Roles of Oxidative Stress in the Pathogenesis of Hepatitis C – Implications for Full-Spectrum Antioxidant Therapy with Phlebotomy

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Abstract

Oxidative stress, mediated largely by NADPH oxidase complexes, and associated with a suppression of heme oxygenase-1 (HO-1) expression, appears to protect the hepatitis C virus (HCV) from endogenous anti-viral activity and lessen responsiveness to interferon-alpha/ribavirin therapy. Oxidative stress also is likely to be a key driver of the hepatocyte damage, cirrhosis, and hepatocarcinogenesis often evoked by chronic HCV infection, and makes HCV more difficult to target therapeutically by accelerating the mutation rate of HCV RNA. A comprehensive strategy for mitigating oxidative stress in the HCV-infected liver may therefore have good therapeutic potential. Agents likely to prove of value in this regard include spirulina – whose chief phytochemical phycocyanobilin can mimic the NADPH oxidase-inhibitory physiological activity of biliverdin/bilirubin – high-dose folate (potent scavenger of peroxynitrite-derived radicals), lipoic acid, N-acetylcysteine, astaxanthin, and silymarin. Reduction of hepatic iron level with repeated phlebotomies is also likely to be protective. Recent pilot studies suggest that administration of multiple complementary antioxidants may indeed have a favorable clinical impact in hepatitis C. Such an approach, while unlikely to be curative, may increase the chance of cure with standard anti-viral therapy, and in any case should reduce risk for cirrhosis and hepatocarcinoma in HCV patients.

Oxidative Stress – A Key Mediator of the Pathogenic Impact of Hepatitis C Infection

Hepatitis C infection is associated with a chronic increase in hepatic oxidative stress. At least two mechanisms appear likely to contribute to this in infected hepatocytes: damage to complex I of the mitochondrial respiratory chain, associated with electron transport that is slow, leaky, and uncoupled, that may be induced by an increase in mitochondrial calcium uptake catalyzed by the HCV core protein;[1-3] and induction of Nox4, triggered by increased autocrine production of TGF-beta.[4, 5] A more moderate induction of Nox1 has also been reported.[5] In addition, activated stellate cells, and possibly Kupffer cells and infiltrating leukocytes, can contribute superoxide generated via NADPH oxidase activity.[6, 7] HCV infection is also typified by an increase in hepatic iron stores,[8, 9] which can render oxidants much more toxic by promoting generation of hydroxyl radical in the Haber-Weiss reaction. And HCV-mediated induction of inducible nitric oxide synthase (iNOS) can likewise potentiate the toxicity of oxidants by promoting peroxynitrite generation.[10, 11]

Oxidative stress appears to drive the pathogenesis – and oppose the therapeutic resolution – of HCV infection in a number of complementary ways. Oxidative stress in hepatocytes contributes to the cellular damage and necrotic or apoptotic cell death associated with elevated serum levels of liver transaminases..[12] It also can promote hepatocyte mutagenesis, mediated by peroxynitrite products or iron-catalyzed hydroxyl radical generation, that may contribute to the high risk for hepatocarcinoma in HCV patients.[13-16] The Nox4 induced by HCV in hepatocytes associates with the nucleus; the superoxide and

hydrogen peroxide which it generates, after interaction with nitric oxide or reduced iron, seems likely to be a key mediator of the DNA damage and hepatocarcinogenesis associated with hepatitis C.[5] Oxidant-mediated mutagenesis of HCV RNA can produce variants which are relatively resistant to interferon-alpha and other therapeutic agents.[17] Recent evidence suggests that hepatocyte oxidative stress may impair autocrine production of interferon-alpha.[18] Moreover, there is considerable reason to believe that oxidative stress impedes the signaling pathway triggered by interferon-alpha, apparently by reducing the ability of the activated interferon-alpha receptor to induce phosphorylation of Tyr-701 in Stat-1, as demonstrated in hepatocytes exposed to hydrogen peroxide or, in hepatocytes transfected with Cyp2e1, ethanol.[19, 20] This may help to explain why concurrent alcohol ingestion and/or hepatic iron overload has been associated with poorer response to interferon-alpha therapy (albeit an impact of iron status on interferon response has not been confirmed in all studies).[21-25] Finally, oxidative stress generated via NADPH oxidase is a key driver of the phenotypic transformation of stellate cells to myofibroblasts, a crucial step in the evolution of hepatic fibrosis and cirrhosis.[26, 27]

In sum, it is reasonable to posit that measures which effectively control oxidative stress in HCV-infected liver may boost the efficacy of concurrent interferon-alpha therapy, increase the effectiveness of endogenous mechanisms for antagonizing viral replication, and, independent of any impact on viral load, notably decrease risk for hepatocellular damage, cirrhosis, and hepatocarcinogenesis. A comprehensive "full-spectrum antioxidant therapy", analogous to that recently proposed for management of non-alcoholic fatty liver disease,[28] incorporating repeated phlebotomies to achieve and maintain low-normal hepatic iron stores, merits serious attention as a strategy for treating HCV infection.

