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Cadmium accumulation in the human retina: Effects of age, gender, and cellular toxicity

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Abstract

Tobacco smoking and aging are among the few factors linked to age-related macular degeneration (AMD), a major cause of blindness in the elderly. Recent studies indicate that cadmium (Cd), an environmental toxic trace metal, is approximately four-fold higher in the retinas of smokers compared to non-smokers. In this study, we determined the effects of age and gender on Cd accumulation in human retinal tissues, specifically the neural retina, retinal pigment epithelium (RPE), and choroid. Cadmium levels in cultured RPE cells or retinal tissues isolated from frozen donor eyes were measured using inductively coupled plasma mass spectrometry (ICP-MS) and graphite furnace atomic absorption spectrophotometry (GF-AAS). Cadmium uptake in cultured human RPE cells (ARPE-19) was also assessed using GF-AAS. Toxic effects of cadmium were determined from cell loss (measured as a decrease in cell density) and lactate dehydrogenase release (an indicator of membrane disruption). In ''young'' eyes (<55 years) Cd was highest in the retinal pigment epithelium and lowest in the neural retina. Cd was higher in all tissues in aged eyes (≥55 years) and was significantly higher in the neural retina and RPE in older females. Cultured RPE cells exposed to Cd showed altered cell morphology, decreased cell survival, elevated ROS levels and concentration-dependent disruption of membrane integrity. We conclude that cadmium is accumulated differently in the neural retinal and RPE of older men and women. The deleterious effects of Cd on RPE cells indicate that this environmental toxin is a potentially important factor in age-related retinal disease.

Keywords: cadmium; neural retina; retinal pigment epithelium; choroid; aging; human; women; graphite furnace atomic absorption spectrophotometry; inductively coupled plasma mass spectrometry; ARPE-19; membrane

1. Introduction

The retinal pigment epithelium (RPE) plays a central role in retinal function and is critical for the survival of photoreceptors. Dysfunction of the RPE underlies numerous retinal diseases and has been implicated in age-related macular degeneration (AMD), a leading cause of blindness. The etiology of AMD is poorly understood. Several studies indicate that the frequency of AMD is higher in women than in men (Klein, 1999). However, tobacco smoking is one of the few known factors clearly linked to this disease.

Tobacco contains harmful substances, including cadmium, a toxic metal and potent carcinogen. Cadmium is used in industrial processes and in fertilizers and is accumulating in the environment. Tobacco plants absorb and concentrate cadmium from contaminated dust and soil (ATSDR, 1999; Satarug and Moore, 2004). In humans, the airways are a primary route of cadmium uptake and approximately 50–95% of inhaled cadmium enters the systemic circulation (Ellis et al., 1979).

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Although cadmium is cleared from the blood, it remains in body tissues with a long half-life, accumulating with age in the kidney and liver (Friberg, 1985).

The effects of cadmium have been extensively studied in the kidney and other tissues, however less is known about the distribution and deleterious effects of this toxin in retinal tissues. Recently, Erie et al. (2005) demonstrated that cadmium is present in the retinal pigment epithelium/choroid at levels higher than in the blood or ocular fluids. Moreover, cadmium was four-higher in retinal tissues from smokers compared to non-smokers. Gender might also influence cadmium absorption. Satarug et al. (2004) reported that women who were non-smokers had nearly the same cadmium body burden as men who smoked an average of nine cigarettes per day for nearly 10 years. At present, there is little information about the relative distribution of cadmium in the human neural retina, retinal pigment epithelium, and choroid, or the effects of age or gender on cadmium distribution in human retinal tissues.

Cadmium has toxic effects on many cells, including neurons (Lopez et al., 2006), lung cells (Yang et al., 1997) and renal tubules (Thevenod et al., 2000). Cadmium-induced damage in most cells is caused in part by increased levels of reactive oxygen species (ROS) (Bertin and Averbeck, 2006). Retinal cells and the retinal pigment epithelium routinely cope with ROS generated by metabolic processes and light. However, during aging, levels of specific antioxidant enzymes in the retina decrease (De La Paz et al., 1996), diminishing the ability of cells to survive oxidative stress. Consequently, it is important to determine whether cadmium contributes to ROS-related cellular damage in retinal cells.

