology, to its latest clinical studies and indications
**Cimetidine**


Abstract: Background/Aims: Cimetidine has been shown to play an important role in the treatment of cancer and the regulation of the immune system. Therefore, we aimed to observe the effects of cimetidine on the systematic immune response in the perioperative period. Methodology: Sixty patients with colorectal cancer were enrolled from Jan 2005 to Dec 2005 from Taizhou Hospital. The patients were administrated with cimetidine (0.8g.d-1 or 1.2g.d-1) or saline from the day of admission to the 10th POD. Venous blood sample was collected and the T-, B- and NK-lymphocyte subsets were determined by flow cytometry. The specimens were subjected to tumor-infiltrating lymphocytes (TILs) response examination. Results: The levels of CD3 and CD4 T-lymphocytes were increased significantly in both low and high dose cimetidine groups 10 days after operation. The number of CD19 B cells was also elevated by cimetidine. However, no significant changes were observed in the CD8, CD4/CD8 value. TIL responses in the cimetidine groups were also enhanced significantly. Conclusions: Cimetidine can alleviate systematic immunosuppression and improve the local immune function of the colorectal cancer patients in the perioperative period

Deva S, Jameson M. Histamine type 2 receptor antagonists as adjuvant treatment for resected colorectal cancer. *Cochrane Database Syst Rev* 2012;8:CD007814.

Abstract: BACKGROUND: Anecdotal reports of tumour regression with histamine type 2 receptor antagonists (H(2)RAs) have lead to a series of trials with this class of drug as adjuvant therapy to try and improve outcomes in patients with resected colorectal cancers. There was a plausible scientific rationale suggesting merit in this strategy. This included improved immune surveillance (by way of increasing tumour infiltrating lymphocytes), inhibiting the direct proliferative effect of histamine as a growth factor for colorectal cancer and, in the case of cimetidine, inhibiting endothelial expression of E-selectin (a cell adhesion molecule thought to be critical for metastatic spread). OBJECTIVES: To determine if H(2)RAs improve overall survival when used as pre- and/or postoperative therapy in colorectal cancer patients who have had surgical resection with curative intent. We also stratified the results to see if there was an improvement in overall survival in terms of the specific H(2)RA used. SEARCH METHODS: Randomised controlled trials were identified using a sensitive search strategy in the following databases: MEDLINE (1964 to present), the Cochrane Central Register of Controlled Trials (CENTRAL, The Cochrane Library 2009), EMBASE (1980 to present) and Cancerlit (1983 to present). SELECTION CRITERIA: Criteria for study selection included: patients with colorectal cancer surgically resected with curative intent, H(2)RAs used i) at any dose, ii) for any length of time, iii) with any other treatment modality and iv) in the pre-, peri- or post-operative period. The results were stratified for the H(2)RA used. DATA COLLECTION AND ANALYSIS: The literature search retrieved 142 articles. There were six studies included in the final analysis, published from 1995 to 2007, including a total of 1229 patients. All patients were analysed by intention to treat according to their initial allocation. Log hazard ratios and standard errors of treatment effects (on overall survival) were calculated using the Cochrane statistical package RevMan Version 5. Hazard ratios and standard errors were recorded from trial publications or, if not provided, were estimated from published actuarial survival curves using a spreadsheet designed for this purpose (http://www.biomedcentral.com/content-supplementary/1745-6215-8-16-S1.xls). MAIN RESULTS: Of the six identified trials, five used cimetidine as the experimental H(2)RA, whereas one used ranitidine. There was a trend towards improved survival when H(2)RAs were utilised as adjuvant therapy in patients having curative-intent surgery for colorectal cancer (HR 0.70; 95% CI 0.48-1.03, P = 0.07). Analysis of the five cimetidine trials (n = 421) revealed a statistically significant improvement in overall survival (HR 0.53; 95% CI 0.32 to 0.87). AUTHORS’ CONCLUSIONS: Of the H(2)RAs
evaluated cimetidine appears to confer a survival benefit when given as an adjunct to curative surgical resection of colorectal cancers. The trial designs were heterogeneous and adjuvant therapy has evolved since these trials were performed. Further prospective randomised studies are warranted.

Yoshimatsu K, Ishibashi K, Yokomizo H et al. [Can the survival of patients with recurrent disease after curative resection of colorectal cancer be prolonged by the administration of cimetidine?]. Gan To Kagaku Ryoho 2006 November;33(12):1730-2.

Abstract: Administration of cimetidine after curative surgery can improve prognosis of patients with colorectal cancer. In this study, we analyzed whether cimetidine can influence the survival of patients with a recurrent disease after colorectal surgery. The subjects were 29 patients with recurrent disease: 14 patients were administered with cimetidine and 15 patients were not. In the cimetidine administered group, seven cases were recurrent in the liver, 5 cases in a local site and 1 case in the lymph node, whereas 7 cases were recurrent in the liver, 4 cases in a local site and 3 cases in the lung for the non-cimetidine administered group. There were no significant differences for both groups in terms of patient's survival after recurrence. Although it was not significant, the patient's survival after curative resection of recurrent disease for the cimetidine administered group was better than the non cimetidine administered group. Although the results did not show cimetidine could influence the overall survival of the patients after recurrence, it might be possible to improve the survival of the patients after resection of the recurrent disease.


Abstract: Cimetidine is known to enhance the survival of gastro-intestinal cancer patients, though the mechanisms involved are incompletely understood. Postulated modes of action include blocking the proliferative effect of tumors and inhibiting T suppressor cell activity, both of which are thought to be mediated by histamine type 2 receptors. Apoptotic cell death may offer an alternative explanation for reduced cell growth. We aimed to examine the effects of histamine, cimetidine, and ranitidine on in vitro proliferation and apoptosis in two human colorectal cancer cell lines, Caco-2 and LoVo. A cell proliferation assay was used as an index of cell growth. Histamine receptor status was determined by quantifying cyclic adenosine monophosphate and apoptosis via DNA fragmentation. Results show that histamine (10(-5) to 10(-9) M) had no effect on the growth of either cell line. The proliferation of Caco-2 was inhibited by ranitidine (10(-7) M) alone and in combination with histamine. Cimetidine (10(-5) M) only suppressed the growth of Caco-2 in the presence of histamine. The H2 antagonists had no effect on LoVo irrespective of histamine. There was no accumulation of cyclic adenosine monophosphate in Caco-2 cells in response to histamine at a similar concentration. Apoptosis was induced in Caco-2 by both antisecretory drugs, and only ranitidine caused apoptotic cell death in LoVo cells. We conclude that cimetidine and ranitidine inhibit Caco-2 cancer cells in vitro, independently of the H2 receptor. In addition, both drugs induce apoptosis in the same cell line. Growth inhibition and apoptosis are likely to contribute to the tumor regressive properties of cimetidine and ranitidine in vivo.


Abstract: Cimetidine is known to suppress the growth of several tumors, including gastrointestinal cancer, in humans and animals. Nonetheless, whether other histamine H(2)-receptor antagonists exert such tumor suppressive effects remains unclear. The effect of roxatidine acetate hydrochloride (roxatidine), an H(2)-receptor antagonist, on the growth of colon cancer implanted in mice was examined and compared with that of cimetidine. Drugs were orally delivered for 26 - 29 days beginning before or after implantation of syngeneic colon cancer (Colon 38) in C57BL/6 mice. Tumor volume was determined throughout and
histrochemical analysis was also performed. Tumor tissue and serum vascular endothelial growth factor (VEGF) levels were measured. In vitro cell growth was assessed by the MTT assay. Both roxatidine and cimetidine significantly suppressed the growth of Colon 38 tumor implants. Histologic analysis revealed that such antagonists markedly increased necrotic areas and decreased the density of microvessels in tumor tissue. Both H(2)-receptor antagonists suppressed VEGF levels in tumor tissue and significantly decreased serum VEGF levels in Colon 38-bearing mice. Such drugs, however, failed to suppress in vitro growth of the cell line. In conclusion, both roxatidine and cimetidine were found to exert suppressive effects on the growth of colon cancer implants in mice by inhibiting angiogenesis via reducing VEGF expression.


Abstract: We herein report the result of a prospective study to investigate the efficacy of cimetidine administration in conjunction with chemotherapy for stage IV colorectal cancer. Sixty-two patients treated with Leucovorin/5-fluorouracil therapy were enrolled from 1996 to 2000. Both groups were well matched for pre-treatment characteristics. There was no difference in survival in cur B patients. However, the cimetidine group had significantly prolonged survival in the patients with cur C or non-resectable carcinoma. This study suggests that cimetidine treatment may improve the survival of patients with non-curative surgery for stage IV colorectal cancer.


Abstract: Thirty-eight colorectal cancer patients were randomly assigned to treatment group, which took cimetidine in the perioperative period, and control group to which no drug was given. Twenty healthy volunteers served as normal controls. NK cells were measured by immunocytochemical technique. The results showed that NK percentages before treatment in both groups of patients were significantly lower than those in normal controls (P < 0.05). NK cell percentages at admission, before operation, on the 2nd and the 10th postoperative days were 14.84 +/- 4.41, 15.74 +/- 3.75, 17.21 +/- 3.69, 21.05 +/- 4.54, respectively, for the treatment group, and 15.00 +/- 2.77, 13.05 +/- 2.46, 14.21 +/- 2.19, 15.58 +/- 1.68, respectively, for control group. The difference was statistically significant (P < 0.01), suggesting that the perioperative administration of cimetidine could help restore NK cells in colorectal cancer patients.


Abstract: Cimetidine, a H(2) receptor antagonist, has been reported to improve survival in gastrointestinal cancer patients. These effects have largely been attributed to the enhancing effects of cimetidine on the host's antitumour cell-mediated immune response, such as inhibition of suppressor T lymphocyte activity, stimulation of natural killer cell activity and increase of interleukin-2 production from helper T lymphocytes. We conducted an in vitro study on the effects of cimetidine on differentiation and antigen presenting capacity of monocyte-derived dendritic cells from advanced colorectal cancer patients and normal controls. As a result, an investigation of expression of surface molecules associated with dendritic cells by flow cytometric analyses showed that cimetidine had no enhancing effect on differentiation of dendritic cells from cancer patients and normal controls. An investigation of [(3)H]thymidine incorporation by allogeneic mixed lymphocyte reactions revealed that cimetidine increased the antigen presenting capacity of dendritic cells from both materials. Moreover, a higher antigen presenting capacity was observed in advanced cancer patients compared to normal controls. These effects might be mediated via specific action of cimetidine and not via H(2) receptors because famotidine did not show similar
effects. Our results suggest that cimetidine may enhance the host's antitumour cell-mediated immunity by improving the suppressed dendritic cells function of advanced cancer patients.


Abstract: Cimetidine has been shown to have beneficial effects in colorectal cancer patients. In this study, a total of 64 colorectal cancer patients who received curative operation were examined for the effects of cimetidine treatment on survival and recurrence. The cimetidine group was given 800 mg day(-1) of cimetidine orally together with 200 mg day(-1) of 5-fluorouracil, while the control group received 5-fluorouracil alone. The treatment was initiated 2 weeks after the operation and terminated after 1 year. Robust beneficial effects of cimetidine were noted: the 10-year survival rate of the cimetidine group was 84.6% whereas that of control group was 49.8% (P<0.0001). According to our previous observations that cimetidine blocked the expression of E-selectin on vascular endothelium and inhibited the adhesion of cancer cells to the endothelium, we have further stratified the patients according to the expression levels of sialyl Lewis antigens X (sL(x)) and A (sL(a)). We found that cimetidine treatment was particularly effective in patients whose tumour had higher sL(x) and sL(a) antigen levels. For example, the 10-year cumulative survival rate of the cimetidine group with higher CSLEX staining, recognizing sL(x), of tumours was 95.5%, whereas that of control group was 35.1% (P=0.0001). In contrast, in the group of patients with no or low levels CSLEX staining, cimetidine did not show significant beneficial effect (the 10-year survival rate of the cimetidine group was 70.0% and that of control group was 85.7% (P=n.s.)). These results clearly indicate that cimetidine treatment dramatically improved survival in colorectal cancer patients with tumour cells expressing high levels of sL(x) and sL(a).


Abstract: Although the beneficial effect of cimetidine on survival in cancer has been clinically demonstrated in colorectal cancer patients, the mode of action of cimetidine has not been elucidated. In this report, we have demonstrated for the first time that cimetidine can block the adhesion of a colorectal tumor cell line to the endothelial cell monolayer in cell culture and that it can suppress the metastasis of the tumor cell in a nude mouse model. We also demonstrated that these antimetastasis effects of cimetidine might occur through down-regulation of the cell surface expression of E-selectin on endothelial cells, a ligand for sialyl Lewis antigens on tumor cells. We found that the cimetidine-mediated down-regulation of E-selectin did not involve down-regulation of E-selectin mRNA or blocking of the nuclear translocation of nuclear factor kappaB, a transcriptional activator of E-selectin gene expression. Because two other histamine type 2 receptor antagonists, famotidine and ranitidine, did not show any similar effect, these actions of cimetidine probably do not occur via blocking of the histamine receptor. These observations support the idea that cancer metastasis can be blocked by cimetidine administration through blocking the adhesion of tumor cells to the endothelium when an interaction between E-selectin and sialyl-Lewis antigens plays a role.


Abstract: We investigated the effects of cimetidine on p-glycoprotein and on the antiproliferative effect of various anticancer drugs which are recognized by p-glycoprotein. We used colon carcinoma SW620 cells and their doxorubicin resistant SW620 Ad300 derived cells, the latter express p-glycoprotein and have multidrug resistance phenotype. To assess the effect of cimetidine on the efflux activity of p-glycoprotein,
we examined its effects on the accumulation of rhodamine123 which is recognized by p-glycoprotein. Cimetidine had no effect on rhodamine123 accumulation on either cell line. Cimetidine did not enhance the antiproliferative effect of any of the anticancer drugs investigated. This indicates that cimetidine was not recognized by p-glycoprotein. However, cimetidine remarkably enhanced the antiproliferation effects of 5-fluorouracil on SW620 cells, not those of 5-fluorouracil on SW620 Ad300, whereas cimetidine itself showed little effect on antiproliferation. This finding indicates that combination therapy using 5-fluorouracil and cimetidine might be useful for cancer patients.


Abstract: BACKGROUND: Previous studies have suggested that cimetidine, a histamine-2 receptor antagonist with immunostimulatory effects, may improve survival in patients with colorectal carcinoma. This effect may be apparent by an increase in the number of peritumoral lymphocytes. A prospective, double blind, randomized, placebo-controlled trial of a short course of preoperative treatment with cimetidine in patients with colorectal carcinoma was performed to assess the effect of cimetidine on survival and on the number of peritumoral lymphocytes. METHODS: One hundred and twenty-five patients who were scheduled to undergo elective colon or rectal excision for carcinoma were randomized to receive either placebo or cimetidine preoperatively for 5 days. In addition to standard histopathology, immunohistochemistry and computer video image analysis were used to assess the number of peritumoral lymphocytes in an objective manner. Interim survival analysis according to the Kaplan-Meier method was performed. RESULTS: A trend toward a survival advantage in the group of patients receiving cimetidine (800 mg twice daily) compared with the placebo group was observed (P = 0.20, log rank test) that was most marked in patients with replication error negative tumors (P = 0.04). Similarly, in these two groups there was a trend toward an increase in the number of patients with a conspicuous lymphocytic infiltration (P = 0.10, chi-square test). However, there was no difference in the number of peritumoral lymphocytes as measured by image analysis. CONCLUSIONS: Based on the results of the current study, a short course of preoperative treatment with cimetidine does appear to have an effect on patient survival; however, the exact mechanism is unknown. The failure of this study to demonstrate a clear increase in the local lymphocyte response does not exclude an immunologic mechanism of action.


Abstract: PURPOSE: To evaluate the influence of a H2 receptor antagonist (cimetidine) on survival in patients with colorectal carcinoma, a randomized, controlled pilot study was performed in three university hospitals in Copenhagen, Denmark. METHODS: A total of 192 patients, who had undergone a resection or an exploratory operation for adenocarcinoma of the colon or rectum between May 1988 and May 1991, were enrolled in the study. After a median observation time of 40 months, outcome was noted for each patient concerning cancer-specific mortality rate. RESULTS: In patients operated with curative intent (n = 148), no difference was found in cancer-specific mortality between the two treatments. However, a tendency toward reduction in mortality rate was found in patients with curatively operated Dukes Stage C
cancer (P = 0.11, log-rank test; difference, 29 percent; 90 percent confidence interval, 2 to 57 percent) in the cimetidine-treated group. In patients with disseminated disease no total difference was found between the two treatment groups. CONCLUSIONS: Cimetidine does not seem to reduce mortality in patients with colorectal cancer, but there seems to be a tendency toward a survival benefit in patients undergoing surgery for Dukes Stage C carcinoma. Results seem to justify trials in this patient category to reveal a benefit of H2 receptor antagonists in adjuvant therapy of colorectal carcinoma.


Abstract: Fifty consecutive patients undergoing resection of colorectal cancer were randomized to either receive cimetidine at a dose of 400 mg bd for a minimum of 5 pre-operative days, then intravenously for 2 postoperative days, or to act as controls. Baseline immune function was determined in all patients by in vitro testing of lymphocyte proliferation (LP) in response to mitogen, skin testing for cell mediated immunity (CMI) and measurement of lymphocyte subsets. Immune function was retested in both groups on the second postoperative day. In control patients the mean postoperative LP value was 41% of pre-operative levels (P < 0.0001) and the mean CMI reduced to 29% (P < 0.0001). Patients treated with cimetidine had no significant fall in these parameters. Numbers of T and natural killer (NK) cells fell after surgery in both groups, and B cell numbers were maintained in the cimetidine group. It is concluded that cimetidine reduces the immunosuppression that follows colonic resection.


