NADPH Oxidase May be a Primary Mediator of the Adverse Effects of Cigarette Smoke and of Complement Activation on Retinal Pigment Epithelium during Induction of Age-Related Macular Degeneration

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Abstract

Cigarette smoking, as well as genetic variants expected to up-regulate activation of the alternative complement pathway, are clearly linked to increased risk for both forms of age-related macular degeneration (AMD). Moderately reactive soluble compounds in cigarette smoke extract (such as acrolein), and the pro-inflammatory complement metabolite C5a, have shown the capacity to activate NADPH oxidase in various tissues. Recent evidence suggests that cadmium (Cd), a key toxin in cigarette smoke, may play a pathogenic role in AMD, consistent with the protective effect of the physiological Cd antagonist zinc in the AREDS1 study; Cd likewise has the potential to activate NADPH oxidase. Retinal pigmented epithelial (RPE) cells express NADPH oxidase activity, and oxidative stress within these cells is suspected to play a mediating role in the pathogenesis of AMD. It is therefore proposed that acrolein, Cd, and C5a all promote activation of NADPH oxidase in RPE cells, and that this phenomenon is a major mediator of the adverse impact of smoking and of the alternative complement pathway on AMD risk. If this hypothesis is correct, increased serum levels of free bilirubin – which functions physiologically to inhibit NADPH oxidase – may be associated with decreased risk for AMD; this prediction is readily testable. Moreover, this hypothesis suggests that spirulina, whose chromophore phycocyanobilin can mimic the NADPH oxidase-inhibitory activity of its chemical relative bilirubin, may have utility for prevention and control of AMD. A corollary of these considerations is that dietary spirulina and supplemental zinc may have potential for alleviating the adverse impact of smoking – and of Cd exposure, to which smoking importantly contributes - on vascular and overall health.

Cigarette Smoke May Promote AMD via NADPH Oxidase Activity in RPE Cells

The adverse effects of smoking on vascular health – linked to greater total mortality than smoking's impact on cancer risk – appear to be mediated primarily, not by nicotine or tars, but by relatively stable organic compounds in the smoke capable of undergoing spontaneous addition reactions with thiols and other nucleophiles within vascular cells.[1] Notably, these compounds include alpha,beta-unsaturated aldehydes or ketones such as acrolein and crotonaldehyde, major constituents of cigarette smoke. A number of studies have observed that exposure of endothelial or vascular smooth muscle cells, *in vitro* or *in vivo*, to cigarette smoke extract (CSE) rich in such compounds leads to induction of oxidative stress generated primarily by NADPH oxidase complexes.[1-7] Concurrent inhibition of these complexes largely alleviates the adverse effects of CSE exposure on endothelial or smooth muscle function. CSE exposure has likewise been shown to boost NADPH oxidase activity in airway epithelia, tracheal smooth muscle, keratinocytes, and glioma cells.[8-16] Activation of various PKC isoforms, and/or c-Src, by the reactive compounds in CSE, appears to mediate NADPH oxidase activation; activation of PKC does not appear to be secondary to activation of phospholipase C and diacylglycerol generation, and so might reflect direct interaction with PKC protein.[15]

It is well documented that smokers are at greatly increased risk for both the dry and wet forms of age-related macular degeneration (AMD).[17, 18] There is also substantial reason to believe that oxidative stress within retinal pigment epithelial (RPE) cells plays a key role in the pathogenesis of both forms of AMD;[19-22] however, few reports have attempted to assess what the primary source of this oxidative stress may be. RPE cells function as phagocytes, engulfing and degrading photoreceptor outer segments; like other phagocytic cells, they express NADPH oxidase activity.[23-25] Moreover, in a mouse model of AMD evoked by laser disruption of Bruch's membrane, viral delivery of small interfering RNA for p22phox to the subretinal space prevents choroidal neovascularization – leading the authors to comment that "NADPH oxidase-mediated ROS production in RPE cells may play an important role in the genesis of neovascular AMD, and this pathway may represent a new target for therapeutic intervention in AMD."[25] Exposure of RPE cells to CSE has been shown to promote oxidative stress in these cells that can induce VEGF expression (a key mediator of neovascular AMD) and also lead to apoptotic cell death; exogenous antioxidants such as N-acetylcysteine are protective in these respects.[26-31] However, how CSE evokes this oxidative stress in RPE cells has not been clarified. In light of the impact of CSE on vascular tissue, it is very reasonable to speculate that this oxidative stress stems primarily from activation of NADPH oxidase.

Cadmium May Also Activate NADPH Oxidase in RPE

High-normal body levels of the toxic heavy metal cadmium (Cd) have been linked to increased risk for vascular disease, osteoporosis, nephropathy, various cancers, and total mortality, and there is ample reason to suspect that these associations are causative.[32-37] Smoking is a key source of Cd exposure, and the body Cd stores of long-time smokers tend to be about twice as high as those of non-smokers. The possibility that Cd accumulation in the retina contributes to the pathogenesis of AMD is suggested by several recent studies. The Cd content of the retina, retinal pigmented epithelium, and the aqueous humor is increased in eyes afflicted with AMD.[38, 39] In a case-control study, urinary levels of Cd (considered a reasonably accurate measure of total body stores) of smokers with AMD were found to be significantly higher than those of smokers without this disorder; in non-smokers, however, urinary Cd did not differ between cases and controls.[40]

