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Iodoacetic acid, but not sodium iodate, creates an inducible swine model of photoreceptor damage

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Abstract

Our purpose was to find a method to create a large animal model of inducible photoreceptor damage. To this end, we tested in domestic swine the efficacy of two chemical toxins, known to create photoreceptor damage in other species: Iodoacetic Acid (IAA) and Sodium Iodate (NaIO₃). Intravenous (IV) administration of NaIO₃ up to 90 mg/kg had no effect on retinal function and 110 mg/kg was lethal. IV administration of IAA (5 – 20 mg/kg) produced concentration dependent changes in visual function as measured by full field and multi-focal electroretinograms (ffERG and mfERG), and 30 mg/kg IAA was lethal. The IAA-induced effects measured at two weeks were stable through eight weeks post-injection, the last time point investigated. IAA at 7.5, 10, and 12 mg/kg produce a concentration-dependent reduction in both ffERG b-wave and mfERG N1 – P1 amplitudes compared to baseline at all post-injection times. Comparisons of dark- and light-adapted ffERG b-wave amplitudes show a more significant loss of rod relative to cone function. The fundus of swine treated with ≥10 mg/kg IAA was abnormal with thinner retinal vessels and pale optic discs, and we found no evidence of bone spicule formation. Histological evaluations show concentration-dependent outer retinal damage that correlates with functional changes. We conclude that NaIO₃ is not an effective toxin in swine. In contrast, IAA can be used to create a rapidly inducible, selective, stable and concentration-dependent model of photoreceptor damage in swine retina. Because of these attributes this large animal model of controlled photoreceptor damage should be useful in the investigation of treatments to replace damaged photoreceptors.

Keywords

Retina; Retinal Degeneration; Iodoacetic Acid; Retinitis Pigmentosa; Porcine Eye; Electrophysiology; Multifocal ERG

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1. Introduction

Most of the anatomical features and functional organization of the retina is conserved across vertebrates, making it possible to create and study models of retinal diseases in diverse species (Joselevitch & Kamermans, 2007). Because of the relative ease of manipulating the rodent genome, many models have been created that recapitulate blinding eye diseases (Baehr & Frederick, 2009), especially Retinitis Pigmentosa (RP). While useful, small animal models have limitations when used in therapeutic approaches, particularly when surgical procedures are required. Further, species differences between small mammals and humans are frequent and, as a consequence, treatment strategies successfully developed for rodents may not prove effective in humans (Pearce-Kelling et al., 2001).

Because of their comparative anatomy and physiology, swine are increasingly utilized as a suitable large mammalian model for many human systemic diseases (Swindle & Smith, 2000) and may in the near future provide cells/tissue for transplantation (Ekser et al., in press). Outside of non-human primates, the swine eye is the most similar to the human in size as well as cone density. Rather than a macula, swine have a cone dense visual streak which runs horizontally across the central retina superior to the optic nerve. In the streak, cone density is similar to man within 5 degrees of the macula (Gerke et al., 1995; Beauchemin, 1974). The cone:rod ratio is 1:3 to 1:5 (Chandler et al., 1999; Gerke et al., 1995) and falls to ~1:17 in the periphery (Hendrickson & Hicks, 2002). Like the human, the high cone density is matched by high ganglion cell density (Garcá et al., 2005).

RP, which affects approximately 100,000 people in the United States, is a heterogeneous group of diseases that all cause rod photoreceptor degeneration. Typical functional changes are noted in humans between adolescence and early adulthood and progression can extend several decades (Dryja & Berson, 1995; Shintani et al., 2009; Berson, 1993). The slow time course of disease progression results in experimental animals that need to be housed over long periods and in large animals, such as the transgenic Pro347Leu mutant domestic swine (Petters et al., 1997) extremely large (>500 lbs at maturity) animals. This is a deterrent to their use in many cases.

For many therapeutic strategies, a rapidly inducible swine model of photoreceptor damage would be advantageous. In small animal models, iodoacetic acid (IAA) has been reported to induce PR cell death (Noell, 1951 & 1953) presumably by inhibiting GAPDH function and inhibiting glycolysis in the highly metabolic PRs. In contrast, sodium iodate (NaIO₃) causes selective necrosis of the RPE, with subsequent PR degeneration (Noell, 1953, Enzmann et al., 2006).