Abstract: The effect of histamine and cimetidine on the growth of four human colon cancer cell lines was studied. Histamine significantly stimulated the uptake of tritiated thymidine in vitro in a dose dependent manner, to a maximum of 120% and 116% of controls for C170 and LIM2412, respectively. This effect was antagonised by cimetidine, but not diphenhydramine. Histamine also stimulated a dose dependent increase in cyclic adenosine monophosphate accumulation in C170 cells, antagonised by cimetidine. When grown as subcutaneous xenografts in Balb/c nu/nu mice, cimetidine had a significant inhibitory effect on the same two cell lines. The final volume of C170 tumours in animals given cimetidine was 44% of controls. This response was dose dependent, plateauing at a cimetidine dose of 50 mg/kg/day. The final volume of LIM2412 tumours in animals given cimetidine was 60% of controls. Histamine administered locally by a mini-osmotic pump stimulated C170 tumour growth to 164% of controls, was antagonised by cimetidine at a dose of 200 mg/kg/day, but not by lower concentrations. Histamine has a trophic effect on at least two colorectal cancer cell lines in vivo and in vitro. As this effect is antagonised by cimetidine, it may be mediated via tumour histamine type 2 receptors.

Modified Citrus Pectin


Abstract: AIM: To discuss the expression of glactin-3 in liver metastasis of colon cancer and its inhibition by modified citrus pectin (MCP) in mice. METHODS: Seventy-five Balb/c mice were randomly divided
into negative control group (n = 15), positive control group (n = 15), low MCP concentration group (n = 15), middle MCP concentration group (n = 15) and high MCP concentration group (n = 15). CT26 colon cancer cells were injected into the subcapsule of mouse spleen in positive control group, low, middle and high MCP concentrations groups, except in negative control, to set up a colon cancer liver metastasis model. The concentration of MCP in drinking water was 0.0%, 0.0%, 1.0%, 2.5% and 5.0% (wt/vol), respectively. Liver metastasis of colon cancer was observed after 3 wk. Enzyme-linked immunosorbent assay (ELISA) was used to detect the concentration of galectin-3 in serum. Expression of galectin-3 in liver metastasis was detected by immunohistochemistry. RESULTS: Except for the negative group, the percentage of liver metastasis in the other 4 groups was 100%, 80%, 73.3% and 60%, respectively. The number of liver metastases in high MCP concentration group was significantly less than that in positive control group (P = 0.008). Except for the negative group, the median volume of implanted spleen tumor in the other 4 groups was 1.51 cm(3), 0.93 cm(3), 0.77 cm(3) and 0.70 cm(3), respectively. The volume of implanted tumor in middle and high MCP concentration groups was significantly smaller than that in positive control group (P = 0.019; P = 0.003). The concentration of serum galectin-3 in positive control and MCP treatment groups was significantly higher than that in the negative control group. However, there was no significant difference between them. Except for the negative control group, the expression of galectin-3 in liver metastases of the other 4 groups showed no significant difference. CONCLUSION: Expression of galectin-3 increases significantly in liver metastasis of colon cancer, which can be effectively inhibited by MCP.


Abstract: OBJECTIVE: To observe the expression of galectin-3 in the liver metastasis of colon cancer in mice and the inhibitory effect of modified citrus pectin (MCP) on galectin-3 expression. METHODS: Seventy-five Balb/c mice were randomized into 5 groups, namely the negative control, positive control, low-concentration MCP, moderate-concentration MCP and high-concentration MCP groups. CT26 colon cancer cells were injected into the subcapsule of the mouse spleen to establish liver metastasis models of colon cancer, but the mice in the negative control group received no tumor cell injection. MCP was added into the drinking water of the mice at the concentrations of 0, 1.0%, 2.5% and 5.0% (m/V). The liver metastasis was observed 3 weeks after tumor cell inoculation. Enzyme-linked immunosorbent assay was performed to determine the serum galectin-3 level. A tissue microarray of the liver metastasis was prepared for immunohistochemical detection of galectin-3 expression in the liver metastasis. RESULTS: In the positive control, low-, moderate- and high-concentration MCP groups, the rates of liver metastasis were 100%, 80%, 73.3% and 60%, respectively. The number of liver metastases in high-concentration MCP group was significantly smaller than that in the positive control group (P<0.05). In the 4 groups with tumor cell inoculation, the median volume of the primary lesions in the spleen was 1.51, 0.93, 0.77 and 0.70 cm(3), respectively, which were significantly smaller in the moderate- and high-concentration MCP groups than in the positive control group (P<0.05). The serum galectin-3 level in the positive control group and MCP-treated groups were significantly higher than that in the negative control group (P<0.01), but similar between the positive control group and the MCP-treated groups (P>0.05). In the positive control and the MCP-treated groups, the expression of galectin-3 in the liver metastases showed no significant differences (P>0.05). CONCLUSION: The expression of galectin-3 is significantly increased in the liver metastasis of colon cancer, and MCP can effectively inhibit the liver metastasis Nangia-Makker P, Hogan V, Honjo Y et al. Inhibition of human cancer cell growth and metastasis in nude mice by oral intake of modified citrus pectin. J Natl Cancer Inst 2002 December 18;94(24):1854-62.

Abstract: BACKGROUND: The role of dietary components in cancer progression and metastasis is an emerging field of clinical importance. Many stages of cancer progression involve carbohydrate-mediated recognition processes. We therefore studied the effects of high pH- and temperature-modified citrus
pectin (MCP), a nondigestible, water-soluble polysaccharide fiber derived from citrus fruit that specifically inhibits the carbohydrate-binding protein galectin-3, on tumor growth and metastasis in vivo and on galectin-3-mediated functions in vitro. METHODS: In vivo tumor growth, angiogenesis, and metastasis were studied in athymic mice that had been fed with MCP in their drinking water and then injected orthotopically with human breast carcinoma cells (MDA-MB-435) into the mammary fat pad region or with human colon carcinoma cells (LSLiM6) into the cecum. Galectin-3-mediated functions during tumor angiogenesis in vitro were studied by assessing the effect of MCP on capillary tube formation by human umbilical vein endothelial cells (HUVECs) in Matrigel. The effects of MCP on galectin-3-induced HUVEC chemotaxis and on HUVEC binding to MDA-MB-435 cells in vitro were studied using Boyden chamber and labeling assays, respectively. The data were analyzed by two-sided Student's t test or Fisher's protected least-significant-difference test. RESULTS: Tumor growth, angiogenesis, and spontaneous metastasis in vivo were statistically significantly reduced in mice fed MCP. In vitro, MCP inhibited HUVEC morphogenesis (capillary tube formation) in a dose-dependent manner. In vitro, MCP inhibited the binding of galectin-3 to HUVECs: At concentrations of 0.1% and 0.25%, MCP inhibited the binding of galectin-3 (10 micro g/mL) to HUVECs by 72.1% (P =.038) and 95.8% (P =.025), respectively, and at a concentration of 0.25% it inhibited the binding of galectin-3 (1 micro g/mL) to HUVECs by 100% (P =.032). MCP blocked chemotaxis of HUVECs toward galectin-3 in a dose-dependent manner, reducing it by 68% at 0.005% (P<.001) and inhibiting it completely at 0.1% (P<.001). Finally, MCP also inhibited adhesion of MDA-MB-435 cells, which express galectin-3, to HUVECs in a dose-dependent manner. CONCLUSIONS: MCP, given orally, inhibits carbohydrate-mediated tumor growth, angiogenesis, and metastasis in vivo, presumably via its effects on galectin-3 function. These data stress the importance of dietary carbohydrate compounds as agents for the prevention and/or treatment of cancer.


Abstract: The health benefits of fruits and vegetables have been the subject of numerous investigations over many years. Two natural substances, quercetin (a flavonoid) and citrus pectin (a polysaccharide found in the cell wall of plants) are of particular interest to cancer researchers. Two modified versions of these substances - quercetin chalcone (QC) and a pH-modified citrus pectin (MCP) - are the focus of this study. Previous research has confirmed that quercetin exhibits antitumor properties, likely due to immune stimulation, free radical scavenging, alteration of the mitotic cycle in tumor cells, gene expression modification, anti-angiogenesis activity, or apoptosis induction, or a combination of these effects. MCP has inhibited metastases in animal studies of prostate cancer and melanoma. To date, no study has demonstrated a reduction in solid tumor growth with MCP, and there is no research into the antitumor effect of QC. This study examines the effects of MCP and QC on the size and weight of colon-25 tumors implanted in balb-c mice. Fifty mice were orally administered either 1 ml distilled water (controls), low-dose QC (0.8 mg/ml), high-dose QC (1.6 mg/ml), low-dose MCP (0. 8 mg/ml) or high-dose MCP (1.6 mg/ml) on a daily basis, beginning the first day of tumor palpation (usually eight days post-implantation). A significant reduction in tumor size was noted at day 20 in all groups compared to controls. The groups given low-dose QC and MCP had a 29-percent (NS) and 38-percent (p<0.02) decrease in size, respectively. The high-dose groups had an even more impressive reduction in size; 65 percent in the QC group and 70 percent in the mice given MCP (both p<0.001). This is the first evidence that MCP can reduce the growth of solid primary tumors, and the first research showing QC has antitumor activity. Additional research on these substances and their effect on human cancers is warranted.
NF-kappaB Inhibitors - Salsalate and Anti-inflammatory Phytochemicals


Abstract: BACKGROUND: Deguelin, a naturally occurring rotenoid, is known to be an Akt inhibitor and to have an anti-tumor effect on several cancers. AIMS: This study was performed to elucidate the effect of deguelin on apoptotic pathways related to NF-kappaB signaling in colon cancer cells and on the anti-tumor effect in colon cancer xenograft mice. METHODS: We studied COLO 205 and HCT116 cells in the presence or absence of deguelin. NF-kappaB signaling was examined by real-time RT-PCR for interleukin (IL)-8, by Western blotting for IkappaB phosphorylation/degradation, and by the electrophoretic mobility shift assay. Cell death was determined by the MTT assay, and apoptosis by Annexin V-FITC staining and caspase-3 activity. We also assessed the expression of antiapoptotic and proapoptotic factors by use of RT-PCR. In colon cancer xenograft mice, we evaluated the effect of deguelin on inoculated tumor growth, and apoptotic index was measured by the in vivo TUNEL assay. RESULTS: Deguelin significantly inhibited IL-8 gene expression, IkappaB phosphorylation/degradation, and DNA binding activity of NF-kappaB in colon cancer cells. Deguelin induced cell death and apoptosis in colon cancer cells in a dose and time-dependent manner. Deguelin down-regulated expression of NF-kappaB-mediated antiapoptotic factors such as cFLIP, Bcl-2, and Bcl-X(L). In the colon cancer xenograft model, the volume of the tumor treated with deguelin was significantly lower than that of the control, and the apoptotic index for deguelin-treated mice was much higher. CONCLUSION: Deguelin might be a potential therapeutic agent for treatment of colorectal cancer.


Abstract: BACKGROUND: Evidence shows a strong relationship between KRAS mutations and the NF-kappaB signaling pathway. In colorectal cancer, however, the study of this subject has been very limited and results are inconsistent. AIMS: To examine the relationship between KRAS mutations and NF-kappaB activation and their effect on chemotherapy response and survival of colorectal cancer patients. MATERIALS AND METHODS: NF-kappaB activation was analyzed by immunohistochemistry in 167 primary colorectal cancer specimens in which the KRAS mutation status was confirmed. Clinical and pathologic data were extracted from the medical records and reviewed. RESULTS: Of 167 tumors screened, 63 (37.7 %) had NF-kappaB activation, 59 (35.3 %) had KRAS mutations, and 30 (18.0 %) had both NF-kappaB activation and KRAS mutations. The frequency of NF-kappaB activation in tumors with KRAS mutations was significantly higher than in tumors with wild type KRAS; 50.8 versus 30.6 %, P = 0.012. Patients with both KRAS mutations and NF-kappaB activation had a lower objective response to first-line chemotherapy than patients with other tumors, 23.8 versus 49.4 % (P = 0.035). Compared to patients with both KRAS mutations and NF-kappaB activation, overall survival of patients in other groups was significantly higher; median overall survival was 28.4 months (95 % CI 21.0-35.8) versus 46.3 months (95 % CI 39.4-53.2), hazard ratio 0.259 (95 % CI 0.125-0.538), P = 0.005. CONCLUSIONS: NF-kappaB activation was associated with KRAS mutation, and both KRAS mutation and NF-kappaB activation were indicative of high tolerance of chemotherapy and poor prognosis for colorectal cancer patients. Tumors with KRAS mutations and NF-kappaB activation may be a unique subtype of colorectal cancer.

Abstract: BACKGROUND: NF-kappaB expression has been shown to be responsible for resistance to antineoplastic agents. AIMS: The aim of our study was to investigate the importance of NF-kappaB expression as prognostic factor in locally advanced rectal cancer patients receiving neoadjuvant radiochemotherapy. METHODS: We retrospectively analysed the immunoreactivity for NF-kappaB in patients with locally advanced rectal cancer who underwent neoadjuvant treatment (chemotherapy and/or radiotherapy) in our Institution between March 2003 and June 2006. RESULTS: Seventy-four consecutive patients were enrolled into this study. Immunohistochemistry analysis for NF-kappaB was performed both in biopsies and in primary tumour samples. NF-kappaB was considered positive when at least 1% of the tumour cells showed nuclear positivity. A significant correlation between a positive NF-kappaB nuclear expression, both in biopsies and in tumour samples, and a worse overall survival was observed. Moreover, median time to progression was significantly shorter in the NF-kappaB-positive subgroup of patients. CONCLUSION: Globally, our findings seem to suggest that NF-kappaB could represent an important parameter able to predict the outcome in patients receiving neoadjuvant treatment for rectal cancer. It also could be useful in order to select patients to receive adjuvant chemotherapy, intensifying the adjuvant therapy and, in the next future, obviating the use of drugs involving NF-kappaB system in their mechanism of action in NF-kappaB-positive patients.


Abstract: INTRODUCTION: The NF-kappaB transcription factor protein family has diverse cellular and biological functions, and posttranslational modification is important to regulate these functions. An important site of phosphorylation of NF-kappaB p65 subunit is at serine-536 (phospho-Ser536-p65), and this phosphorylation is involved in regulation of transcriptional activity, nuclear localization, and protein stability. PATIENTS AND METHODS: In this study, we investigated expression of phospho-Ser536-p65 in colorectal cancers and its relationships with clinicopathological factors. The expression of phospho-Ser536-p65 was examined by immunohistochemistry in 203 primary colorectal cancers, 156 normal mucosa specimens, and 18 metastases in the lymph nodes. RESULTS: The expression of phospho-Ser536-p65 increased from normal mucosa to primary tumor (p < 0.0001). Further, the increased expression of phospho-Ser536-p65 in the cytoplasm of the primary tumors correlated with worse survival of the patients independently of gender, age, tumor location, stage, and differentiation (p = 0.04; hazard ratio, 1.89; 95% CI 1.03-3.47). CONCLUSION: The NF-kappaB p65 subunit phosphorylated at serine-536 is an independent prognostic factor in colorectal cancer patients.


Abstract: Chronic inflammation is one of the primary causes of colorectal cancer (CRC), and major inflammatory pathways implicated in CRC are cyclooxygenase-2 (COX-2) and iNOS; both regulated by nuclear factor-kappa B (NF-kappaB) suggesting that inhibitors of these pathways could be ideal against CRC. Silibinin has shown promising efficacy against various malignancies including CRC, and therefore here we assessed whether silibinin targets NF-kappaB activation and associated signaling as a mechanism of its anti-inflammatory and anti-cancer effects in CRC. Our results indicated that silibinin treatment (50-200 microM) of human CRC SW480, LoVo, and HT29 cells strongly inhibits tumor necrosis factor alpha-induced NF-kappaB activation together with decreased nuclear levels of both p65 and p50 sub-units. Silibinin also significantly increased IkappaBalpha level with a concomitant decrease in phospho-IkappaBalpha, without any effect on TNFR1, TRADD, and RIP2, indicating its inhibitory effect on IkappaB kinase alpha activity. Next we assessed the effect of oral silibinin feeding on NF-kappaB pathway in SW480 (COX-2 negative) and LoVo (COX-2 positive) tumor xenografts in nude mice. Together with its inhibitory efficacy on tumor growth and progression, silibinin inhibited NF-kappaB
activation in both xenografts. The protein levels of various NF-kappaB-regulated molecules such as Bel-2, COX-2, iNOS, VEGF, and MMPs were also decreased by silibinin in both cell culture studies and xenograft analyses, suggesting its potential to alter NF-kappaB transcriptional activity. Together, these findings are highly significant in establishing for the first time that silibinin suppresses CRC growth and progression possibly through its anti-inflammatory activity by interfering with NF-kappaB activation and thus has potential against human CRC. (c) 2011 Wiley Periodicals, Inc


Abstract: BACKGROUND: Extravasation of circulating cancer cells is a key event of metastatic dissemination that is initiated by the adhesion of cancer cells to endothelial cells. It requires interactions between adhesion receptors on endothelial cells and their counter-receptors on cancer cells. Notably, E-selectin, a major endothelial adhesion receptor, interacts with Death receptor-3 present on metastatic colon carcinoma cells. This interaction confers metastatic properties to colon cancer cells by promoting the adhesion of cancer cells to endothelial cells and triggering the activation of the pro-migratory p38 and pro-survival ERK pathways in the cancer cells. In the present study, we investigated further the mechanisms by which the E-selectin-activated pathways downstream of DR3 confer a survival advantage to colon cancer cells. METHODS: Cell survival has been ascertained by using the WST-1 assay and by evaluating the activation of the PI3 kinase/NFkappaB survival axis. Apoptosis has been assayed by determining DNA fragmentation by Hoechst staining and by measuring cleavage of caspases-8 and -3. DR3 isoforms have been identified by PCR. For more precise quantification, targeted PCR reactions were carried out, and the amplified products were analyzed by automated chip-based microcapillary electrophoresis on an Agilent 2100 Bioanalyzer instrument. RESULTS: Interaction between DR3-expressing HT29 colon carcinoma cells and E-selectin induces the activation of the PI3K/Akt pathway. Moreover, p65/RelA, the anti-apoptotic subunit of NFkappaB, is rapidly translocated to the nucleus in response to E-selectin. This translocation is impaired by the PI3K inhibitor LY294002. Furthermore, inhibition of the PI3K/Akt pathway increases the cleavage of caspase 8 in colon cancer cells treated with E-selectin and this effect is still further increased when both ERK and PI3K pathways are concomitantly inhibited. Intriguingly, metastatic colon cancer cell lines such as HT29 and SW620 express higher levels of a splice variant of DR3 that has no trans-membrane domain and no death domain. CONCLUSION: Colon cancer cells acquire an increased capacity to survive via the activation of the PI3K/NFkappaB pathway following the stimulation of DR3 by E-selectin. Generation of a DR3 splice variant devoid of death domain can further contribute to protect against apoptosis.


