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Anatomical evidence of photoreceptor degeneration induced by iodoacetic acid in the porcine eye

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ABSTRACT

Iodoacetic acid (IAA) induces photoreceptor (PR) degeneration in small animal models, however, eye size and anatomic differences detract from the usefulness of these models for studying retinal rescue strategies intended for humans. Porcine eyes are closer in size to human eyes and have a rich supply of rod and cones. This study investigated whether IAA also produced PR degeneration in the porcine retina, whether the damage was preferential for rods or cones, and whether IAA induced remodeling of the inner retina. Pigs were given a single i.v. injection of IAA and were euthanized 2–5 weeks later. Eyes were enucleated and immersed in fixative. Forty-six eyes were studied: Control ($n = 13$), and from pigs that had received the following IAA doses: 5.0 mg/kg ($n = 7$); 7.5 mg/kg ($n = 10$); 10.0 mg/kg ($n = 6$); 12.0 mg/kg ($n = 6$). Tissue was retrieved from four retinal locations: 8 mm and 2 mm above the dorsal margin of the optic disc, and 2 mm and 8 mm below the disc, and was processed for conventional histology, immunohistochemistry, and transmission electron microscopy. At 5.0 mg/kg IAA produced mild, variable cell loss, but remaining cells exhibited normal features. At doses above 5.0 mg/kg, a dose-dependent reduction was observed in the length of PR inner and outer segments, and in the number of PR nuclei. Specific labeling revealed a massive dropout of rod cell bodies with relative sparing of cone cell bodies, and electron microscopy revealed a reduction in the number of PR synaptic terminals. Mild dendritic retraction of rod bipolar cells and hypertrophy of Müller cell stalks was also observed, although the inner nuclear layer appeared intact. The porcine IAA model may be useful for developing and testing retinal rescue strategies for human diseases in which rods are more susceptible than cones, or are affected earlier in the disease process.

1. Introduction

Age-related macular degeneration (AMD) and retinitis pigmentosa (RP), an inherited retinal dystrophy, are two common forms of progressive blindness that share a grossly similar pathology that preferentially damages photoreceptors, while leaving the interneurons (e.g. bipolar and horizontal cells) and output cells (ganglion cells) of the inner retina relatively intact (Humayun et al., 1999; Kim et al., 2002; Medeiros and Curcio, 2001; Santos et al., 1997; Stone et al., 1992). The blindness that ensues is irreversible and current treatments only retard or inhibit progression of the disease and do not restore sight (Sharma and Ehinger, 1999). Because the inner retina and central visual system remain relatively spared in AMD and RP, photoreceptor rescue and replacement strategies such as retinal prostheses, photoreceptor and retinal pigment epithelium transplants, stem cell therapies, genetic modification therapies, and survival factor therapies are being pursued to take advantage of the remaining neural circuitry of surviving neurons in an attempt to restore vision (Musarella and MacDonald, 2011). Many cellular retinal rescue strategies are contingent upon the remaining neural framework of the retina tolerating and responding to these therapies; however, remodeling of the neural retina often accompanies degeneration of photoreceptors (Marc et al., 2003), which may render some therapies unfeasible. Testing of retinal rescue strategies in animal models of photoreceptor degeneration that bear similar resemblance to

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human conditions is critical to determine which therapies have realistic potential.

Transgenic and non-genetic rodent models of photoreceptor degeneration (Chader, 2002; Chang et al., 1993; D'Cruz et al., 2000; Fauser et al., 2002; Jaissle et al., 2001; Li et al., 1996; Olsson et al., 1992; Roof et al., 1994; Sung et al., 1994; Strauss et al., 1998), as well as models developed in canines (Acland et al., 2001; Aguirre and Rubin, 1974), and felines (Ivert et al., 1998) have been invaluable for furthering our understanding of the pathogenesis of inherited photoreceptor disease. However, small eye size and/or differences in retinal anatomy and histology make many established models ill-suited for developing retinal rescue strategies that are to be used in humans. In contrast, the porcine eye is remarkably similar to the human eye in size, retinal anatomy and histology (Beauchemin, 1974; Braekvelt, 1983), and retinal vasculature (De Schaepdrijver et al., 1992; Simoens et al., 1992). The major anatomical difference is that the pig eye lacks a rod-free fovea. Rather, the pig retina contains an 'area centralis' that contains both rods and cones, which is functionally comparable to the human fovea (Chandler et al., 1999; Gerke et al., 1995). Transgenic pigs have been produced that express a mutant rhodopsin transgene, Pro347Leu (Petters et al., 1997). These animals exhibit photoreceptor degeneration that resembles the autosomal dominant form of RP in the human (Li et al., 1998; Petters et al., 1997). However, genetic engineering of Pro347Leu transgenic pigs is intrinsically slow and expensive. Therefore, our group sought to develop an inducible (non-genetic) model of photoreceptor degeneration in the pig using the photoreceptor toxin iodoacetic acid (IAA).