2. Methods

2.1. Animals, surgical and electrophysiological procedures

All methods were approved by the University of Louisville Institutional Animal Care and Use Committee and adhered to the ARVO Statement for Use of Animals in Ophthalmic and Vision Research. Domestic swine ages 6 to 8 weeks were obtained from Oak Hill Genetics (Ewing, IL) and weighed prior to each experimental procedure (range 12–16kg). Table 1 shows the number of swine (eyes) studied with ffERG, mfERG and with histology as a function of toxin concentration and age.

A baseline assessment of retinal structure and function was performed in each swine and included ff- and mfERGs and a clinical fundus examination. The toxin was administered immediately after these assessments. Retinal structure and function were reassessed at 2 and

5 weeks post-toxin administration in one group of swine, and at 2, 6 and 8 weeks post-injection in a second cohort.

Prior to ERG recordings and/or toxin administration, swine were sedated with Telzol (2.0–8.8 mg/kg) and intubated. Anesthesia was induced and maintained with Isoflurane (~2%) in O₂ (Lalonde et al., 2006). A catheter was inserted in the ear vein and lactated Ringer's (Hospira, Lake Forest, IL; cat# 0409-7953-03: 130 mEq Sodium, 4 mEq Potassium, 3 mEq Calcium, 109 mEq Chloride, 28 mEq Lactate, in water; pH 6.6;) was administered throughout the procedure at a rate of 100 ml/hr. Body temperature was monitored every 30 min. with a rectal thermometer and maintained via a heated procedure table. Heart and respiration rates and oxygen saturation were recorded every 10 min. throughout the procedures and anesthesia adjusted to maintain a normal range for these physiological parameters. Pupils were dilated and accommodation relaxed with topical applications of 2.5% phenylephrine hydrochloride and 1% Tropicamide. The cornea was anesthetized with topical proparacaine hydrochloride and covered with contact lenses. Lid specula held the eyelids open.

2.2. Multifocal ERG

To assess the spatial distribution of cone-initiated function across the retina the mfERG was recorded. A DTL electrode was placed on the cornea, looped around the perimeter of the iris and covered with a hard contact lens wetted with artificial tears (Tears Again, OCuSoft, Inc.). Reference and ground electrodes were placed above the eye and behind the ear, respectively. A stay suture was placed in the conjunctiva to keep the eye in place and a retrobulbar block reduced eye drift. For this, a needle was inserted in the intermuscular septum approaching the optic canal and 2 ml of Carbocaine (2%) was injected at the orbital apex, located at the junction of the lateral and middle thirds of the inferior orbital rim. A second bolus of anesthetic (1ml) was deposited while withdrawing the needle. Artificial tears were applied every 2 – 3 minutes to keep the cornea and electrode moist. Similar to previously published methods (Kyhne et al., 2008; Moren et al., 2010) a VERIS System, (Electrodiagnostic, Inc.) was used to record the mfERG. This includes a fundus camera that produces a hexagonal stimulus pattern and simultaneously allows real-time viewing of the pattern on the retina providing feedback of stimulus location relative to the optic disc and retinal blood vessels. A screen-shot image of the fundus was taken before the recording and the optic disc and vessels were used as landmarks to ensure stability of the stimulus and to position the stimulus for recordings at all subsequent assessments after toxin treatment. The average and real time waveforms and the position of the fundus were monitored throughout the recording. The stimulus pattern was positioned so that the optic disc was located in the lower right or left corner and the visual streak in the center of the fundus image. The rotation and position of the streak was determined by the rotation and position of the optic disc, which is oblong in swine. The stimulus covered the entire horizontal extent of the visual streak, which begins 3.6 deg. visual angle above the superior edge of the optic disc and continues 7.2 deg superiorly. mfERG stimuli consisted of 241 elements that subtended 1.7 deg. visual angle and were presented for 9 minutes. We used an un-scaled stimulus pattern with identically sized hexagons across the display because the spatial density profile for swine photoreceptors is not the same as humans.