Abstract: Gallotannin (GT), the polyphenolic hydrolyzable tannin, exhibits anti-inflammatory and anticancer activities through mechanisms that are not fully understood. Several effects modulated by GT have been shown to be linked to interference with inflammatory mediators. Considering the central role of nuclear factor kappa B (NF-kB) in inflammation and cancer, we investigated the effect of GT on NF-kB signaling in HT-29 and HCT-116 human colon cancer cells. DNA binding assays revealed significant suppression of tumor necrosis factor (TNF-alpha)-induced NFkB activation which correlated with the inhibition of IkBalpha phosphorylation and degradation. Sequentially, p65 nuclear translocation and DNA binding were inhibited. GT also down-regulated the expression of NFkB-regulated inflammatory cytokines (IL-8, TNF-alpha, IL-1alpha) and caused cell cycle arrest and accumulation of cells in pre-G1 phase. In vivo, GT (25 mg/kg body weight) injected intraperitoneally (i.p.) prior to or after tumor inoculation significantly decreased the volume of human colon cancer xenografts in NOD/SCID mice. GT-treated xenografts showed significantly lower microvessel density (CD31) as well as lower mRNA
expression levels of IL-6, TNF-alpha and IL-1alpha and of the proliferation (Ki-67) and angiogenesis (VEGFA) proteins, which may explain GTs in vivo anti-tumorigenic effects. Overall, our results indicate that the anti-inflammatory and antitumor activities of GT may be mediated in part through the suppression of NF-kB activation.


Abstract: Colon cancer is the 3rd common malignancy and 4th common cause of cancer death in Korea. Recent studies have shown that abnormal inflammatory response plays a critical role in colon carcinogenesis. A striking example of connection between inflammation and cancer is NF-kappaB, in which key regulator of inflammation and immune response is associated with target for colon cancer treatment. Constitutive NF-kappaB expression in colon cancer is 40-80% in vivo as well as in vitro, and the inactivation of IKKbeta subunit can reduce tumor multiplicity. The possible mechanisms by which NF-kappaB can contribute to colon carcinogenesis include the activator of antiapoptotic gene expression, enhanced cell survival and proliferation, regulation of angiogenesis and promotion of metastasis of cancer cells. Recent insights into the role of NF-kappaB involved in colon cancer development as well as their relevance as therapeutic targets are herein discussed.


Abstract: PURPOSE: Rectal cancer is often clinically resistant to radiotherapy (RT) and identifying molecular markers to define the biologic basis for this phenomenon would be valuable. The nuclear factor kappa-light chain-enhancer of activated B cells (NF-kappaB) is a potential anti-apoptotic transcription factor that has been associated with resistance to RT in model systems. The present study was designed to evaluate NF-kappaB activation in patients with rectal cancer undergoing chemoradiotherapy to determine whether NF-kappaB activity correlates with the outcome in rectal cancer patients. METHODS AND MATERIALS: A total of 22 patients underwent biopsy at multiple points in a prospective study and the data from another 50 were analyzed retrospectively. The pretreatment tumor tissue was analyzed for multiple NF-kappaB subunits by immunohistochemistry. Serial tumor biopsy cores were analyzed for NF-kappaB-regulated gene expression using reverse transcriptase polymerase chain reaction and for NF-kappaB subunit nuclear localization using immunohistochemistry. RESULTS: Several NF-kappaB target genes (Bcl-2, cellular inhibitor of apoptosis protein [cIAP]2, interleukin-8, and tumor necrosis factor receptor-associated-1) were significantly upregulated by a single fraction of RT at 24 h, demonstrating for the first time that NF-kappaB is activated by RT in human rectal tumors. The baseline NF-kappaB p50 nuclear expression did not correlate with the pathologic response to RT. However, an increasing baseline p50 level was prognostic for overall survival (hazard ratio, 2.15; p = .040). CONCLUSION: NF-kappaB nuclear expression at baseline in rectal cancer was prognostic for overall survival but not predictive of the response to RT. Larger patient numbers are needed to assess the effect of NF-kappaB target gene upregulation on the response to RT. Our results suggest that NF-kappaB might play an important role in tumor metastasis but not to the resistance to chemoradiotherapy.


Abstract: Peroxisome proliferator-activated receptor (PPAR)-gamma agonists such as troglitazone, pioglitazone and thiazolidine have been shown to induce apoptosis in human colon cancer cells. The molecular mechanism of PPARgamma agonist-induced apoptosis of colon cancer cells, however, is not
clear. Glycogen synthase kinase-3beta (GSK-3beta) is an indispensable element for the activation of nuclear factor-kappa B (NF-kappaB) which plays a critical role in the mediation of survival signals in cancer cells. To investigate the mechanisms of PPARgamma agonist-induced apoptosis of colon cancer cells, we examined the effect of troglitazone (0-16μM) on the activation of GSK-3beta and NF-kappaB. Our study showed that the inhibitory effect of troglitazone on colon cancer cell growth was associated with inhibition of NF-kappaB activity and GSK-3beta expression in a dose-dependent manner. Cells were arrested in G(0)/G(1) phase followed by the induction of apoptosis after treatment of troglitazone with concomitant decrease in the expression of the G(0)/G(1) phase regulatory proteins; Cdk2, Cdk4, cyclin B1, D1, and E as well as in the anti-apoptosis protein Bcl-2 along with an increase in the expression of the pro-apoptosis-associated proteins; Caspase-3, Caspase-9 and Bax. Transient transfection of GSK-3beta recovered troglitazone-induced cell growth inhibition and NF-kappaB inactivation. In contrast, co-treatment of troglitazone with a GSK-3beta inhibitor (AR-a014418) or siRNA against GSK-3beta, significantly augmented the inhibitory effect of troglitazone on the NF-kappaB activity, the cancer cell growth and on the expression of G(0)/G(1) phase regulatory proteins and pro-apoptosis regulatory proteins. These results suggest that the PPARgamma agonist, troglitazone, inhibits colon cancer cell growth via inactivation of NF-kappaB by suppressing GSK-3beta activity.


Abstract: Hyperactivation of beta-catenin-T-cell-factor (TCF)-regulated gene transcription is a hallmark of colorectal cancer (CRC). The cell-neural adhesion molecule L1CAM (hereafter referred to as L1) is a target of beta-catenin-TCF, exclusively expressed at the CRC invasive front in humans. L1 overexpression in CRC cells increases cell growth and motility, and promotes liver metastasis. Genes induced by L1 are also expressed in human CRC tissue but the mechanisms by which L1 confers metastasis are still unknown. We found that signaling by the nuclear factor kappaB (NF-kappaB) is essential, because inhibition of signaling by the inhibitor of kappaB super repressor (IkappaB-SR) blocked L1-mediated metastasis. Overexpression of the NF-kappaB p65 subunit was sufficient to increase CRC cell proliferation, motility and metastasis. Binding of the L1 cytodomain to ezrin - a cytoskeleton-crosslinking protein - is necessary for metastasis because when binding to L1 was interrupted or ezrin gene expression was suppressed with specific shRNA, metastasis did not occur. L1 and ezrin bound to and mediated the phosphorylation of IkappaB. We also observed a complex containing IkappaB, L1 and ezrin in the juxtamembrane region of CRC cells. Furthermore, we found that L1, ezrin and phosphorylated p65 are co-expressed at the invasive front in human CRC tissue, indicating that L1-mediated activation of NF-kappaB signaling involving ezrin is a major route of CRC progression.

Puvvada SD, Funkhouser WK, Greene K et al. NF-kB and Bcl-3 activation are prognostic in metastatic colorectal cancer. *Oncology* 2010;78(3-4):181-8.

Abstract: PURPOSE: NF-kappaB is an antiapoptotic transcription factor that has been shown to be a mediator of treatment resistance. Bcl-3 is a regulator of NF-kappaB that may play a role in oncogenesis. The goal of this study was to correlate the activation status of NF-kappaB and Bcl-3 with clinical outcome in a group of patients with metastatic colorectal cancer (CRC). METHODS: A retrospective study of 23 patients who underwent surgical resection of CRC at the University of North Carolina (UNC). Activation of NF-kappaB was defined by nuclear expression of select components of NF-kappaB (p50, p52, p65) and Bcl-3. Tissue microarrays were created from cores of normal mucosa, primary tumor, lymph node metastases and liver metastases in triplicate from disparate areas of the blocks, and an intensity score was generated by multiplying intensity (0-3+) by percent of positive tumor cells. Generalized estimating equations were used to note differences in intensity scores among normal mucosa and nonnormal tissues. Cox regression models were fit to see if scores were significantly associated with...
overall survival. RESULTS: p65 NE was significantly higher in primary tumor and liver metastases than normal mucosa (both p < 0.01). p50 nuclear expression was significantly higher for all tumor sites than for normal mucosa (primary tumor and lymph node metastases p < 0.0001, liver metastases p < 0.01). Bcl-3 nuclear expression did not differ significantly between normal mucosa and tumor; however, nuclear expression in primary tumor for each of these components was strongly associated with survival: the increase in hazard for each 50-point increase in nuclear expression was 91% for Bcl-3, 66% for p65, and 52% for p50 (all p < 0.05). CONCLUSIONS: Activation of canonical NF-kappaB subunits p50 and p65 as measured by nuclear expression is strongly associated with survival suggesting NF-kappaB as a prognostic factor in this disease. Primary tumor nuclear expression appears to be as good as, or better than, metastatic sites at predicting prognosis. Bcl-3 nuclear expression is also negatively associated with survival and deserves further study in CRC


Abstract: IMPORTANCE OF THE FIELD: Colorectal cancer (CRC) is the second leading cause of cancer death. Progress has been made in the development of chemotherapy for advanced CRC. Targeted therapies against VEGF or EGFR are now commonly used. Many cases show that tolerance develops to such treatments and thus new strategies are required to replace or complement current therapies. The NF-kappaB signaling pathway plays critical roles in physiological and pathological processes, and the relationship between colon cancer development and NF-kappaB is becoming clear. AREAS COVERED IN THIS REVIEW: We discuss evidence for the participation of activated NF-kappaB in carcinogenesis and consider the possibility of NF-kappaB being a target for CRC treatment. What the reader will gain: NF-kappaB activation might be involved in development of not only colitis-associated cancer, but also sporadic CRC. NF-kappaB activation is associated with hallmarks of cancer. Constitutive NF-kappaB activation is frequently observed in CRC and is associated with angiogenesis and resistance to chemotherapy. Several NF-kappaB inhibitors have proven to be useful. TAKE HOME MESSAGE: Induction of NF-kappaB activation leads to resistance to chemotherapy and constitutively activated NF-kappaB can often be seen in CRC. Anti-NF-kappaB therapy may rescue many cases of CRC and should be examined further for use as a therapy target


Abstract: PURPOSE: Radiation therapy is an integral part of the preoperative treatment of rectal cancers. However, only a minority of patients achieve a complete pathologic response to therapy because of resistance of these tumors to radiation therapy. This resistance may be mediated by constitutively active pro-survival signaling pathways or by inducible/acquired mechanisms in response to radiation therapy. Simultaneous inhibition of these pathways can sensitize these tumors to radiation therapy. METHODS AND MATERIALS: Human colorectal cancer cells were exposed to clinically relevant doses of gamma rays, and the mechanism of their radioresistance was investigated. We characterized the transcription factor nuclear factor-kappaB (NF-kappaB) activation as a mechanism of inducible radioresistance in colorectal cancer and used curcumin, the active ingredient in the yellow spice turmeric, to overcome this resistance. RESULTS: Curcumin inhibited the proliferation and the post-irradiation clonogenic survival of multiple colorectal cancer cell lines. Radiation stimulated NF-kappaB activity in a dose- and time-dependent manner, whereas curcumin suppressed this radiation-induced NF-kappaB activation via inhibition of radiation-induced phosphorylation and degradation of inhibitor of kappaB alpha, inhibition of inhibitor of kappaB kinase activity, and inhibition of Akt phosphorylation. Curcumin also suppressed NF-kappaB-regulated gene products (Bcl-2, Bcl-x(L), inhibitor of apoptosis protein-2, cyclooxygenase-2, and cyclin D1). CONCLUSIONS: Our results suggest that transient inducible NF-kappaB activation
provides a prosurvival response to radiation that may account for development of radioresistance. Curcumin blocks this signaling pathway and potentiates the antitumor effects of radiation therapy.


Abstract: BACKGROUND: Colorectal cancer (CRC) displays intratumoral heterogeneity with less differentiated tumor cells at the invasive front (IF) than in the tumor center (TC). The authors previously observed that several genes were overexpressed at the IF of CRC with relations to inflammatory processes. Because nuclear factor kappaB (NF-kappaB), a dimeric transcription factor, is a major regulator of such processes, and because its target genes are involved in immune response, cell growth control, and cell survival, the expression of NF-kappaB target genes was investigated comparatively in CRC. METHODS: By using gene array profiling, NF-kappaB target gene expression was assessed in CRCs that expressed human mutL homolog 1 (hMLH1), hMSH2, and nuclear beta-catenin by comparing expression at the IF, in the TC, and in normal mucosa. In addition, 5 NF-kappaB target genes with high differential expression were validated by using immunohistochemistry. RESULTS: The expression of NF-kappaB target genes in the TC, at the IF, and in normal mucosa was distinct; whereas, specifically at the IF, most differentially expressed NF-kappaB targets were up-regulated. Moreover, the results indicated that the expression diverged between epithelial tumor cells and inflammatory stromal cells. CONCLUSIONS: Because the results demonstrated that inflammation and the activation of NF-kappaB signaling promoted CRC invasiveness, the current study provided further evidence that downstream targets of NF-kappaB signaling may be specifically relevant in invasion and progression of CRC. Finally, as has been suggested for colitis-associated cancer, the authors of this report concluded that the inhibition of NF-kappaB signaling also may be an additional option for the treatment of sporadic CRC. Cancer 2009. (c) 2009 American Cancer Society


Abstract: Kaurane diterpene compounds have been known to be cytotoxic against several cancer cells through inhibition of nuclear factor-kappaB (NF-kappaB) activity. Here, we showed that inflexinol, a novel kaurane diterpene compound, inhibited the activity of NF-kappaB and its target gene expression as well as cancer cell growth through induction of apoptotic cell death in vitro and in vivo. These inhibitory effects on NF-kappaB activity and on cancer cell growth were suppressed by the reducing agents DTT and glutathione and were abrogated in the cells transfected with mutant p50 (C62S). Sol-gel biochip and surface plasmon resonance analysis showed that inflexinol binds to the p50 subunit of NF-kappaB. These results suggest that inflexinol inhibits colon cancer cell growth via induction of apoptotic cell death through inactivation of NF-kappaB by a direct modification of cysteine residue in the p50 subunit of NF-kappaB


Abstract: Ginsenoside Rg3, the main constituent isolated from Panax ginseng, has been of interest for use as a cancer preventive or therapeutic agent. We investigated here whether Rg3 can inhibit the activity of NF-kappaB, a key transcriptional factor constitutively activated in colon cancer that confers cancer cell resistance to chemotherapeutic agents. To investigate whether RG3 can suppress activation of NF-kappaB, and thus inhibit cancer cell growth, we examined the susceptibility of colon cancer cells (SW620 and HCT116) to treatment with Rg3 (25, 50, 75, 100 microM) and Rg3-induced activation of NF-kappaB. RG3 dose-dependently inhibited cancer cell growth through induction of apoptosis and decreased NF-kappaB activity. In a further study of compounds in colon cancer, we used half of the
IC(50) dose, values in combined treatments of Rg3 (50 microM) with conventional agents - docetaxel (5 nM), paclitaxel (10 nM) cisplatin (10 microM) and doxorubicin (2 microM). Compared to treatment with Rg3 or chemotherapy alone, combined treatment was more effective (i.e., there were synergistic effects) in the inhibition of cancer cell growth and induction of apoptosis and these effects were accompanied by significant inhibition of NF-kappaB activity. NF-kappaB target gene expression of apoptotic cell death proteins (Bax, caspase-3, caspase-9) was significantly enhanced, but the expression of anti-apoptotic genes and cell proliferation marker genes (Bcl-2, inhibitor of apoptosis protein (IAP-1) and X chromosome IAP (XIAP), Cox-2, c-Fos, c-Jun and cyclin D1) was significantly inhibited by the combined treatment compared to Rg3 or docetaxel alone. These results indicate that ginsenoside Rg3 inhibits NF-kappaB, and enhances the susceptibility of colon cancer cells to docetaxel and other chemotherapeutics. Thus, ginsenoside Rg3 could be useful as an anti-cancer or adjuvant anti-cancer agent.


