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Cigarette smoking, oxidative stress, the anti-oxidant response through Nrf2 signaling, and Age-related Macular Degeneration

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ABSTRACT

Age-related Macular Degeneration (AMD) is the leading cause of blindness among the elderly. While excellent treatment has emerged for neovascular disease, treatment for early AMD is lacking due to an incomplete understanding of the early molecular events. Cigarette smoking is the strongest epidemiologic risk factor, yet we do not understand how smoking contributes to AMD. Smoking related oxidative damage during the early phases of AMD may play an important role. This review explores how cigarette smoking and oxidative stress to the retinal pigmented epithelium (RPE) might contribute to AMD, and how the transcription factor Nrf2 can activate a cytoprotective response.

1. Introduction

Age-related Macular Degeneration (AMD) is the leading cause of blindness among the elderly in the United States, representing 54% of legal blindness (Congdon, O'Colmain, et al., 2004). Due to the aging population, the number of people with advanced AMD will increase from 1.75 million now, to 3 million by 2020 (Friedman, O'Colmain, et al., 2004). At present, 7 million people are at risk of developing advanced AMD, and 1 in 3 persons $\geqslant$70 years old with early AMD will develop advanced disease within 10 years (Congdon et al., 2004; Mukesh, Dimitrov, et al., 2004). The vast majority of these people have nonneovascular or dry AMD. Since high dose micronutrient vitamins only slows visual loss in dry AMD, the therapeutic benefit is modest at best (2001). As a result, the impact of AMD to both the individual and the general public is devastating. Improving treatments that reverse, prevent, or even delay the onset of dry AMD would have significant benefit to both the individual and society. Most research has concentrated on neovascular (wet) AMD, which has resulted in the development of effective anti-VEGF treatments. Few studies however, have focused on the factors that are important in the development of dry AMD. Aside from chronological aging, cigarette smoking is the strongest epidemiologic risk factor for AMD yet our understanding of how it contributes to AMD is limited at present. The purpose of this review article is to describe the evidence for how cigarette smoking might contribute to the onset of early AMD.

2. The epidemiologic evidence

The strongest environmental risk factor for AMD is cigarette smoking (Smith, Assink, et al., 2001). Epidemiologic data from several large studies indicate that both AMD onset and disease progression are strongly influenced by smoking (Clemons, Milton, et al., 2005; Khan, Thurlby, et al., 2006; Klein, Knudtson, et al., 2008; Smith et al., 2001). The findings from three continents nicely summarizes the dramatic influence that cigarette smoking has on AMD (Tomany, Wang, et al., 2004). After pooling data, current smoking was associated with an increased incidence of geographic atrophy and late AMD (odds ratios [ORs] relative to nonsmokers: 2.83 and 2.35, respectively; ORs relative to past smokers: 2.80 and 1.82, respectively). Thus, current smokers appear at higher risk of AMD than both past smokers and nonsmokers. In addition, a "dose–response" effect has been established since pack-year smoking strongly correlates with AMD while smoking cessation reduces the risk for dry AMD (Khan et al., 2006). These results provide fundamental evidence for a strong epidemiologic association of cigarette smoking and AMD.

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With aging and early AMD, the retinal pigmented epithelium (RPE) undergoes progressive degeneration. The RPE transitions from its normal cuboidal morphology to irregularly shaped, and then flattened or atrophic when overlying thick basal laminar deposits, or accumulations of heterogeneous material within Bruch membrane (Green, McDonnell, et al., 1985; Sarks, 1976; van der Schaft, Mooy, et al., 1992). With aging and early AMD, the number of RPE cells in the macula declines more than in the periphery (Harman, Fleming, et al., 1997). Cells that overlie drusen are swollen, rounded, and vacuolated (Anderson, Mullins, et al., 2002), but appear distinct from apoptotic cells that are shrunken with nuclear fragmentation. These changes are reminiscent of a prelethal form of oncosis, a pathway of cell death characterized by increased cell volume (Anderson et al., 2002). The strongest evidence however, indicates that apoptosis is the major pathway for RPE cell death. Del Priore, Kuo, et al. (2002) showed that the proportion of apoptotic RPE cells in the macula increases significantly with age while Dunaief, Dentchev, et al. (2002) showed that maculas with AMD have significant increases in TUNEL-positive RPE cells compared with normal eyes. In geographic atrophy, TUNEL-positive RPE cell nuclei appear at the edges of RPE atrophy, which correlates with clinically observed expansion of atrophic areas during disease progression. Epidemiologic studies of AMD suggest that the RPE is a specific target of cigarette smoke induced injury. The Blue Mountains Eye study (Mitchell, Wang, et al., 2002) showed that smoking was associated with increased RPE abnormalities and the AREDS cohort (2001) found that smoking was correlated with geographic atrophy, which is characterized by progressive RPE atrophy and apoptotic cell death.