We recorded the mfERG response sequentially from both eyes to produce a topographic map of the focal retinal response over an area subtending 50 deg of visual angle. The mfERG response amplitude was calculated by averaging the N1-P1 amplitude of each mfERG waveform over the visual streak. To locate the visual streak, raw waveforms were overlaid on the screen shot of the swine fundus using the VERIS software. The waveforms in the visual streak were then grouped and all within the streak were averaged.

2.3. Full-field flash ERG

The fFERG was measured bilaterally using JET electrodes placed on the cornea with methylcellulose (2%; Gonak, Akorn). A ground electrode was placed behind the ear and a reference electrode was on the midline of the forehead. The fFERG was recorded using a UTAS ERG system with a BigShot Ganzfeld (LKC, Technologies, Inc.) stimulator that allowed the swine's head to be placed inside of the ganzfeld bowl. After 30 mins of dark-adaptation the dark-adapted fFERG was recorded to strobe flashes of 0.003, 0.03, and 0.3, 3, and 20 cd s m⁻², referenced to the ISCEV standard flash of 3 cd s m⁻² (Marmor et al., 2009). Dark-adapted responses to ≥ 30 trials were recorded at each flash luminance with an interstimulus interval of 1 s. The animal was then light-adapted with a 20 cd/m² background for 10 min and a series of fFERG responses were recorded at flash luminances of 3, 20 and 140 cd s m⁻². Post hoc analysis of the fFERG responses showed that the dimmest flash, 0.003 cd s m⁻², was frequently below or very near threshold and could not be used to reliably elicit a measurable ERG b-wave. These data were excluded from analysis. For all fFERG responses, the a-wave amplitude was measured from baseline to trough of the waveform. The b-wave amplitude was measured from the trough of the a-wave to the b-wave peak. When no a-wave was present, or when the a- and b-wave were evaluated separately (Figure 1B), the b-wave was computed from baseline to b-wave peak. Implicit time was measured from stimulus onset to a-wave trough or b-wave peak.

2.4. Clinical Examination

The anterior and posterior segments of the eye were examined using a portable slit lamp and indirect ophthalmoscope. Non-invasive imaging of the fundus was obtained with color photography and fluorescein angiography. We assessed and reassessed the health of the swine eyes using these clinical exams before and after toxin administration.

2.5. Toxin administration

IAA and NaIO₃ were dissolved in normal saline to final concentrations that varied from 5 – 30 mg/kg (IAA) and 10–110 mg/kg (NaIO₃) and pH adjusted (7.2–7.4). After baseline ERG function was assessed, swine were administered either IAA or NaIO₃ intravenously via the catheter placed in the ear vein. The toxin was flushed with Ringer's (3 ml) to ensure that the complete dose was administered. 3 swine administered 110 mg/kg NaIO₃ and 2 swine administered 30 mg/kg IAA died or were euthanized due to severe toxic reaction to these drugs.

2.6. Histological evaluation of toxin administration

At a variety of post-injection times (2 – 6 weeks) and after the last ERG assessments, swine were euthanized with Beuthanasia (1ml/5kg) administered through the ear vein catheter; their eyes were enucleated, their retinas fixed and prepared for histological evaluation at the light microscopic level. All histological techniques have been previously described in detail (Liang et al., 2008; Scott et al., 2011) and are described here briefly. Eyes were fixed by immersion in 2% paraformaldehyde/2% glutaraldehyde in phosphate (PO₄) buffer (0.1M, 7.4 pH) at 4°C for ≥ 48 hours. The anterior segment, lens, and vitreous were removed and the posterior eyecups stored in the same fixative at 4°C for ≥ 1 week. A strip of retina was cut that was approximately 3 mm wide and extended from the dorsal to the ventral margin of the eyecup. The strip was bisected at the optic disc and the superior piece and the ventral piece were bisected again. Each of the four pieces was notched on its dorsal edge to preserve orientation. Pieces were dehydrated, infiltrated, and embedded in JB-4 Plus resin (Ted Pella, Redding, CA). Tissue was oriented and cut to produce vertical sections along the dorsal to ventral axis. Sections 4 μ m thick were cut on a rotary microtome (Shandon Lipshaw, Pittsburgh, PA), mounted on slides, dried, and stained with 1% Cresyl violet. Sections were

examined on an 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.

