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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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.

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.32-35 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.36-38 Exposure of RPE cells to C5a boosts their expression of VEGF.38 Increased plasma
levels of C5a are associated with increased risk for AMD. In aggregate, these findings strongly suggest that activation of the alternative 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. 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.

It should be relatively easy to determine whether CSE 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. 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. Hence, if NADPH oxidase mediates much of the adverse impact of CSE 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. 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 supplemental spirulina may have important potential for alleviating the adverse impact of smoking on vascular health. And spirulina may also 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.

References


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