Arguably, these finding might simply reflect the fact that Cd is serving as a marker for intensity of smoking exposure, which mediates AMD by other means. But a pathogenic role for Cd in this regard is also credible. Induction of oxidative stress is believed to be a key mechanism whereby Cd impairs health.[41] Urinary Cd levels correlate strongly with urine levels of 8-oxo-7,8-dihydro-2'-deoxyguanosine, a standard marker for oxidative damage to DNA.[42] In cultured hepatocytes or neurons exposed acutely to low micromolar levels of Cd, NADPH oxidase is strongly activated.[43-45] Moreover, the hypertensive response of rats to chronic administration of Cd in drinking water is substantially blunted when the drug apocynin, which functions to inhibit activation of NADPH oxidase *in vivo*, is administered.[46] And oral administration of spirulina – which can function as an NADPH oxidase antagonist, as discussed below – provides dose-dependent protection from the teratogenicity of Cd in mice.[47] Cd exposure *in vitro* does indeed generate oxidative stress in RPE cells, though the source of this stress has not yet been defined.[48] These findings are consistent with the possibility that Cd exposure promotes AMD by up-regulating NADPH oxidase-mediated oxidative stress in RPE cells.

The mildly favorable results of the AREDS1 study of nutritional supplementation for control of AMD might also be viewed as consistent with this hypothesis. Although Cd has a very long half-life (estimated

as 10-30 years) in the body, and no effective chelation strategies for accelerating its removal are available, the pathogenic impact of the Cd already in the body can be mitigated to some degree by induction of the protein metallothionein.[49] This zinc-inducible protein functions to bind and store zinc and copper ions, but its numerous sulfhydryl groups can also bind Cd ions; in this bound form, Cd appears to be relatively benign. Supplemental zinc intakes in the range of 15-50 daily have been reported to boost the metallothionein content of the monocytes and erythrocytes of young men.[50, 51] The nutritional supplementation tested in the AREDS1 study included an arm in which patients received 80 mg of zinc daily. Risk of progressing to advanced AMD during the study was significantly lower in those subjects received zinc plus antioxidants (20%) was significantly lower than that in this placebo group – but the risk in those receiving zinc alone was only slightly higher (22%).[52] More remarkably, the total mortality over 6.5 years of follow-up was significantly lower in subjects randomized to receive zinc (RR, 0.73; 95% CI, 0.61-0.89)[53] - particularly intriguing in light of evidence that increased Cd stores correlate with increased total mortality. Other recent research suggests that, in subjects with relatively good zinc status, the correlation between body Cd and cancer mortality is attenuated.[37]

Genetic Up-Regulation of Complement Activation May Promote NADPH Oxidase Activity in RPE

AMD is known to be considerably more common in individuals who express alleles of complement factors or complement-related factors which could be expected to up-regulate activation of the alternative complement pathway.[54-57] Complement metabolites are a prominent component of drusen, and RPE cells express receptors for the pro-inflammatory complement metabolites C3a and C5a; moreover, oxidative stress in RPE cells impairs their ability to express inhibitors of complement activation on their plasma membranes.[58-60] Exposure of RPE cells to C5a boosts their expression of VEGF.[60] Increased plasma levels of C5a are associated with increased risk for AMD.[61] In aggregate, these findings strongly suggest that activation of the alterative complement pathway on RPE cells plays a pathogenic role in AMD. In neutrophils, eosinophils, and macrophages, which also express C5a receptors, C5a stimulates NADPH oxidase activity, likely via G protein-triggered PLC-beta/PKC signaling.[62-68] It would be of great interest to know whether C5a likewise can activate the NADPH oxidase in RPE cells; notably, the requisite G proteins, PLC-beta, and PKCs are expressed in these cells.[69, 70]

Antioxidant Activity of Spirulina May Have Potential for Prevention and Control of AMD

It should be relatively easy to determine whether CSE, Cd, and/or C5a can activate NADPH oxidase in RPE cells. A resolution of this issue could be of some practical importance. Recent studies reveal that free bilirubin – generated within cells by heme oxygenase activity – functions physiologically as a feedback inhibitor of NADPH oxidase complexes.[71-73] Although bilirubin is highly insoluble and not readily available for clinical use, its more soluble precursor has more clinical potential. But a more practical alternative in this regard may be offered by the phycobilins – notably phycocyanobilin (PhyCB) – that are major components of cyanobacteria such as spirulina. PhyCB, a derivative and homolog of biliverdin, has recently been shown to mimic the impact of bilirubin/biliverdin on NADPH oxidase activity – likely accounting for the profound and versatile anti-inflammatory effects of oral spirulina (or of phycocyanin, the prominent spirulina protein which contains PhyCB as a chromophore) in rodent studies.[74-76] Hence, if NADPH oxidase mediates much of the adverse impact of CSE, Cd, and/or complement activation on RPE cells, supplementation with spirulina or spirulina extracts may have important potential for the prevention or management of AMD.

A number of studies have linked increased serum bilirubin levels to decreased risk for vascular disorders.[77, 78] In light of the foregoing speculations, it would be intriguing to see whether individuals with relatively high bilirubin levels might be at decreased risk for AMD.

A corollary of these considerations is that supplementation with spirulina – and with zinc, a physiological Cd antagonist - may have important potential for alleviating the adverse impact of smoking on vascular and perhaps overall health. Notably, spirulina may provide some protection for the lungs of smokers – consistent with recent evidence that people with relatively high bilirubin levels are at decreased risk for both lung cancer and chronic obstructive pulmonary disease.[79]

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