The effect of toxin treatment on the retina was evaluated by counting the number of rows of nuclei in vertical sections of the outer (ONL) and inner nuclear layer (INL) in four locations from each retina as described in (Liang et al., 2008; Scott et al., 2011). All quantitative measures were performed with the experimenter blinded to treatment condition. Briefly, the locations evaluated were dorsal periphery and visual streak (8 mm and 2 mm dorsal to the superior margin of the optic disc) and, ventral and ventral periphery (2 mm and 8mm ventral to the inferior margin of the optic disc). Counts of rows of cells in each of the nuclear layers (Outer and Inner) were made at each location from 5 serial sections and 10 adjacent counts per section. Means from the five sections were calculated at each location in each retina. Then an overall mean was computed for each location using each retina as a single observation.

2.7. Statistical analyses

Comparisons were made using two-way ANOVAs, followed by Bonferroni post hoc tests for comparisons between selected groups. A p value of ≤ 0.05 was used to define significant differences between groups.

3. Results

3.1. Intravenous administration of sodium iodate is lethal at concentrations that have no effect on retinal function

Unlike our previous experience using NaIO_3 in other species (rabbits, rats and mice; (Franco et al., 2009), (Li et al., 2006), (Enzmann et al., 2006), (Enzmann et al., 2003); respectively), we were unable to find a non-lethal concentration in the swine that was toxic to retinal pigment epithelial (RPE) cells. NaIO_3 administered intravenously at concentrations from 10 to 90 mg/kg produced no change in fundus or retinal blood vessel morphology, photoreceptor number or ERG response. In two swine the concentration was increased to 110 mg/kg. They experienced severe respiratory distress, gastrointestinal bleeding and extreme lethargy and these animals were euthanized.

3.2. Intravenous administration of iodoacetic acid produces a concentration-dependent decrease in retinal function

Intravenous administration of IAA at concentrations between 5 and 12 mg/kg produced a concentration-dependent decrease in both the a- and b-wave components of the ffERG (Figure 1 A, B) and in the mfERG (Figure 1 C, D) response. We treated one swine with a concentration of 20 mg/kg and found that at 2 weeks post-IAA there was no rod or cone-driven ERG response. As a consequence, this IAA concentration was not pursued. We used a flash luminance of $0.003 \text{ cd s m}^{-2}$, but it frequently did not elicit a response in either control or treated retina and was not used in our comparisons. Experimental swine were treated with IAA concentrations from 5 to 12 mg/kg and evaluated at two weeks post-IAA. Controls were age-matched. Figure 1A compares representative dark- and light-adapted ffERG responses in swine administered IAA 7.5 and 12 mg/kg at two weeks post-injection compared to control. Figure 1B plots the mean a- (baseline to trough) and b-wave (baseline to peak) responses for control and IAA treated swine across flash luminance.

Under dark-adapted conditions (Figure 1B left), a concentration of 5mg/kg did not affect retinal function at any flash luminance. In contrast, concentrations between 7.5 – 12 mg/kg

produced a progressive decline in mean b-wave amplitude ($p < 0.0001$) across flash luminance between 0.003 and 20cd s m⁻². At concentrations of 7.5 – 12 mg/kg, all mean b-wave responses across all flash luminances were significantly diminished compared to control ($p < 0.0001$). Under dark-adapted conditions when the flash was bright, an a-wave was evident ($\geq 0.3\text{cd s m}^{-2}$), its mean amplitude also showed a progressive decline across the three IAA concentrations ($p < 0.0001$). The dramatic decrease in mean a-wave amplitude compared to control ($p < 0.0001$) is consistent with an absence of rod function. In sum, all IAA concentrations $\geq 7.5\text{mg/kg}$ produce a decrement in ffERG a- and b-wave amplitudes compared to control ($p = 0.001$). Under our light-adapted conditions the ffERG mean a- and b-wave amplitudes in swine treated with 7.5mg/kg differed significantly from those treated with 12 mg/kg across flash luminance ($p = 0.001$). Responses in swine treated with 10mg/kg were similar to those of swine treated with 7.5 mg/kg.