Abstract: NF-kappaB interferes with the effect of most anti-cancer drugs through induction of anti-apoptotic genes. Targeting NF-kappaB is therefore expected to potentiate conventional treatments in adjuvant strategies. Here we used a pharmacological inhibitor of the IKK2 kinase (AS602868) to block NF-kappaB activation. In human colon cancer cells, inhibition of NF-kappaB using 10 microM AS602868 induced a 30-50% growth inhibitory effect and strongly enhanced the action of SN-38, the topoisomerase I inhibitor and CPT-11 active metabolite. AS602868 also potentiated the cytotoxic effect of two other antineoplastic drugs: 5-fluorouracil and etoposide. In xenografts experiments, inhibition of NF-kappaB potentiated the antitumoural effect of CPT-11 in a dose-dependent manner. Eighty-five and 75% decreases in tumour size were observed when mice were treated with, respectively, 20 or 5 mg kg(-1) AS602868 associated with 30 mg kg(-1) CPT-11 compared to 47% with CPT-11 alone. Ex vivo tumour analyses as well as in vitro studies showed that AS602868 impaired CPT-11-induced NF-kappaB activation, and enhanced tumour cell cycle arrest and apoptosis. AS602868 also enhanced the apoptotic potential of TNFalpha on HT-29 cells. This study is the first demonstration that a pharmacological inhibitor of the IKK2 kinase can potentiate the therapeutic efficiency of antineoplastic drugs on solid tumours.


Abstract: Many natural compounds have been shown to prevent cancer cell growth through the redox regulation of transcription factors. NF-kappaB, a redox transcription factor, has been implicated in the apoptotic cell death of several cancer cells. This study examined whether or nor 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyranone (DDMP) isolated from onions can modulate the activity of NF-kappaB, thereby induce the apoptotic cell death of colon cancer cells. Treatment with different DDMP concentrations (0.5-1.5 mg/mL) for various periods (0-48 h) inhibited the growth of colon cancer cells (SW620 and HCT116) followed by the induction of apoptosis in a dose dependent manner. It was also found that DDMP modulated tumor necrosis factor-alpha (TNF-alpha) and tetradeanoyl phorbol acetate (TPA)-induced NF-kappaB transcriptional and DNA binding activity. Moreover, DDMP suppressed the NF-kappaB target anti-apoptotic genes (Bcl-2), whereas it induced the expression of the apoptotic genes (Bax, cleaved caspase-3 and cleaved PARP). These results suggest that DDMP from onions inhibit colon cancer cell growth by inducing apoptotic cell death through the inhibition of NF-kappaB.

Abstract: The efficiency of radiotherapy for rectal cancer treatment is limited because of radioresistance. Transcription factor nuclear factor-kappaB (NF-kappaB), which has antiapoptotic properties, may play an important role in this process. Recent studies indicate that behavior of the tumor is done not only by oncogenic events in tumor cells but also by microenvironment surrounding the tumor. Therefore we tested anti-inflammatory cytokines IL-4 and IL-10 occurring in tumor stroma whether they can modulate response of colorectal cancer cells to irradiation and whether this potential effect is associated with NF-kappaB. SW620 colorectal cancer cells were used for all experiments. Cell growth and clonogenicity were determined by cell proliferation assay and clonogenic assay, respectively. Activation of NF-kappaB was assessed by ELISA-based transcription factor assay and luciferase reporter assay. Apoptosis was determined by measuring caspase 3 activity. Irradiation (2-8 Gy) inhibited growth and clonogenicity of SW620 cells, induced apoptosis, and activated NF-kappaB, predominantly its subunits p50, p65, and RelB. IL-4 or IL-10 (1, 10, 100 ng/ml) neither inhibited growth and clonogenicity nor activated NF-kappaB, but they sensitized cells to irradiation in a dose dependent manner. Radiosensitization by IL-4 or IL-10 was associated with inhibition of NF-kappaB, predominantly its subunits p50 and p65 and increased apoptosis. In conclusion, modulation of the intestinal microenvironment, high local concentration of anti-inflammatory cytokines such as IL-4 and IL-10 may help to overcome resistance of colorectal tumors to radiotherapy. In this process NF-kappaB may be employed.


Abstract: PURPOSE: NF-kB expression has been shown to be responsible for resistance to antineoplastic agents and it also plays a part in the activation of the epidermal growth factor receptor downstream signaling pathway in colorectal tumors. The aim of our analysis was to investigate a correlation between NF-kB expression, response rate, time to progression, and survival in advanced colorectal cancer patients receiving cetuximab and irinotecan. PATIENTS AND METHODS: We analyzed retrospectively the immunoreactivity for NF-kB in irinotecan-refractory patients receiving cetuximab and irinotecan. Results Seventy-six patients were analyzed. Cetuximab and irinotecan were administered as second-line chemotherapy in 19 patients and after > or = two lines of chemotherapy in the remaining 57 patients. We observed a partial response (PR) in 16 patients for an overall response rate of 24%. Thirty-two patients (48%) experienced progressive disease; median time to progression (TTP) was 3.6 months and median overall survival was 10.3 months. NF-kB was positive in 46 patients (60%). All main clinical characteristics were well balanced between NF-kB-positive and NF-kB-negative patients. The response rate was 10% (four PRs) versus 48% (12 PRs; P = .0007) in NF-kB-positive and NF-kB-negative tumors, respectively. Median TTP in NF-kB-positive patients was 3 v 6.4 months in the remaining patients (P = .021). Median overall survival was 9.5 v 15.8 months for NF-kB-positive and NF-kB-negative patients, respectively (P = .036) CONCLUSION: The difference in median TTP, overall survival, and response rate seem to confirm that NF-kB may play a crucial role in predicting the efficacy of cetuximab and irinotecan in advanced colorectal tumors.


Abstract: Compounds such as S-allylmercaptocysteine, diallyl disulfide, and S-trityl-L-cysteine isolated from garlic have been known to be effective in chemoprevention. Nuclear transcription factor-kappaB (NF-kappaB) has been known to be an implicated factor in apoptotic cell death of several cancer cells. In this study, we investigated whether a sulfurcompound (named thiacremonone) isolated from garlic could modulate NF-kappaB activity and thereby induce apoptotic cell death of colon cancer cells. Treatment
with different concentrations (30 - 150 microg/ml) of thiacremonone for various periods (0 - 48 h) inhibited colon cancer cell (SW620 and HCT116) growth followed by induction of apoptosis in a dose-dependent manner. We also found that thiacremonone modulated tumor necrosis factor-alpha (TNF-alpha) and tetradecanoyl phorbol acetate (TPA)-induced NF-kappaB transcriptional and DNA binding activity. Moreover, thiacremonone suppressed NF-kappaB target anti-apoptotic genes (Bel-2, cIAP1/2, and XIAP) and inflammatory genes (iNOS and COX-2), whereas it induced apoptotic genes (Bax, cleaved caspase-3, and cleaved PARP) expression. These results suggest that a novel sulfurocompound from garlic inhibited colon cancer cell growth through induction of apoptotic cell death by modulating of NF-kappaB


Abstract: Constitutive NF-kappaB activity has been found in many cancer cells of different origin. In our study we focused on constitutive NF-kappaB activity and its impact on radiation-induced NF-kappaB activity, intrinsic radiosensitivity, and apoptosis. Using colorectal cancer cell lines (Caco-2, SW480, SW620) we demonstrated that each cell line expresses different level of constitutive NF-kappaB activity. Moreover, irradiation caused secondary NF-kappaB activation which differed in each cell line. The cell lines tested displayed also different intrinsic radiosensitivities as was determined by clonogenic assay, and different spontaneous and radiation-induced apoptosis determined by activity of caspase 3. Complex analysis of our results revealed that there was a strong correlation between constitutive NF-kappaB activity and radiation-induced NF-kappaB activity (r=0.835), the level of constitutive NF-kappaB activity predicted the level of secondary, radiation-induced NF-kappaB activity. Furthermore, SW620 cells with the highest level of constitutive NF-kappaB activity displayed the lowest radiosensitivity and the lowest level of spontaneous apoptosis; Caco-2 cells with almost undetectable level of constitutive NF-kappaB activity displayed the highest radiosensitivity and even highest level of spontaneous apoptosis. SW480 cells showed intermediate level of constitutive NF-kappaB activity, intermediate radiosensitivity and intermediate level of spontaneous apoptosis. Our data suggest that the level of constitutive NF-kappaB activity may predict radiosensitivity of colorectal cancer cells. Such prediction may allow the individualization of patient treatment by radiotherapy


Abstract: BACKGROUND: 5-fluorouracil (5-FU), the most common antimetabolite used for the treatment of colorectal cancer, exerts its cytotoxic effects through the induction of apoptosis. Folinic acid potentiates the effect of 5-FU. Drug activity is currently limited as a result of inducible chemoresistance. Limited research suggests that the transcription factor nuclear factor kappa-B (NF-kappaB), which has antiapoptotic properties, may play a major role in inducible chemoresistance. MATERIALS AND METHODS: SW48 colon cancer cells were used for all experiments. Cell growth was determined by cell proliferation assay. Apoptosis was assessed by measuring caspase 3 activity. Activation of NF-kappaB was ascertained by electrophoretic mobility shift assay, luciferase reporter assay, and Western blot analysis. RESULTS: Treatment with 5-FU (0.001-10 mm), not only inhibited growth and induced apoptosis but significantly activated NF-kappaB in SW48 cells. Folinic acid alone (0.01-100 mg/L) did not inhibit growth but improved the cytotoxic effect of 5-FU in a dose-dependent manner. Likewise, folic acid alone did not activate NF-kappaB or induce apoptosis but enhanced 5-FU-mediated NF-kappaB activation and cell apoptosis. Transfection with adenovirus IkappaBalpha super-repressor strongly inhibited constitutive activation of NF-kappaB and significantly enhanced 5-FU and 5-FU/Folinic acid-mediated growth inhibition (P < 0.05). CONCLUSIONS: Treatment with 5-FU activates NF-kappaB. Folinic acid enhances 5-FU-mediated activation of NF-kappaB. Inhibition of NF-kappaB enhances the cytotoxic effect of 5-FU with or without Folinic acid in colon cancer cells

Abstract: The ubiquitous NF-kappaB transcription factor has been reported to inhibit apoptosis and to induce drug resistance in cancer cells. Drug resistance is the major reason for cancer therapy failure and neoplastic cells often develop multiple mechanisms of drug resistance during tumor progression. We observed that NF-kappaB or P-glycoprotein inhibition in the HCT15 colon cancer cells led to increased apoptotic cell death in response to daunomycin treatment. Interestingly, NF-kappaB inhibition through transfection of a plasmid coding for a mutated IkappaB-alpha inhibitor increased daunomycin cell uptake. Indeed, the inhibition of NF-kappaB reduced mdr1 mRNA and P-glycoprotein expression in HCT15 cells. We identified a consensus NF-kappaB binding site in the first intron of the human mdr1 gene and demonstrated that NF-kappaB complexes could bind with this intronic site. Moreover, NF-kappaB transactivates an mdr1 promoter luciferase construct. Our data thus demonstrate a role for NF-kappaB in the regulation of the mdr1 gene expression in cancer cells and in drug resistance.


Abstract: The transcription factor nuclear factor kappaB (NFkappaB) is constitutively active in many types of cancercells and regulates the expression of several antiapoptotic genes. Previous studies demonstrated a role for the inhibition of NFkappaB in cancer therapy using a transgenic approach in mice. We found that NFkappaB was transiently activated much greater than background constitutive levels during colon cancer cell readhesion, which rendered the readhering colon cancer cells exquisitely susceptible to apoptosis in the presence of soluble NFkappaB inhibitors. These compounds greatly reduced colon cancer cell implantation in an in vivo seeding model of metastasis. The ability of soluble NFkappaB inhibitors to significantly induce apoptosis of readherent colon cancer cells makes them prospective candidates for preventing colon cancer metastasis.

McCarty MF, Block KI. Preadministration of high-dose salicylates, suppressors of NF-kappaB activation, may increase the chemosensitivity of many cancers: an example of proapoptotic signal modulation therapy. *Integr Cancer Ther* 2006 September;5(3):252-68.

Abstract: NF-kappaB activity is elevated in a high proportion of cancers, particularly advanced cancers that have been treated previously. Cytotoxic treatment selects for such up-regulation inasmuch as NF-kappaB promotes transcription of a large number of proteins that inhibit both the intrinsic and extrinsic pathways of apoptosis; NF-kappaB also boosts expression of mdr1, which expels many drugs from cells. Indeed, high NF-kappaB activity appears to be largely responsible for the chemo- and radioresistance of many cancers. Thus, agents that suppress NF-kappaB activity should be useful as adjuvants to cytotoxic cancer therapy. Of the compounds that are known to be NF-kappaB antagonists, the most practical for current use may be the nonsteroidal anti-inflammatory drugs aspirin, salicylic acid, and sulindac, each of which binds to and inhibits Ikappa kinase- beta, a central mediator of NF-kappa activation; the low millimolar plasma concentrations of salicylate required for effective inhibition of this kinase in vivo can be achieved with high-dose regimens traditionally used to manage rheumatic disorders. The gastrointestinal toxicity of such regimens could be minimized by using salsalate or enteric-coated sodium salicylate or by administering misoprostol in conjunction with aspirin therapy. Presumably, best results would be seen if these agents were administered for several days prior to a course of chemo- or radiotherapy, continuing throughout the course. This concept should first be tested in nude mice bearing xenografts of chemoresistant human tumors known to have elevated NF-kappa activity. Ultimately, more complex adjuvant regimens can be envisioned in which salicylates are used in conjunction with other NF-kappa antagonists and/or agents that target other mediators of down-regulated apoptosis in cancer, such as Stat3; coadministration of salicylate and organic selenium may have intriguing potential in this regard.
These strategies may also have potential as adjuvants to metronomic chemotherapy, which seeks to suppress angio-genesis by targeting cycling endothelial cells in tumors


Abstract: NF-κB comprises a family of cellular transcription factors that are involved in the inducible expression of a variety of cellular genes that regulate the inflammatory response. NF-κB is sequestered in the cytoplasm by inhibitory proteins, I(κ)B, which are phosphorylated by a cellular kinase complex known as IKK. IKK is made up of two kinases, IKK-alpha and IKK-beta, which phosphorylate I(κ)B, leading to its degradation and translocation of NF-κB to the nucleus. IKK kinase activity is stimulated when cells are exposed to the cytokine TNF-alpha or by overexpression of the cellular kinases MEKK1 and NIK. Here we demonstrate that the anti-inflammatory agents aspirin and sodium salicylate specifically inhibit IKK-beta activity in vitro and in vivo. The mechanism of aspirin and sodium salicylate inhibition is due to binding of these agents to IKK-beta to reduce ATP binding. Our results indicate that the anti-inflammatory properties of aspirin and salicylate are mediated in part by their specific inhibition of IKK-beta, thereby preventing activation by NF-κB of genes involved in the pathogenesis of the inflammatory response

**Silibinin**


Abstract: Silibinin, a flavonolignan, is the major active component of the milk thistle plant (*Silybum marianum*) and has been shown to possess anti-neoplastic properties. TNF-related apoptosis-inducing ligand (TRAIL) is a promising anti-cancer agent which selectively induces apoptosis in cancer cells. However, resistance to TRAIL-induced apoptosis is an important and frequent problem in cancer treatment. In this study, we investigated the effect of silibinin and TRAIL in an in vitro model of human colon cancer progression, consisting of primary colon tumor cells (SW480) and their derived TRAIL-resistant metastatic cells (SW620). We showed by flow cytometry that silibinin and TRAIL synergistically induced cell death in the two cell lines. Up-regulation of death receptor 4 (DR4) and DR5 by silibinin was shown by RT-PCR and by flow cytometry. Human recombinant DR5/Fe chimera protein that has a dominant-negative effect by competing with the endogenous receptors abrogated cell death induced by silibinin and TRAIL, demonstrating the activation of the death receptor pathway. Synergistic activation of caspase-3, -8, and -9 by silibinin and TRAIL was shown by colorimetric assays. When caspase inhibitors were used, cell death was blocked. Furthermore, silibinin and TRAIL potentiated activation of the mitochondrial apoptotic pathway and down-regulated the anti-apoptotic proteins Mcl-1 and XIAP. The involvement of XIAP in sensitization of the two cell lines to TRAIL was demonstrated using the XIAP inhibitor embelin. These findings demonstrate the synergistic action of silibinin and TRAIL, suggesting chemopreventive and therapeutic potential which should be further explored


Abstract: INTRODUCTION: Perioperative anticancer therapy that does not impair wound healing is needed to counter the persistent proangiogenic plasma compositional changes that occur after colorectal resection. Polyphenon E (PolyE), a green tea derivative (main component EGCG), and Siliphos (main component silibinin), from the milk thistle plant, both have antitumor effects. This study assessed the impact of PolyE/Siliphos (PES) on wound healing and the growth of CT-26 colon cancer in several
murine models. METHODS: One wound healing and three tumor studies were performed. Tumor Study (TS)1 assessed the impact of PES on subcutaneous tumor growth, whereas TS2 assessed PES's impact on subcutaneous growth when given pre- and post-CO(2) pneumoperitoneum (pneumo), sham laparotomy, or anesthesia alone. TS3 determined the ability of PES to limit hepatic metastases (mets) after portal venous injection of tumor cells. In the final study, laparotomy and gastrotomy wound healing were assessed several ways. BALB/c mice were used for all studies. The drugs were given via drinking water (PolyE) and gavage (Siliphos), daily, for 7-9 days preprocedure and for 7-21 days postoperatively. Tumor mass, number/size of hepatic mets, and proliferation and apoptosis rates were assessed. The abdominal breaking strength and energy to failure were measured postmortem as was gastric bursting pressures. RESULTS: PES significantly inhibited subcutaneous growth in the nonoperative setting. PES also significantly decreased the number/size of liver mets when given perioperatively. Abdominal wound breaking strength, energy to wound failure, and collagen content were not altered by PES; gastrotomy bursting strength also was not affected by PES. Neither drug alone had a significant impact on tumor growth. CONCLUSIONS: The PES combination inhibited subcutaneous and hepatic tumor growth yet did not impair wound healing. PES holds promise as a perioperative anticancer therapy.