3. RPE cell apoptosis and other early features of AMD develop in mice exposed to cigarette smoke

Our laboratory recently showed that mice exposed to chronic cigarette smoke develop features of early AMD (Fujihara, Nagai, et al., 2008). Mice exposed to cigarette smoke developed oxidative damage and ultrastructural degeneration to the RPE and Bruch membrane, as well as RPE cell apoptosis. Two month old C57Bl6 mice were exposed to either filtered air or cigarette smoke in a smoking chamber for 5 h/day, 5 days/week for 6 months. Fig. 1 (Fujihara et al., 2008) shows mice exposed to cigarette smoke had immunolabeling for 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-OHdG), a marker of oxidative DNA damage, in 85 ± 3.7% of RPE cells counted compared to 9.5 ± 3.9% in controls ($p < 0.00001$). Using regression analysis, the two most pronounced ultrastructural changes (severity grading scale from 0 to 3) seen were a loss of basal infoldings (mean difference in grade = 1.98; $p < 0.0001$), and an increase in intracellular vacuoles (mean difference in grade = 1.7; $p < 0.0001$; Fig. 2 (Fujihara et al., 2008)). These ultrastructural changes to the RPE have been identified in AMD. Bruch membrane was thicker in mice exposed to smoke (1086 ± 332 nm) than those raised in air (543 ± 132 nm; $p = 0.0069$), which is suggestive of accelerated aging. Ultrastructural changes to Bruch membrane in cigarette-smoke exposed mice were smaller in magnitude than changes in the RPE, but consistently demonstrated significantly higher grade injury in cigarette-exposed mice, including basal laminar deposits (mean difference in grade = 0.54; $p < 0.0001$), increased outer collagenous layer deposits (mean difference in grade = 0.59; $p = 0.002$), and increased basal laminar deposit continuity (mean difference in grade = 0.4; $p < 0.0001$). A higher percentage of apoptotic RPE cells from mice exposed to cigarette smoke (average 8.0 ± 1.1%) than room air (average 0 ± 0%; $p = 0.043$) was identified using TUNEL staining (Fig. 3, Fujihara et al., 2008). We plan to use this model for studying the mechanism of smoke induced changes during early AMD.

Espinosa-Heidmann, Suner, et al. (2006) found that a shorter duration, higher concentration of cigarette smoke in 16 month old C57Bl6 mice induced ultrastructural changes to Bruch membrane and the choriocapillaris endothelium that are compatible

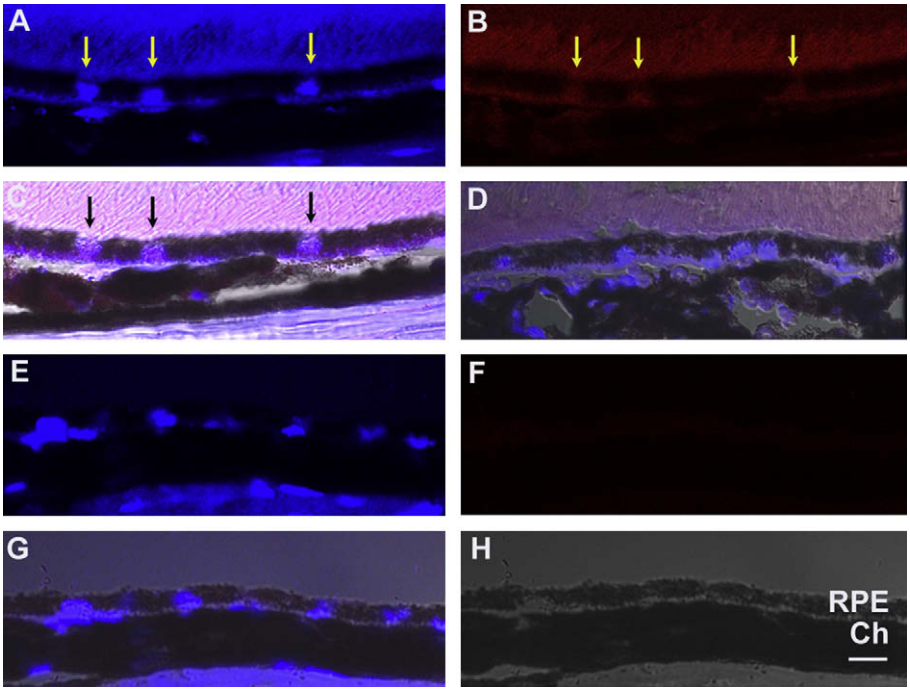

Fig. 1. Immunohistochemistry of 8-OHdG nuclear labeling of the RPE. Two month C57Bl6 mouse exposed to smoke for 6 months showing (A) DAPI labeled nuclei (arrows); (B) 8-OHdG labeled RPE nuclei (arrows); (C) merged image of A and B with the Brightfield image showing violet nuclei (arrows); (D) merged image of DAPI and IgG1 control image with Brightfield image overlay. Eight month old C57Bl6 mouse raised in air showing DAPI labeled RPE nuclei in (E) and 8-OHdG immunostaining in (F); (G) Merged DAPI and 8-OHdG immunostained image with Brightfield image overlay showing blue nuclei in H. Brightfield image. RPE, retinal pigmented epithelium; Ch, choroid. Bar = 15 mm. Figure shows representative images from $N = 10$ mice (50 samples/mouse).