Figure 1C shows representative light-adapted mfERG response maps for a control and for swine administered IAA 7.5 and 10 mg/kg at two weeks post-injection (unshaded region is the visual streak). Insets to the right show the mfERG mean response over the entire visual streak. Figure 1D plots the mean N1-P1 amplitudes for control mfERGs and swine administered 5 – 10 mg/kg. The mfERG also shows a concentration-dependent amplitude change. Similar to the ffERG, 5mg/kg IAA produced no discernable effect, whereas concentrations $\geq 7.5\text{ mg/kg}$ significantly reduced the mean N1-P1 amplitude compared to control (ANOVA, $p < 0.0001$).

We also noted a concentration dependence in the number of swine affected by IAA treatment using the ffERG. Table 2 shows that the percent of swine with significantly lower b-wave amplitudes compared to control increases with IAA concentration. The rest of our results include only swine significantly affected by IAA administration.

3.3. IAA induces concentration dependent morphological damage in the outer but not the inner nuclear layer

Figure 2A shows representative transverse retinal sections of eyes from control and IAA-treated swine (5 – 12.0 mg/kg) whose ERGs were evaluated at two weeks post-IAA. Sections represent 8 mm and 2 mm (within the visual streak) dorsal to the optic nerve. Consistent with our ERG results, a concentration of 20 mg/kg completely eliminated all ONL nuclei (data not shown). We found no difference between locations and as a consequence, Figure 2B plots the average number photoreceptor nuclei rows over all locations as a function of IAA concentration and time post-IAA. As a control for the plane of section, we evaluated and found that the average number of INL nuclei was consistent across IAA concentration and time. In addition to demonstrating that our plane of section was perpendicular to the retinal laminae, these results show that IAA selectively damages nuclei in the ONL. A concentration of 5 mg/kg had no effect on the number of ONL nuclei at 2 weeks post-injection, although a small but significant decrease was observed at 5 weeks post-injection. All concentrations $\geq 7.5\text{ mg/kg}$ reduced the number of ONL nuclei compared to control ($p < 0.0001$). The decrease in overall ONL nuclei was similar across IAA concentration. The absence of a dose-dependent decrease results from the survival of cone photoreceptors; when rod nuclei are counted they show a concentration dependent decrease Scott et al., (2011).

In addition to the changes in photoreceptor nuclei, we also observed a concentration dependent change in the fundus of swine administered $\geq 10\text{ mg/kg}$ IAA. Figure 3 shows representative fundus images of both eyes in one swine administered 10 mg/kg IAA prior to IAA and 5 weeks post-IAA. There is a subtle paling of the optic nerve head and a narrowing of the blood vessels. We found similar changes in 25% and 50% of swine administered 10

and 12 mg/kg, respectively. We did not observe blood vessel leakage in fluorescein angiograms in any swine at any concentration or evaluation time.

3.4. Glycemic Control Improves the ERG Signal

In some swine, glucose levels were monitored during ERG evaluations and we found that the majority were hypoglycemic (≤ 60 mg/dL). In four untreated swine, we adjusted glucose levels intravenously to keep glycemia levels within the normal range (60–140 mg/dL). We found that glycemic control significantly elevated the light-adapted ffERG and mfERG response (Figure 4B & C) amplitudes, but not the dark-adapted ffERG (Figure 4A).

3.5. IAA-induced decline in dark- and light-adapted ffERG is stable

We assessed the dark- and light-adapted ffERGs at 2 through 6 weeks post-IAA and compared swine administered 7.5 and 12 mg/kg. Figure 5 plots the ratio of the ERG response amplitude for each swine at each IAA post-injection time to its control amplitude prior to IAA administration. We estimated this ratio in controls, using responses recorded in the same swine at similar time intervals. Figures 5A and 5B plot the dark and light-adapted ffERG b-wave, respectively. For both 7.5 and 12 mg/kg, all post-injection evaluation times were significantly lower than control ($p < 0.0001$) and the reduction in the response is similar across time.