Abstract: Silibinin, an effective chemo-preventive agent in various cancer types, suppresses cancer cell growth, but its effects on cancer stem-like cells (CSCs) remain unclear. This study aimed to examine whether silibinin inhibited the development of CSCs and disclose the underlying signaling. The colorectal cancer spheroid culture system was used for enriching CSCs. The effects of silibinin on CSCs were evaluated by counting sphere numbers, and calculating the percentage of CD133+ cells by flow cytometry and immunofluorescence both in the absence and presence of different concentrations of silibinin. The results showed the sphere number of CCS was 36 +/- 9.6 after 15 days of CSC enrichment in spheroid culture, and the percentage of CD133+ cells increased to 18 +/- 6.4% compared to 3 +/- 0.8% before enrichment. Treatment with silibinin reduced the sphere formation to 5 +/- 3.3 and decreased the CD133+ percentage to 8 +/- 2.3%. Interestingly, treatment of silibinin suppressed the activation of the AKT Ser473/mTOR pathway in spheroid culture through suppressing the activity of protein phosphatase 2Ac subunit (PP2Ac). In a xenograft tumor model, treatment with silibinin also inhibited tumor formation rate and tumor growth. Silibinin, which inhibits colon CSCs self-renewal and sphere formation by suppressing the PP2Ac/AKT Ser473/mTOR pathway, may be a compound for developing new strategies in modulating CSCs in cancer therapy.


Abstract: Chronic inflammation is one of the primary causes of colorectal cancer (CRC), and major inflammatory pathways implicated in CRC are cyclooxygenase-2 (COX-2) and iNOS; both regulated by nuclear factor-kappa B (NF-kappaB) suggesting that inhibitors of these pathways could be ideal against CRC. Silibinin has shown promising efficacy against various malignancies including CRC, and therefore here we assessed whether silibinin targets NF-kappaB activation and associated signaling as a mechanism of its anti-inflammatory and anti-cancer effects in CRC. Our results indicated that silibinin treatment (50-200 microM) of human CRC SW480, LoVo, and HT29 cells strongly inhibits tumor necrosis factor alpha-induced NF-kappaB activation together with decreased nuclear levels of both p65 and p50 sub-units. Silibinin also significantly increased IkappaBalpha level with a concomitant decrease in phospho-IkappaBalpha, without any effect on TNFR1, TRADD, and RIP2, indicating its inhibitory effect on IkappaB kinase alpha activity. Next we assessed the effect of oral silibinin feeding on NF-kappaB pathway in SW480 (COX-2 negative) and LoVo (COX-2 positive) tumor xenografts in nude mice.
Together with its inhibitory efficacy on tumor growth and progression, silibinin inhibited NF-kappaB activation in both xenografts. The protein levels of various NF-kappaB-regulated molecules such as Bel-2, COX-2, iNOS, VEGF, and MMPs were also decreased by silibinin in both cell culture studies and xenograft analyses, suggesting its potential to alter NF-kappaB transcriptional activity. Together, these findings are highly significant in establishing for the first time that silibinin suppresses CRC growth and progression possibly through its anti-inflammatory activity by interfering with NF-kappaB activation and thus has potential against human CRC. (c) 2011 Wiley Periodicals, Inc


Abstract: PURPOSE: Earlier, we reported the strong preventive efficacy of silibinin against colorectal cancer (CRC), but its usefulness against established CRC or effect of its withdrawal on CRC growth remained unknown. Present study focused on these important issues by employing two different treatment protocols in advanced human CRC SW480 xenograft in nude mice. METHODS: In the first treatment protocol, silibinin was fed for 28 days (200 mg/kg body weight, 5 days/week) to mice with growing SW480 xenograft; thereafter, tumor growth was monitored for additional 3 weeks without silibinin treatment. In the second protocol, silibinin treatment was started after 25 days of SW480 cells injection (established tumors), and tumor growth was studied 4 days, 8 days and 16 days after silibinin treatment. RESULTS: In both treatment protocols, silibinin had strong and sustained inhibitory effect on xenograft growth. Detailed xenograft analyses showed that silibinin, in both treatment protocols, exerts anti-proliferative, pro-apoptotic and anti-angiogenic effects. Further, silibinin reduced the expression of beta-catenin and phospho-GSK3beta in xenograft tissues. Silibinin also targeted signaling molecules involved in CRC proliferation and survival (cyclin D1, c-Myc and survivin) as well as angiogenesis regulators (VEGF and iNOS). CONCLUSIONS: Collectively, these findings substantiate silibinin's therapeutic efficacy against CRC, advocating its translational potential


Abstract: Mutations in APC/beta-catenin resulting in an aberrant activation of Wnt/beta-catenin pathway are common in colorectal cancer (CRC), suggesting that targeting the beta-catenin pathway with chemopreventive/anticancer agents could be a potential translational approach to control CRC. Using human CRC cell lines harboring mutant (SW480) versus wildtype (HCT116) APC gene and alteration in beta-catenin pathway, herein we performed both in vitro and in vivo studies to examine for the first time whether silibinin targets beta-catenin pathway in its efficacy against CRC. Silibinin treatment inhibited cell growth, induced cell death, and decreased nuclear and cytoplasmic levels of beta-catenin in SW480 but not in HCT116 cells, suggesting its selective effect on the beta-catenin pathway and associated biologic responses. Other studies, therefore, were performed only in SW480 cells where silibinin significantly decreased beta-catenin-dependent T-cell factor-4 (TCF-4) transcriptional activity and protein expression of beta-catenin target genes such as c-Myc and cyclin D1. Silibinin also decreased cyclin-dependent kinase 8 (CDK8), a CRC oncoprotein that positively regulates beta-catenin activity, and cyclin C expression. In a SW480 tumor xenograft study, 100- and 200-mg/kg doses of silibinin feeding for 6 weeks inhibited tumor growth by 26% to 46% (P < .001). Analyses of xenografts showed that similar to cell culture findings, silibinin decreases proliferation and expression of beta-catenin, cyclin D1, c-Myc, and CDK8 but induces apoptosis in vivo. Together, these findings suggest that silibinin inhibits the growth of SW480 tumors carrying the mutant APC gene by down-regulating CDK8 and beta-catenin signaling and, therefore, could be an effective agent against CRC

Abstract: Colorectal cancer is one of the leading causes of cancer-related morbidity and mortality. The use of nontoxic phytochemicals in the prevention and intervention of colorectal cancer has been suggested as an alternative to chemotherapy. Here we assessed the anticancer efficacy of silibinin against advanced colorectal cancer LoVo cells both in vitro and in vivo. Our results showed that silibinin treatment strongly inhibits the growth of LoVo cells (P < 0.05-0.001) and induces apoptotic death (P < 0.01-0.001), which was associated with increased levels of cleaved caspases (3 and 9) and cleaved poly(ADP-ribose) polymerase. Additionally, silibinin caused a strong cell cycle arrest at G(1) phase and a slight but significant G(2)-M-phase arrest at highest concentration (P < 0.01-0.001). Molecular analyses for cell cycle regulators showed that silibinin decreases the level of cyclins (D1, D3, A and B1) and cyclin-dependent kinases (1, 2, 4, and 6) and increases the level of cyclin-dependent kinase inhibitors (p21 and p27). Consistent with these results, silibinin treatment also decreased the phosphorylation of retinoblastoma protein at Ser(780), Ser(795), and Ser(807)/Ser(811) sites without significantly affecting its total level. In animal studies, oral administration of silibinin for 6 weeks (at 100 and 200 mg/kg/d for 5 days/wk) significantly inhibited the growth of LoVo xenograft (P < 0.001) in athymic nude mice without any apparent toxicity. Analyses of xenograft tissue showed that silibinin treatment inhibits proliferation and increases apoptosis along with a strong increase in p27 levels but a decrease in retinoblastoma phosphorylation. Together, these results suggest the potential use of silibinin against advanced human colorectal cancer.


Abstract: Herein, for the first time, we investigated in vivo efficacy and associated molecular biomarkers and mechanisms of a chemopreventive agent, silibinin, against human colorectal carcinoma (CRC) HT29 xenograft growth. Nude mice were implanted with HT29 cells and fed with vehicle (carboxymethyl cellulose or phosphatidylcholine) or 200 mg/kg/d dose of silibinin or 100 and 200 mg/kg/d doses of silybin-phytosome (5 days per week) for 32 days. Silibinin inhibited tumor growth that accounted for 48% (P = 0.002) decrease in tumor volume and 42% (P = 0.012) decrease in tumor weight at the end of the experiment without any adverse health effect. A stronger antitumor efficacy was observed with silybin-phytosome preparation. Silibinin decreased proliferation index by 40% (P < 0.001), increased apoptotic index by approximately 2-fold (P = 0.001), and reduced microvessel density by 36% (P = 0.001) in tumors. Antiproliferative and proapoptotic effects of silibinin were associated with down-regulation of extracellular signal-regulated kinase 1/2 (ERK1/2) and Akt phosphorylation as well as cyclin D1 expression. Antiangiogenic effect of silibinin was coupled with a strong decrease in inducible nitric oxide synthase (NOS) and NOS3, cyclooxygenase-1 (COX-1) and COX-2, and hypoxia-inducing factor-1 alpha (HIF-1 alpha) and vascular endothelial growth factor (VEGF). These findings suggest in vivo antitumor efficacy of silibinin against CRC involving its antiproliferative, proapoptotic, and antiangiogenic activities. The inhibition of ERK1/2 and Akt signaling may account for antiproliferative and proapoptotic effects, whereas down-regulation of NOS, COX, HIF-1 alpha, and VEGF expression could lead to antiangiogenic effect of silibinin against CRC. Overall, potential use of silibinin against human CRC could be suggested.


Abstract: Silibinin, a flavonolignan from milk thistle, has intestinal cancer chemopreventive efficacy in
rodents. It is a strong antioxidant and modulates the insulin-like growth factor (IGF) system by increasing circulating levels of IGF-binding protein 3 (IGFBP-3) and decreasing levels of IGF-I. Here, the hypothesis was tested that administration of oral silibinin generates agent levels in human blood and colorectal and hepatic tissues consistent with pharmacologic activity. Patients with confirmed colorectal adenocarcinoma received silibinin formulated with phosphatidylcholine (silipide) at dosages of 360, 720, or 1,440 mg silibinin daily for 7 days. Blood and biopsy samples of normal and malignant colorectum or liver were obtained before dosing, and blood and colorectal or hepatic tissues were collected at resection surgery after the final silipide dose. Levels of silibinin were quantified by high-pressure liquid chromatography-UV, and plasma metabolites were identified by liquid chromatography-mass spectrometry. Blood levels of IGFBP-3, IGF-I, and the oxidative DNA damage pyrimidopurinone adduct of deoxyguanosine (M1dG) were determined. Repeated administration of silipide was safe and achieved levels of silibinin of 0.3 to 4 micromol/L in the plasma, 0.3 to 2.5 nmol/g tissue in the liver, and 20 to 141 nmol/g tissue in colorectal tissue. Silibinin monoglucuronide, silibinin diglucuronide, silibinin monosulfate, and silibinin glucuronide sulfate were identified in the plasma. Intervention with silipide did not affect circulating levels of IGFBP-3, IGF-I, or M1dG. The high silibinin levels achieved in the human colorectal mucosa after consumption of safe silibinin doses support its further exploration as a potential human colorectal cancer chemopreventive agent.

**Diindolylmethane**


Abstract: 3,3'-Diindolylmethane (DIM) is a major in vivo condensation product of indole-3-carbinol, which is present in cruciferous vegetables. Although these compounds have been widely implicated in antitumorigenic and proapoptotic properties in animal as well as in vitro models of cancer, the underlying cellular mechanisms regulated by DIM are only partially understood. Activating transcription factor 3 (ATF3) is a member of the ATF/c-AMP response element-binding (CREB) subfamily of the basic-region leucine zipper family and has been known to induce apoptosis in human colorectal cancer (CRC) cells. The present study was performed to elucidate the molecular mechanism of ATF3 induction by DIM in human CRC cells. The DIM treatment induced apoptosis and induced ATF3 gene expression at protein and messenger RNA levels. DIM increased ATF3 promoter activity, and the region of -84 to +34 within ATF3 promoter was responsible for promoter activation by DIM. This region contained an ATF binding site. Deletion and point mutation of the ATF binding site (-23 to -16) abolished ATF3 promoter activation by DIM, and overexpression of ATF4 enhanced ATF3 transactivation. Chromatin immunoprecipitation assay confirmed the binding of ATF4 in the ATF3 promoter. Inhibition of ATF4 expression by small interference RNA results in repression of DIM-induced ATF3 expression. The current study demonstrates that DIM stimulates ATF3 expression through ATF4-mediated pathway and subsequently induces apoptosis in human CRC cells.


Abstract: N-myc downstream regulated gene-1 participates in carcinogenesis, angiogenesis, metastases, and anticancer drug resistance. In the present study, we analyzed the expression pattern of N-myc downstream regulated gene-1 following treatment of human colonic cancer cell lines; HCT-116 (well differentiated with wild-type p53 gene) and Colo-320 (poorly differentiated with mutant p53 gene), with
3,3'-diindolylmethane, a well-established proapoptotic agent product derived from indole-3-carbinol. Treatment of Colo-320 and HCT-116 with 3,3'-diindolylmethane disclosed inhibition of cell viability in a dose-dependent manner, mediated through apoptosis induction. The increased expression of N-myc downstream regulated gene-1 was detected only in poorly differentiated colon cancer cells, Colo-320 cell line. Our results suggest that N-myc downstream regulated gene-1 expression is enhanced by 3,3'-diindolylmethane in poorly differentiated cells and followed by induction of apoptosis. 3,3'-diindolylmethane induced apoptosis may represent a new regulator of N-myc downstream regulated gene-1 in poorly differentiated colonic cancer cells.


Abstract: Isothiocyanates (ITCs) and indoles derived from cruciferous vegetables possess growth-inhibiting and apoptosis-inducing activities in cancer cell lines in vitro. ITCs like sulforaphane (SFN) are cytotoxic, whereas indoles including indole-3-carbinol or its condensation product 3,3'-diindolylmethane (DIM) are acting by cytostatic mechanisms in human colon cancer cell lines. In the present study, we have investigated the impact of defined combinations of SFN and DIM (ratio 1:4, 1:2, 1:1, 2:1 and 4:1) on cell proliferation, cell-cycle progression and apoptosis induction in cultured 40-16 colon carcinoma cells. Calculations of combination effects were based on the method of Chou et al. (1984) *Adv. Enzyme Regul.*, 22, 27-55, and were expressed as a combination index (CI) with CI < 1, CI = 1 or CI > 1 representing synergism, additivity or antagonism, respectively. Interestingly, at a total drug concentration of 2.5 microM, all combinations of SFN and DIM were antagonistic. With increasing concentrations, the antagonistic effect gradually turned into a synergistic interaction at the highest combined cytotoxic concentration of 40 microM. Cell-cycle analyses with SFN:DIM ratios of 1:1, 1:2 and 1:4 and total concentrations between 10 and 25 microM confirmed antagonism at low and additive effects at higher doses. SFN (10 microM) in combination with DIM (10 microM) resulted in strong G(2)/M cell-cycle arrest, which was not observed with either compound alone. Our results indicate that cytotoxic concentrations of SFN:DIM combinations affect cell proliferation synergistically. At low total concentrations (below 20 microM), which are physiologically more relevant, the combined broccoli compounds showed antagonistic interactions in terms of cell growth inhibition. These data stress the need for elucidating mechanistic interactions for better predicting beneficial health effects of bioactive food components.


Abstract: 3,3'-Diindolylmethane (DIM) is the major in vivo product of acid-catalyzed oligomerization of indole-3-carbino1, which is a promising anticancer agent present in cruciferous vegetables and has itself been reported to have anticarcinogenic properties. This study examined DIM-mediated regulation of apoptosis in the HCT116 (wild-type p53) and HT-29 (mutant p53) human colon cancer cell lines. DIM (0-30 micromol/L) substantially decreased the number of viable cells and induced apoptosis of HCT116 and HT-29 cells in a concentration-dependent manner. Western-blot analyses of total cell lysates revealed that DIM increased the activation of caspase-3, -7, -8, and -9 and enhanced poly(ADP-ribose) polymerase cleavage in both HCT116 and HT-29 cells. In addition, DIM increased the translocation of cytochrome c and Smac/Diablo from the mitochondria to the cytoplasm. In concert with the caspase-8 activation by DIM, increased levels of Fas and truncated Bid were observed. DIM did not affect the protein levels of p53, Bcl-2, Bax, or Fas ligand (FasL) in HCT116 cells. In HT-29 cells, however, DIM decreased Bcl-2 levels, although the protein levels of Bax or FasL were not affected. The caspase-8 inhibitor Z-IETD-FMK attenuated the DIM-induced apoptosis, indicating that increased activation of this enzyme contributed to the increase in p53-independent apoptosis that was observed in colon cancer cells. We have
demonstrated that DIM induces apoptosis in colon cancer cells, providing insights into the mechanisms underlying its antitumorigenic activities.