IAA inhibits anaerobic glycolysis by reacting with the sulfhydryl group of glyceraldehyde-3-phosphate dehydrogenase (Winkler et al., 2003) causing lower production of metabolic energy that ultimately leads to impaired function and cell death. In the retina, IAA toxicity may have the greatest effect on photoreceptors because these cells have the highest rate of glycolytic activity in the body (Noell, 1953). Previous studies in the rat (Graymore and Tansley, 1959), rabbit (Lasansky and De Robertis, 1959; Liang et al., 2008; Noell, 1953; Orzalesi et al., 1970), ground squirrel (Farber et al., 1983), and monkey (Noell, 1952, 1953), suggest that IAA preferentially damages photoreceptors, while sparing the neurons of the inner retina. In the rabbit retina, the photoreceptor loss was greatest in a region inferior to the visual streak, which may be related to the unusual vascular patterns in the rabbit eye (Liang et al., 2008).

In this study we examined retinae from pigs after systemic injection of IAA to determine whether the toxin induced photoreceptor degeneration in this species, whether there was a topographical pattern of damage across the retina, and to determine whether the damage was preferential for rods or cones. A range of doses of IAA was used to determine the effective dose for producing photoreceptor loss, and to determine whether a dose that was not lethal to photoreceptors would induce remodeling in the retina, which is a hallmark of the retinal response to genetically-mediated photoreceptor loss (Marc et al., 2003), and which has been detected in other retinal injury models (Fisher and Lewis, 2003; Linberg et al., 2006; Marc et al., 2008; Nagar et al., 2009; Peichl and Bolz, 1984). Cell loss and the topographical distribution of photoreceptor damage were assessed using standard histological techniques, specific cell populations were examined using immunohistochemistry and confocal microscopy, and ultrastructure was examined using transmission electron microscopy.

2. Methods and materials

2.1. Animals

Domestic pigs (age range, 6–8 weeks; weight range, 12–16 kg) were acquired from Oak Hill Genetics (Ewing, IL) and cared for by our collaborators in the Department of Ophthalmology and Visual Sciences, University of Louisville, Louisville, KY. Pigs were given a single intravenous injection of sterile IAA (Sigma, St. Louis, MO) dissolved in normal saline at a dosage of 5.0 mg/kg, 7.5 mg/kg, 10.0 mg/kg, and 12.0 mg/kg, via a catheter placed in the ear vein. After a 2–5 week survival period, pigs were euthanized with Beuthanasia-D (1 ml/5 kg, i.v.), the eyes were enucleated and immersed in fixative. Whole globes were shipped to our laboratory for anatomical studies. Upon arrival, each eye was confirmed to be grossly normal by examination under a dissecting microscope and then processed for histological studies. A total of 46 eyes were examined. Thirteen eyes were normal controls, while the remaining 33 eyes were from pigs treated with IAA (5.0 mg/kg, $N = 7$; 7.5 mg/kg, $N = 10$; 10.0 mg/kg, $N = 10$; 12.0 mg/kg, $N = 6$). All protocols involving pigs were performed in accordance with the tenets of the Association for Research in Vision and Ophthalmology statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the University of Louisville IACUC.

2.2. Fixation

Pig eyes were fixed by immersion in either 4% paraformaldehyde in phosphate (PO_4) buffer (0.1 M, pH 7.4) or 2% paraformaldehyde/2% glutaraldehyde in PO_4 buffer (0.1 M, pH 7.4) at 4 °C for 48 h. Eyes fixed with 4% paraformaldehyde were used for conventional histology and immunohistochemistry, while eyes fixed with 2% paraformaldehyde/2% glutaraldehyde were used for transmission electron microscopy as well as conventional histology. Eyes were dissected to remove the anterior segment, lens, and vitreous, leaving a posterior eyecup that contained the sclera, choroid, RPE, and neural retina. Every effort was made to inhibit separation of the retina from the RPE during dissection and processing of the posterior eyecup; however, this occurred in several specimens. Posterior eyecups were then placed back in the same fixative and stored at 4 °C for at least 1 week to ensure adequate fixation of retinal tissue.

2.3. Plastic sections

A 3 mm wide strip of tissue containing the retina, RPE, choroid, and sclera was retrieved from the posterior eyecup. The optic disc, which is located inferotemporally in the porcine eye, was used as a readily identifiable landmark during tissue retrieval. Vertical cuts extending from the dorsal to ventral margin of the posterior eyecup were made along both the nasal and temporal margin of the optic disc. The tissue strip was then bisected along the horizontal plane through the optic disc and the dorsal and ventral halves were again bisected along the horizontal plane. Each of the four pieces was notched on its dorsal edge to preserve orientation. The pieces were dehydrated in ascending ethanol concentrations, infiltrated, and embedded in JB-4 Plus resin (Ted Pella, Redding, CA). Tissue was oriented within the block so that vertical sections could be obtained along the dorsal to ventral axis and the tissue blocks were trimmed to preserve this orientation information. Sections 4 microns thick were cut on a rotary microtome (Shandon Lipshaw, Pittsburgh, PA), mounted on slides, dried, and stained with 1% cresyl violet (Sigma, St. Louis, MO). Sections were examined at 40 or 100× using a Zeiss Axioskop microscope (Zeiss, Thornwood, NY). Photomicrographs were taken on a Q-Color3 high-resolution camera (Olympus America Inc., Center Valley, PA) and digitally processed using Adobe Photoshop (Adobe Systems, San Jose, CA) to adjust brightness and contrast.

2.4. Nuclear profile counts and statistics

Nuclear profile counts were performed in order to quantify photoreceptor loss in the outer nuclear layer (ONL) as well as to