3.6. IAA administration spares light-adapted function relative to dark-adapted function

Figure 5C plots the percent change in the dark- vs light-adapted ffERG b-wave amplitudes of individual swine for both IAA concentrations across post-injection assessment times. All of the data points fall to the right of a line with a slope of 1 defining a function where rod and cone function would be similarly affected. These data show that all conditions produce a larger effect on the dark- relative to the light-adapted response and that the majority of swine administered 7.5 mg/kg show a reduced effect of IAA. The results are consistent with the idea that rod function is more susceptible to IAA administration compared to cone function.

mfERG response ratios for swine administered 7.5 mg/kg are plotted in (Figure 5D) and show a decline in cone function in the visual streak similar to the light-adapted ffERG. Namely, average N1-P1 amplitudes are lower than control at all time points tested (2 – 8 weeks post injection). It should be noted that we found a significant increase in the mfERG responses in swine administered 7.5 mg/kg between 2 and 5–6 weeks of age ($p < 0.01$).

4. Discussion

At sub-lethal concentrations, we found no effect of NaIO_3 on swine RPE two weeks post treatment. In contrast, NaIO_3 causes degeneration of the zonula occludens, basal membrane, swelling of organelles and finally patchy, necrotic loss of RPE in other mammals, including humans in this time frame (Mecklenburg & Schraermeyer, 2010; Enzmann et al., 2006). The specificity of NaIO_3 toxicity on the RPE is not well understood. There are several factors, studied in a variety of species other than swine that potentially contribute. NaIO_3 pools selectively in rabbit RPE cells (Grignolo, 1969). It chemically reacts with cuttlefish melanin leading to an increased rate of conversion of glycine to glyoxylate. This reaction is likely to lead to degradation of the melanosome membrane and the release of oxidized toxins into the RPE cell (Baich and Ziegler, 1992). Iodate inhibits the lysosomal enzyme acid phosphatase, which probably contributes to a reduction in RPE degradation of shed PR outer segments and a buildup of toxic products (Hayasaka et al., 1988). Other general increases in toxicity may be produced by inhibition of the metabolic enzymes triose phosphate dehydrogenase (rabbits, Ashburn et al., 1980) succinic dehydrogenase and lactate dehydrogenase (Birr,

1970). Although none of these are specific to RPE they may be the cause of the systemic toxic effects that we observe at 110 mg/kg.

Why swine RPE appears to be resistant to NaIO_3 within the time frame tested here is unknown. Swine and human RPE and choroidal capillaries appear morphologically similar (Beauchemin, 1974). One difference that has been noted is that swine RPE has an increased regenerative capacity after surgical debridement compared to the non-human primate. Specifically, hypopigmented, but fully differentiated swine RPE cells replace debrided areas within seven days (Del Priore et al., 1995) compared to 21 days in non-human primates (Valentino et al., 1995). Since we examined the effects of NaIO_3 at two weeks after treatment, it is possible that there is an initial insult that is followed by healing, which mitigated the outcome of our experiments. Regardless of the cause, NaIO_3 would not be a toxin of choice to create an inducible model of photoreceptor damage.

In contrast, retinal dysfunction and photoreceptor damage are rapidly induced in a concentration-dependent manner with IAA. Low IAA concentrations diminished both light- and dark-adapted ERG responses and higher concentrations completely eliminated the dark-adapted ERG and severely diminish the light-adapted ERG. These functional changes are established by two weeks post-administration and are stable through eight weeks. Morphological evaluation shows that damage is confined to photoreceptors.

We show that IAA can be effectively used to create a rapidly inducible and stable model of photoreceptor damage in swine. IAA concentrations between 7.5 and 20 mg/kg create moderate to severe damage to photoreceptors, which can be reliably evaluated using full-field and multifocal ERGs. IAA at 10 mg/kg creates more variable results and we conclude that 7.5 or 12 mg/kg are the appropriate concentrations for creating the most consistent concentration dependent models of photoreceptor damage. At all concentrations ≥ 7.5 mg/kg, the effects of IAA are evident at two weeks post-injection and are stable through 6 weeks.