In the present study, we assessed the relative distribution of cadmium in the human neural retina, RPE, and choroid and the effects of donor age and gender on cadmium content. In addition, we tested the hypothesis that cadmium has toxic effects in RPE cells that disrupt cell growth, alter membrane integrity and permeability, and that these effects are enhanced during conditions of oxidative stress.

2. Methods

2.1. Specimens

Human eyes from donors aged 1.5 to 87 years were obtained from the Montana Eye Bank (Missoula, MT) and University of Texas Lions Eye Bank (Galveston, TX) in adherence with the tenets of the Declaration of Helsinki and institutional review board approval. The causes of death of the subjects were as follows: 11 acute myocardial/cardiac arrests, 5 respiratory arrest/failures, one cerebrovascular accident, one drowning, 1 motor vehicle accident, 3 traumas, and 1 pancreatitis. The donors ranged in age from 1.5 to 87 years (21 eye pairs and 2 unpaired eyes comprising 122 retinal tissue samples). Eyes were prepared as described by van Kuijk et al. (1991). The eyecups were stored at −85 °C under argon gas. The time between death of the donors and storage ranged from 3 to 26 h. Donors with evidence of macular degeneration or other ocular disease were excluded from the study.

2.2. Sample preparation

For metal quantification, eyes were removed from the freezer and thawed for ~30 min at room temperature. The neural retina was removed with forceps and homogenized in 1 ml of deionized trace metal free water (Milli-Q). Trace metal free MilliQ water was used instead of buffered saline solutions to avoid possible trace metal contamination and to lyse the cells. Two methods were used to remove RPE cells. In the first method, 500 µl of Milli-Q water was placed in the eyecup and the interior was gently stroked with the tip of a sable brushes to release RPE cells. The fluid was then collected and the procedure was repeated to obtain a 1 ml sample. The yield with this method was low. Therefore, in subsequent experiments, after the neural retina was removed, the choroid with RPE cells attached, was gently detached with forceps, cut into small 4–5 mm pieces and transferred to a scintillation vial with a Teflon cap containing 2 ml of Milli-Q water. The vial was then vortexed for 5–10 s to separate the RPE cells from the choroid. The pieces of choroid were removed from the suspension, excess water on the choroid pieces was blotted, and they were then transferred to a homogenizing tube containing 1 ml of Milli-Q water. Following homogenization, samples were placed in pre-weighed polypropylene tubes (Falcon). Aliquots (200 µl) were transferred to microcentrifuge tubes for protein concentration measurements using a Bradford assay kit (Pierce Biotechnology, Inc, Milwaukee, WI). Except for yield of RPE cells, the two methods gave similar results.

2.3. Digestion

Methods for analysis of cadmium in human ocular tissues followed protocols for measurements in human tissues that have been previously developed in the trace metal facility at UTMB (Alcock, 1987; Ramanujam et al., 1999). Digestion of tissue samples were carried out using 0.5–1.0 ml of 30% hydrogen peroxide (GFS Chemicals; Powell, OH) at 70–80 °C in a drying oven for 18–24 h followed by 0.1 ml of Ultra-pure nitric acid (GFS Chemicals) until completely ashed. The digested ash was dissolved in 0.25 N double-distilled ultra-pure trace-metal free nitric acid (1–4 ml). Each sample was further diluted 1:5 and 1:10 (v/v) using 1% 0.25 N Ultra-pure nitric acid prior to analysis (Alcock, 1987).

2.4. Graphite furnace atomic absorption spectrophotometry (GF-AAS)

Concentrations of Cd in the digested samples were determined by GF-AAS and results were compared with inductively coupled plasma-mass spectrometry (ICP-MS). GF-AAS was performed using a Perkin-Elmer Model-5100 atomic absorption spectrophotometer equipped with a Perkin-Elmer HGA-600 graphite furnace with a Zeeman-5100 deuterium arc correction and a Perkin-Elmer-60 auto-sampler attached to the graphite