Abstract: 3,3'-Diindolylmethane (DIM) is an anticancer agent that induces cell cycle arrest and apoptosis through unknown mechanisms. Here, we report that DIM can selectively induce proteasome-mediated degradation of class I histone deacetylases (HDAC1, HDAC2, HDAC3, and HDAC8) without affecting the class II HDAC proteins. DIM induced downregulation of class I HDACs in human colon cancer cells in vitro and in vivo in tumor xenografts. HDAC depletion relieved HDAC-mediated transcriptional inhibition of the cyclin-dependent kinase inhibitors p21WAF1 and p27KIP2, significantly increasing their expression and triggering cell cycle arrest in the G(2) phase of the cell cycle. Additionally, HDAC depletion was associated with an induction of DNA damage that triggered apoptosis. Our findings indicate that DIM acts to selectively target the degradation of class I HDACs.


Abstract: BACKGROUND: 3,3'-Diindolylmethane (DIM), an indole derivative produced in the stomach after the consumption of broccoli and other cruciferous vegetables, has been demonstrated to exert anti-cancer effects in both in vivo and in vitro models. We have previously determined that DIM (0 - 30 micromol/L) inhibited the growth of HT-29 human colon cancer cells in a concentration-dependent fashion. In this study, we evaluated the effects of DIM on cell cycle progression in HT-29 cells. METHODS: HT-29 cells were cultured with various concentrations of DIM (0 - 30 micromol/L) and the DNA was stained with propidium iodide, followed by flow cytometric analysis. [3H]Thymidine incorporation assays, Western blot analyses, immunoprecipitation and in vitro kinase assays for cyclin-dependent kinase (CDK) and cell division cycle (CDC)2 were conducted. RESULTS: The percentages of cells in the G1 and G2/M phases were dose-dependently increased and the percentages of cells in S phase were reduced within 12 h in DIM-treated cells. DIM also reduced DNA synthesis in a dose-dependent fashion. DIM markedly reduced CDK2 activity and the levels of phosphorylated retinoblastoma proteins (Rb) and E2F-1, and also increased the levels of hypophosphorylated Rb. DIM reduced the protein levels of cyclin A, D1, and CDK4. DIM also increased the protein levels of CDK inhibitors, p21CIP1/WAF1 and p27KIP1. In addition, DIM reduced the activity of CDC2 and the levels of CDC25C phosphatase and cyclin B1. CONCLUSION: Here, we have demonstrated that DIM induces G1 and G2/M phase cell cycle arrest in HT-29 cells, and this effect may be mediated by reduced CDK activity.

Tocotrienols


Abstract: Tocotrienol (T3), unsaturated vitamin E, has recently gained considerable attention as a potent antiangiogenic agent minimizing tumor growth, the exact intracellular mechanisms of which remain poorly understood. Because hypoxia-inducible factor-1alpha (HIF-1alpha), its downstream target vascular endothelial growth factor (VEGF), and other angiogenic factors such as interleukin-8 (IL-8) and cyclooxygenase 2 (COX-2) play critical roles in neovascularization, we tested the hypothesis that the inhibitory effect of T3 on tumor angiogenesis is via regulation of these angiogenic factors. We used 2
cancer cell lines, human colorectal adenocarcinoma cells (DLD-1) and human hepatoma cells (HepG2). T3 isomers (2 micromol/L) inhibited hypoxia-induced VEGF secretion from DLD-1, with delta-T3 showing potent inhibition. Delta-T3 suppressed hypoxia-induced VEGF and IL-8 expression in DLD-1 at both mRNA and protein levels, and we found the inhibitory mechanism of delta-T3 by reducing HIF-1alpha protein expression or increasing HIF-1alpha degradation. Also, delta-T3 (2 micromol/L) did not affect hypoxia-induced COX-2 mRNA expression; however, delta-T3 tended to suppress (P = 0.044) hypoxia-induced COX-2 protein expression, implying a possible post-transcriptional mechanism by delta-T3. Overall, our results confirmed that T3 has an inhibitory effect on angiogenic factor secretion from cancer cells and revealed the possible mechanisms, providing new information about the antiangiogenic effects of T3.


Abstract: As high telomerase activity is detected in most cancer cells, inhibition of telomerase by drug or dietary food components is a new strategy for cancer prevention. Here, we investigated the inhibitory effect of vitamin E, with particular emphasis on tocotrienol (unsaturated vitamin E), on human telomerase in cell-culture study. As results, tocotrienol inhibited telomerase activity of DLD-1 human colorectal adenocarcinoma cells in time- and dose-dependent manner, interestingly, with delta-tocotrienol exhibiting the highest inhibitory activity. Tocotrienol inhibited protein kinase C activity, resulting in down-regulation of c-myc and human telomerase reverse transcriptase (hTERT) expression, thereby reducing telomerase activity. In contrast to tocotrienol, tocopherol showed very weak telomerase inhibition. These results provide novel evidence for the first time indicating that tocotrienol acts as a potent candidate regulator of telomerase and supporting the anti-proliferative function of tocotrienol.

Low-Dose Naltrexone

Donahue RN, McLaughlin PJ, Zagon IS. Low-dose naltrexone targets the opioid growth factor-opioid growth factor receptor pathway to inhibit cell proliferation: mechanistic evidence from a tissue culture model. Exp Biol Med (Maywood) 2011 September 1;236(9):1036-50.

Abstract: Naltrexone (NTX) is an opioid antagonist that inhibits or accelerates cell proliferation in vivo when utilized in a low (LDN) or high (HDN) dose, respectively. The mechanism of opioid antagonist action on growth is not well understood. We established a tissue culture model of LDN and HDN using short-term and continuous opioid receptor blockade, respectively, in human ovarian cancer cells, and found that the duration of opioid receptor blockade determines cell proliferative response. The alteration of growth by NTX also was detected in cells representative of pancreatic, colorectal and squamous cell carcinomas. The opioid growth factor (OGF; [Met(5)]-enkephalin) and its receptor (OGFr) were responsible for mediating the action of NTX on cell proliferation. NTX upregulated OGF and OGFr at the translational but not at the transcriptional level. The mechanism of inhibition by short-term NTX required p16 and/or p21 cyclin-dependent inhibitory kinases, but was not dependent on cell survival (necrosis, apoptosis). Sequential administration of short-term NTX and OGF had a greater inhibitory effect on cell proliferation than either agent alone. Given the parallels between short-term NTX in vitro and LDN in vivo, we now demonstrate at the molecular level that the OGF-OGFr axis is a common pathway that is essential for the regulation of cell proliferation by NTX.

Abstract: Native opioid peptides serve as growth factors in a number of normal and neoplastic cells and tissues, including the prevention and delayed growth of human colon cancer xenografts in nude mice. This study examined the hypothesis that opioids exert a direct inhibitory influence on tumor cell growth by the use of a tissue culture model. The naturally occurring pentapeptide [Met5]enkephalin depressed growth of HT-29 human colon cancer cells from 17 to 41% at 12-72 h after administration of 10(-6)M concentration; consistent with previously defined nomenclature, this peptide was termed opioid growth factor (OGF). OGF action exhibited a dose-response relationship, was reversible and not cytotoxic, and was opioid receptor mediated. Growth inhibition by OGF was not dependent on serum, and was noted in the two other human colon cancer cell lines examined WiDr and COLO 205. This peptide continually repressed growth because an increase in cell number was noted when cells were exposed to the potent opioid antagonist naltrexone or an antibody to OGF. Both OGF and its receptor, zeta (zeta), were found in colon cancer cells by immunocytochemistry, and receptor binding assays revealed a nuclear-associated receptor with a dissociation constant of 8.9 nM and a maximum binding capacity of 43 fmol/mg of protein. OGF was produced and secreted by the tumor cells. These results lead to the suggestion that OGF has a direct, tonic, inhibitory action on the growth of human colon cancer cells and contribute to our understanding of the mechanisms underlying the marked antitumor effect of this peptide in nude mice inoculated with human colon cancer cells.


Abstract: Nude mice inoculated with human colon cancer (HT-29) and receiving 0.1 mg/kg naltrexone (NTX) beginning immediately after tumor cell injection exhibited a marked retardation in tumorigenicity. This dosage of NTX, which blocked opioid receptors for 6-8 h/day, resulted in a delay of 2.4-fold in tumor appearance compared to control subjects. At the time (10 days) when all control mice had tumors, 80% of the mice in the 0.1 mg/kg NTX group had no signs of neoplasia. Binding capacity, but not affinity, of [3H][Met5]-enkephalin was reduced 85% of control levels in tumor tissue from mice of the 0.1 NTX group. Plasma, but not tumor tissue levels of [Met5]-enkephalin were elevated (2.5-fold) in contrast to control values. These results suggest that daily intermittent opioid receptor blockade with NTX provokes the interaction of opioids and receptors in the interval following drug availability, with opioids serving to inhibit tumorigenicity of human colon cancer.


Abstract: Opioid growth factor (OGF) is a native endogenous opioid peptide ([Met5]-enkephalin) that interacts with the OGF receptor (OGFr), and serves as a tonically active negative growth factor in neoplasia. To inquire whether OGF modulates anchorage-independent growth, HT-29 human colon cancer cells were grown in soft agar and subjected to this peptide. In contrast to controls, HT-29 cells exposed to OGF had 57% fewer colonies, and these colonies were reduced in area by 75%. The changes induced by OGF were abolished by concomitant treatment with nalozone, indicating a receptor-mediated mechanism for peptide activity. Continuous blockade of opioid-receptor interactions with the potent and long-acting opioid antagonist, naltrexone (NTX), revealed an increase of 81 and 49% in the number and area, respectively, of colonies compared to control levels. These data suggest that OGF is tonically active in neoplastic cells growing in soft agar medium. HT-29 cells studied under anchorage-independent conditions were not influenced in growth by a variety of natural and synthetic opioids, including those selective for micro, delta, and kappa opioid receptors. Similar effects on anchorage-independent growth by OGF and NTX observed for HT-29 cells were recorded in pancreatic adenocarcinoma cells (Mia...
PaCa-2, Panc-1) and squamous cell carcinoma of the head and neck (CAL-27). These results using anchorage-independent conditions are consistent with previous data showing that OGF can markedly influence tumor growth in xenografts, and suggest that clonogenic assays can be utilized as indicators of tumorigenicity when tumor transplantation experiments are restricted.

**Macrophage Activating Factor - GcMAF**


Ref ID: 38989

Abstract: Serum vitamin D binding protein (Gc protein) is the precursor for the principal macrophage-activating factor (MAF). The MAF precursor activity of serum Ge protein of colorectal cancer patients was lost or reduced because Ge protein is deglycosylated by serum alpha-N-acetylgalactosaminidase (Nagalase) secreted from cancerous cells. Deglycosylated Ge protein cannot be converted to MAF, leading to immunosuppression. Stepwise treatment of purified Ge protein with immobilized beta-galactosidase and sialidase generated the most potent macrophage-activating factor (GcMAF) ever discovered, but it produces no side effect in humans. Macrophages treated with GcMAF (100 microg/ml) develop an enormous variation of receptors and are highly tumoricidal to a variety of cancers indiscriminately. Administration of 100 nanogram (ng) human maximally activates systemic macrophages that can kill cancerous cells. Since the half-life of the activated macrophages is approximately 6 days, 100 ng GcMAF was administered weekly to eight nonanemic colorectal cancer patients who had previously received tumor-resection but still carried significant amounts of metastatic tumor cells. As GcMAF therapy progressed, the MAF precursor activities of all patients increased and conversely their serum Nagalase activities decreased. Since serum Nagalase is proportional to tumor burden, serum Nagalase activity was used as a prognostic index for time course analysis of GcMAF therapy. After 32-50 weekly administrations of 100 ng GcMAF, all colorectal cancer patients exhibited healthy control levels of the serum Nagalase activity, indicating eradication of metastatic tumor cells. During 7 years after the completion of GcMAF therapy, their serum Nagalase activity did not increase, indicating no recurrence of cancer, which was also supported by the annual CT scans of these patients.

**Intravenous Ascorbate Therapy**


Abstract: Ascorbic acid is an essential nutrient commonly regarded as an antioxidant. In this study, we showed that ascorbate at pharmacologic concentrations was a prooxidant, generating hydrogen-peroxide-dependent cytotoxicity toward a variety of cancer cells in vitro without adversely affecting normal cells. To test this action in vivo, normal oral tight control was bypassed by parenteral ascorbate administration. Real-time microdialysis sampling in mice bearing glioblastoma xenografts showed that a single pharmacologic dose of ascorbate produced sustained ascorbate radical and hydrogen peroxide formation selectively within interstitial fluids of tumors but not in blood. Moreover, a regimen of daily pharmacologic ascorbate treatment significantly decreased growth rates of ovarian (P < 0.005), pancreatic (P < 0.05), and glioblastoma (P < 0.001) tumors established in mice. Similar pharmacologic concentrations were readily achieved in humans given ascorbate intravenously. These data suggest that ascorbate as a prodrug may have benefits in cancers with poor prognosis and limited therapeutic options.
Abstract: Ascorbate (ascorbic acid, vitamin C), in pharmacologic concentrations easily achieved in humans by i.v. administration, selectively kills some cancer cells but not normal cells. We proposed that pharmacologic ascorbate is a prodrug for preferential steady-state formation of ascorbate radical (Asc(•-)) and H(2)O(2) in the extracellular space compared with blood. Here we test this hypothesis in vivo. Rats were administered parenterally (i.v. or i.p.) or oral ascorbate in typical human pharmacologic doses (approximately 0.25-0.5 mg per gram of body weight). After i.v. injection, ascorbate baseline concentrations of 50-100 microM in blood and extracellular fluid increased to peaks of >8 mM. After i.p. injection, peaks approached 3 mM in both fluids. By gavage, the same doses produced ascorbate concentrations of <150 microM in both fluids. In blood, Asc(•-) concentrations measured by EPR were undetectable with oral administration and always <50 nM with parenteral administration, even when corresponding ascorbate concentrations were >8 mM. After parenteral dosing, Asc(•-) concentrations in extracellular fluid were 4- to 12-fold higher than those in blood, were as high as 250 nM, and were a function of ascorbate concentrations. By using the synthesized probe peroxyxanthone, H(2)O(2) in extracellular fluid was detected only after parenteral administration of ascorbate and when Asc(•-) concentrations in extracellular fluid exceeded 100 nM. The data show that pharmacologic ascorbate is a prodrug for preferential steady-state formation of Asc(•-) and H(2)O(2) in the extracellular space but not blood. These data provide a foundation for pursuing pharmacologic ascorbate as a prooxidant therapeutic agent in cancer and infections

Abstract: Human pharmacokinetics data indicate that i.v. ascorbic acid (ascorbate) in pharmacologic concentrations could have an unanticipated role in cancer treatment. Our goals here were to test whether ascorbate killed cancer cells selectively, and if so, to determine mechanisms, using clinically relevant conditions. Cell death in 10 cancer and 4 normal cell types was measured by using 1-h exposures. Normal cells were unaffected by 20 mM ascorbate, whereas 5 cancer lines had EC(50) values of <4 mM, a concentration easily achievable i.v. Human lymphoma cells were studied in detail because of their sensitivity to ascorbate (EC(50) of 0.5 mM) and suitability for addressing mechanisms. Extracellular but not intracellular ascorbate mediated cell death, which occurred by apoptosis and pyknosis/necrosis. Cell death was independent of metal chelators and absolutely dependent on H(2)O(2) formation. Cell death from H(2)O(2) added to cells was identical to that found when H(2)O(2) was generated by ascorbate treatment. H(2)O(2) generation was dependent on ascorbate concentration, incubation time, and the presence of 0.5-10% serum, and displayed a linear relationship with ascorbate radical formation. Although ascorbate addition to medium generated H(2)O(2), ascorbate addition to blood generated no detectable H(2)O(2) and only trace detectable ascorbate radical. Taken together, these data indicate that ascorbate at concentrations achieved only by i.v. administration may be a pro-drug for formation of H(2)O(2), and that blood can be a delivery system of the pro-drug to tissues. These findings give plausibility to i.v. ascorbic acid in cancer treatment, and have unexpected implications for treatment of infections where H(2)O(2) may be beneficial

Valproate and Histone Deacetylase Inhibitors

Abstract: Valproate (VPA) is a well-characterized histone deacetylase inhibitor with anti-neoplastic properties. We analyzed the growth blocking effects and the molecular mode of action of this compound in colorectal cancer cells in vitro and in vivo. Caco-2, SW-480, CX-1 or WIDR cell lines were exposed to VPA (0.25-2 mM) for various time periods. Cell growth, cell cycle progression and apoptosis were analyzed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide dye reduction assay and flow cytometry. Cell cycle- and apoptosis-regulating proteins and histone acetylation were assessed by Western blotting. In vivo tumor growth and regulating protein expression under VPA were investigated in a subcutaneous xenograft tumor model. VPA inhibited the growth of all cell lines with cell cycle arrest paralleled by the up-regulation of H3 and H4 acetylation. In vivo tumor growth was substantially depressed by VPA (200 mg/kg bw). Cell cycle proteins (cdk1, cdk2, cdk4, cyclin D, cyclin E, p19, p21 and p27) were differentially altered by VPA. Predominantly cdk1 was decreased and p27 was up-regulated in all models. Apoptosis-related proteins were altered in vivo with up-regulation of bax and down-regulation of bcl-2. VPA exerts anti-neoplastic activity in colorectal tumor cell lines in vitro and in vivo by altering cell cycle regulation.