Although a concentration of 5 mg/kg produces photoreceptor morphological damage, it is not detected functionally. This may indicate a lower limit of photoreceptor damage without consequences to visual function. Although there is some variability in the functional outcome at any single IAA dose, the model can be used successfully if our experimental protocol is followed. (1) Baseline functional evaluations should be performed in every animal to reduce variability in the evaluation of normal function across animals. (2) Individual animals should be tracked over most time points. (3) Comparisons should be based on a cohort of at least four eyes within a concentration. (4) Because lower IAA concentrations yield some swine unaffected by IAA administration, more swine should be included in this group. (5) Glycemic levels should be monitored and adjusted as needed. With these methodological limitations in mind, the damage created by IAA is rapid, stable and concentration dependent and can be evaluated non-invasively, which significantly adds to the value of this toxin-induced approach.

Our assessments show that IAA has a concentration dependent effect on all photoreceptors, but preferentially impairs rod function relative to cone function regardless of concentration. Our results also show that both dark- and light-adapted ffERG changes are stable over time. This conclusion differs from Wang et al. (2011), whose results showed an improvement in ffERG cone function between two and five weeks in swine administered both 7.5 and 12 mg/kg IAA. Given our findings of variability in IAA-induced effects and their small sample size, especially at 7.5 mg/kg (e.g., two eyes), we believe that our conclusion is more representative of the model. We have observed a small, but significant recovery of cone function using the mfERG between 2 and 5–6 weeks post IAA, but only at 7.5 mg/kg. Such

an increase may reflect an initial, transient damage to synaptic connections between cones and cone bipolar cells in the IPL, which subsequently heal (Scott et al., 2011) and thereafter is stable.

We and others (Wang et al., 2011; Scott et al., 2011; Liang et al., 2008) show that IAA directs damage to swine PRs, having little or no effect on other neuroretinal cells. The mechanism of IAA action and specificity likely resides in its inhibition of glycolysis. IAA inhibits glyceraldehyde 3-phosphate dehydrogenase (Winkler et al., 2003) which decreases metabolic energy production, and combined with the high metabolic rate of PRs, death occurs within 2–3 days (Orzalesi et al., 1970). IAA also induces ischemic damage in other highly metabolic tissues such as the GI tract (Corbett & Lees, 1997) and vasculature smooth muscle (Barron et al., 1989).

We show that the morphological damage created by IAA is similar across a broad retinal area (between 2 and 8mm dorsal and ventral to the visual streak). This conclusion also was reached in the more extensive morphological examination of this IAA swine model by Scott et al., (2011). Noell (1953) in rhesus monkey and Wang et al. (2011) in swine concluded that there is a central to peripheral gradient of IAA induced photoreceptor damage when central and far peripheral retina are compared (1 mm from the ora serrata). It is possible that the spared photoreceptors observed in these reports are malformed cones found at the retinal margin in human (Williams, 1991) and monkey retina (Chen et al., 2000). These marginal cones lack outer segments and if they are incapable of phototransduction, this could render them resistant to IAA. This issue remains unresolved, as their presence in normal swine has not been reported. Regardless of whether IAA induces a gradient of effect in the far periphery (> 8mm radius from the optic nerve head), it is unlikely these spared photoreceptors contribute significantly to the light-adapted ffERG and they cannot contribute to the mfERG which is confined to the central visual streak.

We observed that NaIO₃ is not a useful toxin in swine, although we and others have used it successfully in rodents and in other species (Franco et al., 2009; Li et al., 2006; Enzmann et al., 2006; Enzmann et al., 2003). In rodents, a NaIO₃ concentration of 15 – 30 mg/kg creates moderate to severe RPE damage with subsequent photoreceptor loss. We found that concentrations up to 90 mg/kg have no effect on the swine visual function and higher concentrations are lethal. IAA has previously been reported to have variable interspecies effects (Rabbit, Liang et al., 2008 & Orzalesi et al., 1970; Ground squirrel, Farber et al., 1983; Rat, Graymore & Tansley, 1959; Cat, monkey, & rabbit; Noell, 1951 & 1953). In swine, concentrations of IAA that induce reliable photoreceptor damage create variable changes in rabbit and concentrations that were much higher than the lethal dose in swine were necessary to induce PR damage in ground squirrel, rat, and monkey. Consistent across all studies, cones are more resistant to IAA damage than rod photoreceptors.