Clinical Potential of Biliverdin and Spirulina

There is recent evidence that the biliverdin generated by induction of heme oxygenase-1 (HO-1) exerts its potent antioxidant activity in large part via inhibition of certain isoforms of NADPH oxidase; bilirubin, rapidly generated within biliverdin-exposed cells, appears to mediate this inhibition.[29-31] HO-1 induction, however, is suppressed in HCV infected hepatocytes, and there is reason to suspect that this effect aids viral replication and survival.[32, 33] Transfection or induction of HO-1 in HCV replicon cells lines has been shown to slow HCV replication, and this effect seems to be primarily attributable to HO-1-generated biliverdin.[34, 35] Biliverdin per se dose-dependently suppresses this replication, whereas CO-releasing drugs and modulation of hepatocyte iron level has minimal impact in this regard. In addition to biliverdin's antioxidant potential, this agent might also interfere with HCV replication by inhibiting the protease activity of the nonstructural HCV protein 3/4A; whether this effect contributes importantly to HO-1's impact on viral replication is not yet clear.[36] Since activation of NAPDH oxidase in hepatocytes, Kuppfer cells, stellate cells, and infiltrating leukocytes likely contributes importantly to the oxidative stress and pathogenicity associated with chronic HCV infection, practical strategies for boosting hepatic biliverdin levels – or administration of biliverdin mimics – could have important clinical potential.

Oral administration of biliverdin has recently been shown to inhibit glomerulosclerosis and oxidative stress in diabetic mice; a suppression of glomerular Nox4 induction is associated with this effect, possibly reflecting a direct inhibitory impact of biliverdin on pre-existing NADPH oxidase complexes.[37] Although biliverdin is currently expensive to synthesize and not available for clinical use, the biliverdin derivative phycocyanobilin (PhyCB) constitutes about 0.6% (dry weight) of the food cyanobacterium spirulina;

within cells, it is efficiently converted to the bilirubin analog phycocyanorubin, which likewise can potently inhibit certain NADPH oxidase complexes.[38, 39] Indeed, it has been suggested that the versatile anti-inflammatory and anti-oxidant utility of oral spirulina (or of oral phycocyanin, which incorporates PhyCB as a chromophore) is attributable to the NADPH oxidase-inhibitory activity of PhyCB.[38] In that regard, it is notable that oral administration of either phycocyanin or free PhyCB mimics the impact of oral biliverdin on glomerulosclerosis and Nox4 induction in diabetic mice.[39] It is therefore reasonable to posit that oral administration of spirulina or of phycocyanin may be useful in the clinical management of HCV infection.

In this regard, a very recent pilot clinical study, in which HCV patients received either 1.5 g spirulina daily or the hepatoprotective phytochemical silymarin, showed a trend toward greater reduction of viral load in the spirulina-treated group (20% of patients achieved a complete or partial reduction of viral load), and improvements in serum transaminases and quality of life scores were significantly greater in the spirulina group.[40] It seems likely that the dose of spirulina tested was suboptimal from an antioxidant standpoint;[38] immunomodulatory mucopolysaccharides in the spirulina may have contributed to the observed response.[40, 41] In any case, further evaluation of whole spirulina, phycocyanin, or PhyCB-enriched spirulina extracts in clinical HCV appears warranted.