Abstract: The beta-amyloid precursor protein (APP) represents a type I transmembrane glycoprotein that is ubiquitously expressed. In the brain, it is a key player in the molecular pathogenesis of Alzheimer disease. Its physiological function is however less well understood. Previous studies showed that APP is up-regulated in prostate, colon, pancreatic tumor, and oral squamous cell carcinoma. In this study, we show that APP has an essential role in growth control of pancreatic and colon cancer. Abundant APP staining was found in human pancreatic adenocarcinoma and colon cancer tissue. Interestingly, treating pancreatic and colon cancer cells with valproic acid (VPA, 2-propylpentanoic acid), a known histone deacetylase (HDAC) inhibitor, leads to up-regulation of GRP78, an endoplasmic reticulum chaperone immunoglobulin-binding protein. GRP78 is involved in APP maturation and inhibition of tumor cell growth by down-regulation of APP and secreted soluble APPalpha. Trichostatin A, a pan-HDAC inhibitor, also lowered APP and increased GRP78 levels. In contrast, treating cells with valpromide, a VPA derivative lacking HDAC inhibitory properties, had no effect on APP levels. VPA did not modify the level of epidermal growth factor receptor, another type I transmembrane protein, and APLP2, a member of the APP family, demonstrating the specificity of the VPA effect on APP. Small interfering RNA-mediated knockdown of APP also resulted in significantly decreased cell growth. Based on these observations, the data suggest that APP down-regulation via HDAC inhibition provides a novel mechanism for pancreatic and colon cancer therapy.


Abstract: Agents that inhibit histone deacetylases (HDAC inhibitors) have been shown to enhance radiation response. The aim of this study was to evaluate the effects of low, minimally cytotoxic concentrations of the HDAC inhibitor, valproic acid (VPA), on radiation response of colorectal cancer cells. Cell lines LS174T and an isogenic pair of HCT116, which differed only for the presence of wild-type p53, were exposed to ionizing radiation (IR) alone, VPA alone, or the combination. Clonogenic survival, gamma-H2AX induction, apoptosis, changes in mitochondrial membrane potential, and mitochondrial levels of p53 and Bcl-2 family proteins were assessed. In vivo studies monitored tumor growth suppression after therapy in mice bearing HCT116/p53(+/+) and HCT116/p53(-/-) tumor xenografts. VPA led to radiosensitization, which was dependent on p53 status. A decrease in clonogenic survival, an increase in apoptosis, and an increase in levels of gamma-H2AX were observed after...
VPA+IR, compared to IR alone, in wild-type p53 cells (LS174T and HCT116/p53(+/+)), as opposed to p53 null cells (HCT116/p53(-/-)). Exposure to VPA resulted in enhancement of IR-induced mitochondrial localizations of Bax and Bcl-xL, mitochondrial membrane potential, and cytochrome c release only in wild-type p53 cell lines. VPA also enhanced tumor growth suppression after IR only in wild-type p53 xenografts. These data suggest that VPA may have an important role in enhancing radiotherapy response in colorectal cancer, particularly in tumors with the wild-type p53 genotype.


Abstract: Histone deacetylase (HDAC) inhibitors such as suberoylanilide hydroxamic acid (SAHA, Vorinostat), valproic acid (VPA), and FK228 are members of a relatively novel class of small molecular weight chemicals that have high antineoplastic activity. They cause growth inhibition and apoptosis specifically in tumor cells, and they act also as chemo- and radio-sensitizers. In the present study, the potential of SAHA and VPA to induce resistance was studied. To that aim HDAC inhibitor-resistant sublines were generated by stepwise exposure of colon tumor cells to increasing concentrations of these compounds. Clonogenic data demonstrated that the...


Abstract: Valproic acid has been demonstrated to mediate cytotoxic effects against tumor cells by acting as a histone-deacetylase inhibitor. However, to date, there are only limited data on the effects of valproic acid in colon cancer. Moreover, information regarding combinations of the drug with chemotherapeutic agents is very limited. The latter is of interest as there is increasing evidence for synergism between so-called "molecular targeting drugs" and chemotherapy. We first demonstrated that valproic acid dose-dependently reduced the viability of adenocarcinoma cell lines. After co-incubation with a variety of chemotherapeutic agents, only valproic acid in combination with mitomycin C consistently induced synergistic growth inhibition in all cell lines. To confirm these results in an ex vivo situation, five samples of fresh colon cancer cells were studied. Again, the effect of valproic acid on the viability of the fresh tumor cells was dose dependent. In four of five samples of freshly isolated colon cancer cells, the synergistic effect of valproic acid and mitomycin C on the inhibition of cell growth was confirmed by calculation of the combination index by multiple drug effect analysis. In conclusion, this is the first demonstration that valproic acid as a model substance for histone-deacetylase inhibitors is effective in tumor cells freshly isolated from patients with colon cancer and that the combination of mitomycin C and valproic acid synergistically decreases viability of colon cancer cells.


Abstract: BACKGROUND: Epigenetic aberrations lead to chemotherapy resistance; hence, their reversal by inhibitors of DNA methylation and histone deacetylases may overcome it. PATIENTS AND METHODS: Phase II, single-arm study of hydralazine and magnesium valproate added to the same schedule of chemotherapy on which patients were progressing. Schedules comprised cisplatin, carboplatin, paclitaxel, vinorelbine, gemcitabine, pemetrexed, topotecan, doxorubicin, cyclophosphamide, and anastrozole. Patients received hydralazine at 182 mg for rapid, or 83 mg for slow, acetylators, and magnesium valproate at 40 mg/kg, beginning a week before chemotherapy. Response, toxicity, DNA methylation, histone deacetylase activity, plasma valproic acid, and hydralazine levels were evaluated.
RESULTS: Seventeen patients were evaluable for toxicity and 15 for response. Primary sites included cervix (3), breast (3), lung (1), testis (1), and ovarian (7) carcinomas. A clinical benefit was observed in 12 (80%) patients: four PR, and eight SD. The most significant toxicity was hematologic. Reduction in global DNA methylation, histone deacetylase activity, and promoter demethylation were observed. 

CONCLUSIONS: The clinical benefit noted with the epigenetic agents hydralazine and valproate in this selected patient population progressing to chemotherapy and re-challenged with the same chemotherapy schedule after initiating hydralazine and valproate lends support to the epigenetic-driven tumor-cell chemoresistance hypothesis (ClinicalTrials.gov Identifier: NCT00404508)

**Ambrisentan – Endothelin Receptor Antagonist**


Abstract: AIM: Prognostic indicators from clinical, laboratory and pathological data of patients with colorectal cancer are essential to identify high-risk groups in whom adjuvant therapy could be beneficial. Endothelin-1 (ET-1), a growth factor, has been associated with the development and spread of solid tumours. This prospective study was performed to determine whether preoperative plasma big ET-1 concentrations might be useful as a prognostic indicator in patients with colorectal carcinoma.

METHODS: Overall, 65 consecutive patients with colorectal cancer confirmed by biopsy were included prospectively in this study from 1998 to 2001. Plasma samples from a peripheral vein were obtained prior to surgery. Univariate analysis of survival used age (less than or more than 70 years), gender, Dukes' stage (A/B vs C), tumour size (less than or more than 50 mm), vascular invasion, and plasma big ET-1 concentrations, and significant factors were then analyzed using a Cox regression model. RESULTS: Three variables, age, Dukes' tumour stage and plasma big ET-1 concentration, and prognostic significance (p < 0.05). Factors associated with a poorer prognosis were age more than 70 years (p = 0.02), Dukes' C (p = 0.04) and plasma big ET-1 concentration more than 4.2 pg/mL (p = 0.02). The Cox regression model identified the same three variables as having independent prognostic value for overall survival. CONCLUSION: Preoperative plasma big ET-1 concentration may be useful in predicting overall survival in patients with colorectal cancer. Plasma big ET-1 concentrations may be useful in the selection of high-risk, lymph node-negative patients with colorectal cancer for adjuvant therapy


Abstract: Endothelin (ET)-1 can act as an autocrine/paracrine growth factor or an antiapoptotic factor in human cancers. To study the role of ET-1 in human colon cancer, proliferation and apoptosis of colon carcinoma cells was investigated using human HT-29 and SW480 colon carcinoma cells. ET-1 was secreted by these cells. Treatment of cells with bosentan, a dual ET(A/B)-receptor antagonist, decreased cell number. Inhibition of DNA synthesis by bosentan was observed only in the presence of serum. Exogenously added ET-1 did not increase DNA synthesis in serum-deprived cells. SW480 cells were sensitive and HT-29 cells were resistant to FasL-induced apoptosis. Bosentan sensitised resistant HT-29 cells to FasL-induced, caspase-mediated apoptosis, but not to TNF-alpha-induced apoptosis. Bosentan and/or FasLigand (FasL) did not modulate the expression of caspase-8 or FLIP. Bosentan sensitisation to apoptosis was reversed by low concentrations (10(-13)-10(-10) M), but not by high concentrations (10(-9)-10(-7) M) of ET-1. These results suggest that the binding of ET-1 to high-affinity sites inhibits FasL-induced apoptosis, while the binding of either ET-1 or receptor antagonists to low-affinity sites promotes FasL-induced apoptosis. In conclusion, endothelin signalling pathways do not induce human colon cancer cell proliferation, but are survival signals controlling resistance to apoptosis

Abstract: Endothelin-1 (ET-1) is a vasoconstrictor peptide which stimulates proliferation in vitro in different cell types, including colorectal cancer cells. Raised ET-1 levels have been detected both on tissue specimens and in the plasma of patients with cancers. To investigate the role of ET-1 in colorectal cancer: (i) ET-1 plasma levels in patients with colorectal cancer were measured by radioimmunoassay: group 1 = controls (n = 22), group 2 = primary colorectal cancer only (n = 39), group 3 = liver metastases only (n = 26); (ii) ET-1 expression in primary colorectal cancer specimens (n = 10) was determined immunohistochemically and (iii) the effect of intraportally infused antagonists to the two ET-1 receptors, ET(A) and ET(B), on the growth of liver metastases in a rat model was assessed. ET-1 plasma levels were significantly increased in both patients with primary tumour and patients with metastases, compared to controls (P < 0.01, 3.9 +/- 1.4, 4.5 +/- 1.5, vs. 2.75 +/- 1.37 pg/ml, respectively). Immunohistochemically, strong expression of ET-1 was found in the cytoplasm, stroma and blood vessels of cancers, unlike the normal colon where only the apical layer of the epithelium, vascular endothelial cells and surrounding stroma were positively stained. In the rat model, there was significant reduction in liver tumour weights compared to controls, following treatment with the ET(A) antagonist (BQ123) 30 min after the intraportal inoculation of tumour cells (P < 0.05). These results suggest ET-1 is produced by colorectal cancers and may play a role in the growth of colorectal cancer acting through ET(A) receptors. ET(A) antagonists are indicated as potential anti-cancer agents.


Abstract: An imbalance between proliferation and apoptosis is important in tumor progression. Endothelin-1 (ET-1) has vasoconstricting and mitogenic activities and may be involved in apoptosis regulation. We found that ET-1 and FasL systems were colocalized in human colon tumors and that ET-1 was secreted by human (HT-29, SW480) and rat (PROb, REGb) colon carcinoma cell lines. Bosentan, a mixed endothelin-A- and -B- (ET(A)/ET(B)) receptor antagonist, potentiated FasL- (APO-1, CD95) induced apoptosis in these cells. The specific inhibition of enzymes involved in ceramide production did not restore survival of cells exposed to FasL and bosentan. Inhibition of PKC with bisindolylmaleimide IX enhanced FasL-induced apoptosis in HT-29, PROb and REGb cells in the absence of bosentan. These results suggest that ET-1 is an autocrine survival factor able to protect colon carcinoma cells against FasL-induced apoptosis, involving the protein kinase C (PKC) but not the sphingomyelin-ceramide signaling transduction pathways.


Abstract: BACKGROUND AND OBJECTIVES: Endothelin-1 (ET-1), a potent vasoconstricting peptide, plays an important role in carcinogenesis. Previous in vitro studies have shown that colorectal cancer cells produce ET-1. METHODS: ET-1 and its receptors ET-A (ET(A) R) and ET-B (ET(B) R) were analyzed in colorectal cancer cell lines and tumors by Western blot and immunohistochemistry. Also, ET-1 levels were measured by ELISA in blood samples collected before and after tumor resection. RESULTS: ET-1 was immunohistochemically expressed by tumor cells at a variable level in 39 cases tested. The adjacent normal mucosa was negative for ET-1 expression. Strong ET(A) R expression observed in the deeper infiltrating areas at the periphery of neoplastic tissue correlated significantly with tumor stage. ET(B) R levels were very low or undetectable. Western blot analysis in paired (normal, tumor) fresh-frozen samples of colorectal cancers and in four colon carcinoma cell lines confirmed these findings. In addition, lower levels of ET-1 in the peripheral circulation after the tumor resection were found by ELISA as
compared to those observed before surgery. CONCLUSIONS: ET-1 and ET(A) R, but not ET(B) R, are expressed at a higher level in primary and cultured colon carcinoma cells as compared to normal colon mucosa cells. Further functional studies are needed to explore the role of ET-1/ET(A) R axis in colon carcinogenesis.


Abstract: BACKGROUND: The endothelin axis has recently emerged as an important factor in tumour metastasis. The aim of this study was to investigate the endothelin axis and its downstream pathways related to metastasis in colon carcinoma. MATERIALS AND METHODS: mRNA expression of 36 genes associated with the endothelin axis in 18 non-metastatic and 20 metastatic colon carcinomas with individual-matched normal mucosa were evaluated using real-time reverse transcription polymerase chain reaction. RESULTS: Seventeen out of 36 genes, including endothelin A receptor, were significantly overexpressed in the tumour tissue compared to the individual-matched normal mucosa. Seven out of 36 genes, including endothelin B receptor, were significantly down-regulated in tumour tissue. Phosphatase and tensin homolog (PTEN) was significantly down-regulated in the metastatic patients compared to the non-metastatic patients. CONCLUSION: This study indicated that central genes in the endothelin axis are overexpressed when colon tissue becomes malignant. Down-regulation of PTEN may promote a progressive phenotype of colon carcinomas.


Abstract: The endothelin-1 antagonist, Atrasentan (ABT-627) was used to modify perfusion in the human tumor xenograft model, HT29, growing in nude mice. Atrasentan produced a significant increase in perfusion, as measured in vivo by Gd-DTPA DCE-MRI. Changes in tumor hypoxia were assessed by comparing the binding of two hypoxia tracers, pimonidazole and EF5 given before and after Atrasentan administration. In vehicle-treated controls, the distribution of EF5 and pimonidazole was very similar. However, Atrasentan treatment was associated with decreased uptake of the second hypoxia tracer (EF5), relative to the first (pimonidazole). Although Atrasentan had no independent effect on the growth of HT29 tumors, Atrasentan combined with 20 Gy radiation led to a modest but significant increase in tumor growth delay compared to radiation alone.


Abstract: The peptide endothelin (ET) 1 promotes proliferation in a number of epithelial cancers. The aim of this study was to identify the mechanism of ET-1-stimulated proliferation in colorectal cancer cells in vitro. METHODS: The effects of ET-1 on colorectal cancer cell lines HT29, LIM1215 and SW620 were studied. Cells were cultured with ET-1 plus antagonists/inhibitors to ET(A) or ET(B) receptors, G protein subtypes, phosphoinositide 3-kinase (PI3K) or protein kinase C (PKC). DNA replication and apoptosis were investigated by 5-bromo-2'-deoxyuridine incorporation and Annexin V staining. Transactivation of the epidermal growth factor (EGF) receptor was investigated by blockade of the receptor in the presence of ET-1, measurement of levels of phosphorylated EGF receptor in the presence of ET-1, and comparing the effects of ET-1 and EGF on cell proliferation. RESULTS: ET-1 significantly stimulated growth of all cell lines via ET(A) receptors. ET-1 stimulated DNA replication, not apoptosis. ET-1-stimulated growth was inhibited by antagonist of pertussis toxin-sensitive G proteins, PI3K and PKC. Inhibition of the EGF receptor reduced the effect of ET-1. ET-1 increased levels of phosphorylated EGF receptor via the ET(A) receptor. CONCLUSION: ET-1 increased DNA.
replication in colorectal cancer cells via the ET(A) receptor. This mitogenic action was mediated via pertussis toxin-sensitive G proteins, PI3K, PKC and transactivation of the EGF receptor


Abstract: Endothelin-1 (EDN1) is a growth factor that is frequently produced by cancer cells and plays a critical role in tumorigenesis. However, the molecular mechanism controlling the expression of EDN1 in cancers is unknown. Constitutive activation of beta-catenin pathway is responsible for the initiation of the vast majority of colon cancers. Here we show that the EDN1 gene is directly regulated by beta-catenin in colon cancer cells. A specific DNA element within the EDN1 promoter is required for activation, and is associated with beta-catenin's cognate DNA binding partner, TCF4, in vivo. Inhibition of beta-catenin signaling results in lowered expression of EDN1, while enhancement of beta-catenin signaling leads to further activation of the gene. Significantly elevated EDN1 expression occurs in 80% of primary human colon cancers, consistent with it being a direct target of beta-catenin. Furthermore, EDN1 is able to rescue colon cancer cells from growth arrest and apoptosis resulting from inhibition of beta-catenin signaling, implicating a key role of EDN1 in promoting the oncogenic function of beta-catenin. These results indicate EDN1 overexpression as a major cause in colon cancers and reveal further details of the genetic programs responsible for tumorigenesis of colon cancers


Abstract: The distribution of endothelin-A- and B- (ET(A), ET(B)) receptor subtypes was compared in colorectal cancer to that in normal colon and their expression in the colorectal cancer cell lines LIM1215, HT29, SKCO1, SKCO17 and LoVo was determined, using gross and high resolution autoradiography and quantified by densitometry. ET(A) and ET(B) binding sites were expressed by all the cell lines. There was significantly (p = 0.008) higher expression of ET(A)-receptors by cancers (205.95 dpm x 1000/mm²) compared normal colon (129.19 dpm x 1000/mm²). However, for ET(B)-receptors, this was reversed, with significantly (p = 0.008) higher expression of ET(B) binding in normal colon (207.00 dpm x 1000/mm²) than in tumours (122.35 dpm x 1000/mm²)


Abstract: BACKGROUND: The vasoactive peptide endothelin 1 (ET-1) acts via two receptors, endothelin receptors A (ET(A)) and B (ET(B)). ET-1 is overexpressed by human cancers in vivo and in vitro and may be mitogenic for cancer cells. METHOD: To elucidate if ET-1 is a growth regulator the following were investigated in human colorectal cancer cell lines (LIM1215 and HT29): ET-1 production by ELISA; ET receptor expression using radioligand autoradiographic techniques; and responsiveness to ET-1, and to ET(A) and ET(B) antagonism by growth measurements. RESULTS: ET-1 was produced by LIM1215 and HT29 cells (21.3 and 41.7 fmol/ml/10(6) cells (24 hours); 22.6 and 71.7 fmol/ml/10(6) cells (48 hours), respectively). ET(A) and ET(B) receptors were expressed by both cell lines. Addition of ET-1 resulted in a dose dependent increase in cell numbers which was significant at 10(-8)-10(-9) M for LIM1215, with the greatest increase at 10(-9) M (32.7% and 28.4% increase above controls at 48 hours and 72 hours; p<0.05) and at 10(-8)-10(-9) M for HT29, with the greatest increase at 10(-9) M (13.4% and 15.7% increase above controls at 48 hours and 72 hours; p<0.05). ET(A) antagonists BQ123 and BQ610, but not the ET(B) antagonist BQ788, inhibited ET-1 induced proliferation of both LIM1215 and HT29 (p<0.05). CONCLUSION: ET-1 can stimulate the proliferation of colorectal cancer cell lines via the ET(A), but not the ET(B), receptor
Zileuton (5-Lipoxygenase Inhibitor)


Abstract: ABSTRACT: BACKGROUND: Arachidonic acid metabolite, generated by cyclooxygenase (COX), is implicated in the colorectal cancer (CRC) pathogenesis. Inhibiting COX may therefore have anti-carcinogenic effects. Results from use of non-steroidal anti-inflammatory drugs inhibiting only COX have been conflicting. It has been postulated that this might result from the shunting of arachidonic acid metabolism to the 5-lipoxygenase (5-LOX) pathway. Cancer cell viability is promoted by 5-LOX through several mechanisms that are similar to those of cyclooxygenase-2 (COX-2). Expression of 5-LOX is upregulated in colorectal adenoma and cancer. The aim of this study was to investigate the shunting of arachidonic acid metabolism to the 5-LOX pathway by cyclooxygenase inhibition and to determine if this process antagonizes the anti-cancer effect in colorectal cancer cells. METHODS: Three colorectal cancer cell lines (HCA7, HT-29 & LoVo) expressing 5-LOX and different levels of COX-2 expression were used. The effects of aspirin (a non-selective COX inhibitor) and rofecoxib (COX-2 selective) on prostaglandin E2 (PGE2) and leukotriene B4 (LTB4) secretion were quantified by ELISA. Proliferation and viability were studied by quantifying double-stranded DNA (dsDNA) content and metabolic activity. Apoptosis was determined by annexin V and propidium iodide staining using confocal microscopy, and caspase-3/7 activity by fluorescent substrate assay. RESULTS: COX inhibitors suppressed PGE2 production but enhanced LTB4 secretion in COX-2 expressing cell lines (P <0.001). The level of COX-2 expression in colorectal cancer cells did not significantly influence the anti-proliferative and pro-apoptotic effects of COX inhibitors due to the shunting mechanism. CONCLUSIONS: This study provides evidence of shunting between COX and 5-LOX pathways in the presence of unilateral inhibition, and may explain the conflicting anti-carcinogenic effects reported with use of COX inhibitors.


Abstract: Colorectal cancer (CRC) is a complex disease with genetic and epigenetic alterations in many key oncogenes and tumor suppressor genes. The active principle of a gum resin from Boswellia serrata, 3-acetyl-11-keto-beta-boswellic acid (AKBA), has recently gained attention as a chemopreventive compound due to its ability to target key oncogenic proteins such as 5-lipoxygenase and nuclear factor-kappaB. AKBA has been shown to inhibit the growth of CRC cells; however, the precise molecular mechanisms underlying its anticancer activities in CRC remain unclear. We hypothesized that boswellic acids may achieve their chemopreventive effects by modulating specific microRNA (miRNA) pathways. We found that AKBA significantly up-regulated expression of the let-7 and miR-200 families in various CRC cell lines. Both let-7 and miR-200 are putative tumor-suppressive miRNAs. AKBA modulated the expression of several downstream targets of the let-7 and miR-200 families, such as CDK6, vimentin and E-cadherin. These data were further strengthened by miRNA knockdown studies, which revealed that inhibition of let-7i facilitated enhanced cancer cell proliferation, migration and invasion. In addition, AKBA also induced similar modulation of the let-7 and miR-200 downstream genes in CRC tumors orthotopically implanted in nude mice. These results indicate that AKBA-induced antitumor effects in CRC occur, at least partly through the up-regulation of specific miRNA pathways. Our data provide novel evidence that anticancer effects of boswellic acids are due in part to their ability to regulate cellular epigenetic machinery and further highlight the promise for this phytochemical in the preventative and therapeutic applications of CRC.

Abstract: PURPOSE: Arachidonic acid metabolism via the cyclooxygenase (COX) and 5-lipoxygenase (5-LOX) pathways modulates cell growth and apoptosis. Many studies have examined the effects of COX inhibitors on human colorectal cancer, but the role of 5-LOX in colonic cancer development has not been well studied. The purpose of this study was to evaluate the expression of 5-LOX in colon polyps and cancer and the effect of 5-LOX inhibition on colon cancer cell proliferation. EXPERIMENTAL DESIGN: Colonic polyps, cancer, and normal mucosa were evaluated for 5-LOX expression by immunohistochemistry. Reverse transcription-PCR was used to establish 5-LOX expression in colon cancer cells. Thymidine incorporation and cell counts were used to determine the effect of the nonspecific LOX inhibitor Nordihydroguaiaretic Acid and the 5-LOX inhibitor Rev5901 on DNA synthesis. A heterotopic xenograft model in athymic mice using HT29 and LoVo human colon cancer cells was used to evaluate the effect of the 5-LOX inhibitor zileuton on tumor growth. RESULTS: 5-LOX is overexpressed in adenomatous polyps and cancer compared with that of normal colonic mucosa. LOX inhibition and 5-LOX inhibition decreased DNA synthesis in a concentration- and time-dependent manner in the Lovo cell line (P < 0.05). Inhibition of 5-LOX in an in vivo colon cancer xenograft model inhibited tumor growth compared with that of controls (P < 0.05). CONCLUSIONS: This study showed that 5-LOX is up-regulated in adenomatous colon polyps and cancer compared with normal colonic mucosa. The blockade of 5-LOX inhibits colon cancer cell proliferation both in vitro and in vivo and may prove a beneficial chemopreventive therapy in colon cancer.


Abstract: CONTEXT: 5-Lipoxygenase (5-LO) is an arachidonic acid- metabolizing enzyme, which has been demonstrated to exert a role in colorectal cancer tumorigenesis. Its activity in promoting neoangiogenesis in colorectal malignancies has been also recently theorized on the basis of in vitro studies. OBJECTIVE: To investigate whether any correlation existed between 5-LO immunexpression amount and the quantity of neoangiogenesis, as reflected by microvessel density (MVD) in human sporadic surgically resected colorectal adenocarcinomas. DESIGN: A total of 45 formalin-fixed, paraffin-embedded colorectal adenocarcinomas were submitted to the immunohistochemical procedures for 5-LO and CD105, which represent specific markers for neoangiogenesis and which were used in the assessment of MVD. RESULTS: CD105-positive, intratumoral, newly formed vessels were present in 45 of 45 cases with variable MVD values. A 5-LO-positive immunohistochemical reaction was also found in 45 of 45 cases. A significantly higher MVD was evident in cases displaying a high 5-LO amount in comparison with those characterized by a low 5-LO expression (28.33 vs 19.44 vessels per mm(2); P = .02). In addition, a positive significant correlation emerged between 5-LO immunexpression amount and the MVD counts (r = 0.2986, P = .04). CONCLUSIONS: Our study demonstrates the existence of a relationship between 5-LO expression and the neoangiogenesis process as reflected by intratumoral MVD in human sporadic colorectal adenocarcinomas, thus suggesting that 5-LO may modulate the formation of blood vessels in these neoplasias.


Abstract: We investigated whether leukotriene B4 (LTB4) and its signaling pathway play an important role in the progression of human colon cancer via a direct stimulation of cancer cell proliferation. Remarkable expression of LTB4 receptor 1 (BLT1) in human colon cancer tissues was detected by
immunohistochemistry, and Western blot analysis revealed the BLT1 expression in cultured human colon cancer cell lines, Caco2 and HT29. The 5-lipoxygenase inhibitor AA-861 and LTB(4)-receptor antagonist U75302 showed negative effects on survival and proliferation of both Caco2 and HT-29 cells. The inhibition of cell proliferation is due to the apoptosis because nuclear condensation and increased annexin V expression were observed in the cells treated with AA-861 and U75302. Knockdown of BLT1 by small interfering RNA caused the suppression of BLT1 protein, resulting in the inhibition of cancer cell proliferation. Blockade of BLT1 by the receptor antagonist significantly suppresses the LTB(4)-stimulated extracellular signal-regulated kinase (ERK) activation in colon cancer cells. These results indicate that the blockade of the LTB(4)-signaling pathway induces apoptosis via the inhibition of ERK activation in colon cancer cells. The LTB(4)-signaling pathway might be a new therapeutic target for colon cancer.


Abstract: Cyclooxygenase (COX)-2 and 5-lipoxygenase (5-LOX) are key enzymes involved in arachidonic acid metabolism. Their products, prostaglandins and leukotrienes, are involved in colorectal tumor development. We aimed at evaluating whether combined blocking of the COX-2 and 5-LOX pathways might have additive antitumor effects in colorectal cancer. The expression/activity of COX-2 and 5-LOX were assessed in 24 human colorectal cancer specimens. The effects of the COX-2 inhibitor celecoxib and the 5-LOX inhibitor MK886 on prostaglandin E(2) and cysteinyl leukotriene production, tumor cell proliferation, cell apoptosis, and Bcl-2/Bax expression were evaluated in the Caco-2 and HT29 colon cancer cells. We also investigated the effect of the enzymatic inhibition on mitochondrial membrane depolarization, one of the most important mechanisms involved in ceramide-induced apoptosis. Up-regulation of the COX-2 and 5-LOX pathways was found in the tumor tissue in comparison with normal colon mucosa. Inhibition of either COX-2 or 5-LOX alone resulted in activation of the other pathway in colon cancer cells. Combined treatment with 10 micromol/L celecoxib and MK886 could prevent this activation and had additive effects on inhibiting tumor cell proliferation, inducing cell apoptosis, decreasing Bcl-2 expression, increasing Bax expression, and determining mitochondrial depolarization in comparison with treatment with either inhibitor alone. The administration of the ceramide synthase inhibitor fumonisin B1 could prevent some of these antineoplastic effects. In conclusion, our study showed that inhibition of 5-LOX by MK886 could augment the antitumor activity of celecoxib in human colorectal cancer.


Abstract: AIM: To evaluate the 5-lipoxygenases (Loxs) expression level in human colorectal cancer specimens in order to determine its clinicopathologic significance in human tumorigenesis. METHODS: The relative quantity of 5-Lox mRNA in paired 91 colorectal tumor and adjacent normal mucosa samples was determined by real time quantitative PCR. Additionally, the expression of 5-Lox and cyclooxygenase (Cox)-2 proteins was also examined using immunohistochemical staining methods. RESULTS: There was a marked increase in 5-Lox mRNA levels in the tumor compared with paired normal mucosa samples (P < 0.0001). Sixty six (72.5%) tumors showed high 5-Lox mRNA levels. The positivity rate of 5-Lox and Cox-2 protein expression was 68.7% and 79.1% respectively. There was a significant association between tumoral 5-Lox mRNA level and tumor size (Rho = 0.392, P = 0.0002), depth or vessel invasion. CONCLUSION: These results suggest that 5-Lox is up-regulated in colorectal cancer and that inhibition of its expression might be valuable in the prevention and treatment of colorectal cancer.

**Chloroquine/Hydroxychloroquine**


Abstract: Autophagy is a conserved catabolic process that degrades cytoplasmic proteins and organelles for recycling. The role of autophagy in tumorigenesis is controversial because autophagy can be either protective or damaging to tumor cells, and its effects may change during tumor progression. A number of cancer cell lines have been exposed to chloroquine, an anti-malarial drug, with the aim of inhibiting cell growth and inducing cell death. In addition, chloroquine inhibits a late phase of autophagy. This study was conducted to investigate the anti-cancer effect of autophagy inhibition, using chloroquine together with 5-fluorouracil (5-FU) in a colon cancer cell line. Human colon cancer DLD-1 cells were treated with 5-FU (10 μM) or chloroquine (100 μM), or a combination of both. Autophagy was evaluated by western blot analysis of microtubule-associated protein light chain 3 (LC3). Proliferative activity, alterations of the cell cycle, and apoptosis were measured by MTT assays, flow cytometry, and western blotting. LC3-II protein increased after treatment with 5-FU, and chloroquine potentiated the cytotoxicity of 5-FU. MTT assays showed that 5-FU inhibited proliferation of the DLD-1 cells and that chloroquine enhanced this inhibitory effect of 5-FU. The combination of 5-FU and chloroquine induced G1 arrest, up-regulation of p27 and p53, and down-regulation of CDK2 and cyclin D1. These results suggest that chloroquine may potentiate the anti-cancer effect of 5-FU via cell cycle inhibition. Chloroquine potentiates the anti-cancer effect of 5-FU in colon cancer cells. Supplementation of conventional chemotherapy with chloroquine may provide a new cancer therapy modality.

Sasaki K, Tsuno NH, Sunami E et al. Resistance of colon cancer to 5-fluorouracil may be overcome by combination with chloroquine, an in vivo study. *Anticancer Drugs* 2012 August;23(7):675-82.

Abstract: Autophagy is a complex of adaptive cellular response that enhances cancer cell survival in the face of cellular stresses such as chemotherapy. Recently, chloroquine diphosphate (CQ), a widely used antimalarial drug, has been studied as a potential inhibitor of autophagy. Here, we aimed to investigate the role of CQ in potentiating the effect of 5-fluorouracil (5-FU), the chemotherapeutic agent of first choice for the treatment of colorectal cancer, in an animal model of colon cancer. The mouse colon cancer cell line colon26 was used. For the in-vivo study, colon26 cells were injected subcutaneously into BALB/c mice, which were treated with saline as a control, CQ (50 mg/kg/day), 5-FU (30 mg/kg/day), or the combination therapy (CQ plus 5-FU). The tumor volume ratio and body weight were monitored. After the sacrifice, tumor tissue protein extracts and tumor sections were prepared and subjected to immunoblotting for the analysis of autophagy-related and apoptosis-related proteins, and the terminal transferase uridylyl end labeling assay. The combination of CQ resulted in the inhibition of 5-FU-induced autophagy and a significant enhancement in the 5-FU-induced inhibition of tumor growth. Furthermore, the combined treatment of CQ and 5-FU resulted in a significant increase in the ratio of apoptotic cells compared with other treatments. The expression levels of the proapoptotic proteins, namely Bad and Bax, were increased by the CQ treatment in the protein extracts from tumors. Our findings suggest that the combination therapy of CQ and 5-FU should be considered as an effective strategy for the treatment of colorectal cancer.

Abstract: BACKGROUND: Chloroquine (CQ), the worldwide used anti-malarial drug, has recently been focused as a potential anti-cancer agent as well as a chemosensitizer when used in combination with anti-cancer drugs. It has been shown to inhibit cell growth and/or to induce cell death in various types of cancer. 5-Fluorouracil (5-FU) is the chemotherapeutic agent of first choice in colorectal cancer, but in most cases, resistance to 5-FU develops through various mechanisms. Here, we focused on the combination of CQ as a mechanism to potentiate the inhibitory effect of 5-FU on human colon cancer cells. METHODS: HT-29 cells were treated with CQ and/or 5-FU, and their proliferative ability, apoptosis, and autophagy induction effects, and the affection of the cell cycle were evaluated. The proliferative ability of HT-29 was analyzed by the MTS assay. Apoptosis was quantified by flow-cytometry after double-staining of the cells with AnnexinV/PI. The cell cycle was evaluated by flow-cytometry after staining of cells with PI. Autophagy was quantified by flow-cytometry and Western blot analysis. Finally, to evaluate the fate of the cells treated with CQ and/or 5-FU, the colony formation assay was performed. RESULTS: 5-FU inhibited the proliferative activity of HT-29 cells, which was mostly dependent on the arrest of the cells to the G0/G1-phase but also partially on apoptosis induction, and the effect was potentiated by CQ pre-treatment. The potentiation of the inhibitory effect of 5-FU by CQ was dependent on the increase of p21Cip1 and p27Kip1 and the decrease of CDK2. Since CQ is reported to inhibit autophagy, the catabolic process necessary for cell survival under conditions of cell starvation or stress, which is induced by cancer cells as a protective mechanism against chemotherapeutic agents, we also analyzed the induction of autophagy in HT-29. HT-29 induced autophagy in response to 5-FU, and CQ inhibited this induction, a possible mechanism of the potentiation of the anti-cancer effect of 5-FU. CONCLUSION: Our findings suggest that the combination therapy with CQ should be a novel therapeutic modality to improve efficacy of 5-FU-based chemotherapy, possibly by inhibiting autophagy-dependent resistance to chemotherapy.
usefulness of an oral anti-cancer drug low dose metronomic chemotherapy as an alternative cancer
therapy in elderly cancer patients requiring palliation