Ancillary Antioxidant Strategies

As noted, there is reason to suspect that iNOS may contribute to the pathogenesis of HCV infection, in part through generation of peroxynitrite.[42, 43] In cells which actively concentrate folic acid, high-dose folate administration promotes scavenging of peroxynitrite-derived radicals, reflecting the avid scavenging activity of tetrahydrofolates.[44-46] This appears to explain the ability of high-dose folate to reverse peroxynitrite-mediated uncoupling of endothelial nitric oxide synthase in clinical disorders associated with endothelial dysfunction.[45-48] A recent study in rats subjected to cardiac ischemia-reperfusion damage, in which pre-administration of high-dose folate was markedly protective, suggests that folate can ameliorate oxidative damage in ischemic mitochondria;[49] thus, high-dose folate may also have potential for providing protection from the mitochondrial oxidants generated in HCV infection. With respect to folate's potential to act as an antioxidant in the liver, there are very recent reports that supra-physiological intakes of folate exert a protective antioxidant effect on the livers of rats fed high levels of ethanol or dietary fat.[50, 51]

Glutathione production can be enhanced in hepatocytes and many other tissues by phase 2 inducer compounds – of which lipoic acid is a clinically well documented example[52-54] – as well as by administration of N-acetylcysteine or other tolerable cysteine sources.[55, 56] Lipoic acid has traditionally been employed in a range of hepatopathies,[57] and some but not all studies suggest that concurrent N-acetylcysteine supplementation can improve therapeutic outcomes in HCV patients treated with interferon-alpha.[58-62] Nocturnal melatonin supplementation, which, like phase 2 inducers, can boost tissue expression of glutathione and various antioxidant enzymes,[63, 64] may also have potential in this regard, though it has received little study in the context of hepatitis C.

Silymarin, and its chief active component silibinin, have long been used with varying degrees of success in liver disorders; in viral hepatitis, standard regimens appear to have achieved modest reductions in serum transaminases, without influence on viral load.[65-67] The molecular basis of silymarin's

hepatoprotective utility is still unclear. However, a recent study reveals that silymarin dose-dependently increases expression of the key antioxidant enzyme heme oxygenase-1- which generates biliverdin from heme - in hepatocyte-derived cells cultures.[68] The concentrations of silymarin employed in this study (100 µM or more) were high relative to concentrations achievable with standard regimens, so it is not clear whether this finding is clinically relevant. The failure of standard silymarin regimens to influence viral load appears inconsistent with a clinically significant impact on HO-1 activity. Perhaps the multigram daily doses of well-absorbed phytosome preparations, now being assessed in cancer therapy,[69] would have a better chance to achieve such an effect in vivo. Silymarin is not known to be a phase 2 inducer, so the basis of its impact on HO-1 expression remains obscure. In any case, administration of ample doses of silymarin/silibinin in preparations that assure optimal bioavailability may be warranted in HCV, in light of silymarin's cytoprotective impact in liver disorders.

The carotenoid astaxanthin appears to be the most bioactive of lipid-soluble scavenging antioxidants for protection of biological membranes – many times more effective than alpha-tocopherol in this regard.[70-72] Although astaxanthin did not influence HCV replication or interferon-alpha responsiveness in a replicon cell line,[73] it is reasonable to suspect that astaxanthin treatment might lessen the membrane damage (including pro-oxidant damage to the mitochondrial respiratory chain) associated with oxidative stress in clinical HCV infection.

There are many clinical reports that reduction of elevated hepatic iron stores with repeated phlebotomies and low-iron diets tends to quell hepatocyte cell damage in patients infected with HCV, as reflected by notable reduction in serum transaminases.[74-76] Even more impressive is a long-term controlled (but not randomized) study concluding that long-term iron reduction therapy is associated with a marked reduction in subsequent risk for hepatic cancer; in the iron reduction group, incidence of hepatic cancer over ten years of follow-up was only a quarter as high as in the control group.[77] This may reflect a key role for iron-catalyzed mutagenesis in HCV-associated hepatic cancer.[15, 16]

Overview

Although clinical evaluations of single-antioxidant therapy in hepatitis C have in the main observed rather paltry (if any) benefits, a recent open study employing a complex antioxidant regimen (incorporating lipoic acid, silymarin, schisandra, and other agents) noted favorable effects on serum transaminases, viral load, and liver histology in 25-44% of patients.[12] Several case histories of resolution of hepatitis C in patients treated with silymarin, lipoic acid, and selenium have also been published.[78] These reports, although hardly definitive, suggest that regimens which achieve an important antioxidant impact on the liver by combining complementary antioxidant measures may indeed have clinically useful potential in the management of hepatitis C.

In summation, there is reason to suspect that a full-spectrum antioxidant strategy – preferably incorporating spirulina (or phycocyanobilin-enriched spirulina extracts), high-dose folate, lipoic acid, N-acetylcysteine, astaxanthin, and silibinin phytosome – complemented by phlebotomy-induced iron depletion, may have utility for lessening hepatocellular damage, fibrosis and carcinogenesis in hepatitis C patients, while rendering them more responsive to therapy with interferon-alpha/ribavarin.

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