IAA-induced functional changes in swine mimic functional changes in patients with RP, where the initial complaint is reduced night vision (rod function) and in later stages diminished day vision (cone function). Thus, moderate concentrations of IAA can mimic more initial stages of RP and ≥12 mg/kg, late disease states. Clinical examination of the swine fundus shows that higher concentrations of IAA induce optic nerve pallor and thinning of retinal vessels, other hallmarks of late disease progression. We never observed bone spicules and fluorescence angiography showed that blood vessels are intact. Not surprisingly, inner retinal remodeling seen in late stages of RP in human and genetic models (Marc et al., 2003) was not evident within our 5 week histological analysis. We do not believe that the IAA model of photoreceptor damage is useful for these analyses, because the time frame required could be very long and the swine will become very large.

Two publications reporting the effects of IAA on swine retinal morphology have preceded this report (Scott et al., 2011 & Wang et al., 2011). Our results extend their findings and present the first extensive characterization of the concentration dependent changes in retinal function induced by IAA in swine. While genetic models of RP are most similar to the human disease, our IAA swine model has the advantage of rapid induction and stability. In addition, it represents a reliable approach to create a variety of functional phenotypes that emulate the progression of retinal dysfunction in human RP. Induction of damage can be accomplished in young swine and the rapid effects keep the swine a manageable size. This opens the use of this large animal model of photoreceptor damage to many laboratories exploring treatment strategies to replace lost photoreceptors and restore visual function.

Highlights

IAA treatment produces dose-dependent damage of photoreceptors in swine

IAA treatment produces preferential damage in rods compared to cones

In swine, IAA produces a rapidly inducible large animal model of photoreceptor damage that is useful for therapeutic research

Sodium Iodate has no effect on RPE or photoreceptor morphology or retinal function in swine at sub-lethal doses

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Figure 1. IAA reduces dark- and light-adapted retinal function in a concentration dependent manner at two weeks post-injection

(A) Representative dark-adapted (top) and light-adapted (bottom) fERG waveforms from swine administered 7.5 (solid grey line) or 12 (black line) mg/kg IAA compared to an untreated control (dashed grey line). Although both IAA concentrations reduce the ERG response, 12 mg/kg produces a more severe deficit. (B) Mean fERG a- (plotted below 0) and b-wave (plotted above 0) responses recorded at two weeks post IAA are plotted across flash luminance for every concentration administered. Only 5 mg/kg is similar to control. All other concentrations produce a dose-dependent reduction in both the a- and b-waves relative to control. (C) Representative mfERG waveforms recorded at two weeks post-

injection from two different swine administered 7.5 or 10 mg/kg IAA and an untreated control. The reduction in the mfERG response also is concentration dependent. The inset shows the response averaged over the visual streak (unshaded area). The average waveform in swine administered 5 mg/kg is similar to control. (D) Mean mfERG N1 - P1 response amplitude is plotted as a function of IAA concentration. All concentrations >5 mg/kg are significantly reduced compared to control. (** $p < 0.01$).

Figure 2. IAA reduces photoreceptor nuclei in a concentration dependent fashion consistent with changes in retinal function

(A) Representative light micrographs of cresyl violet stained retinal transverse sections from a control swine and swine administered IAA from 5 – 12 mg/kg sacrificed at 2 weeks post-injection. Images in the top and bottom rows are from 8 and 2 mm dorsal to the optic disc, respectively. Scale bar = 50 μ m (B) The mean number of rows of nuclei were counted in both areas and the results were similar and have been pooled. The numbers of rows were evaluated at 2 and 5 weeks post-injection and at IAA concentrations between 5 and 12 mg/kg. The data show a decrease in the mean number of rows comprised of photoreceptor nuclei (ONL) at all concentrations and at both time points compared to control, except 5mg/

kg. There are no changes in nuclear counts in the inner nuclear layer (INL). The effect of IAA on photoreceptor nuclei does not change between 2 and 5 weeks post-IAA injection, except for 5 mg/kg where 5 weeks shows a significant decrease (* $p < 0.05$).

Pig 2995

Pre-injection Control

5 weeks Post-injection (10 mg/kg)

Figure 3. IAA administration alters the fundus

Representative fundus photographs from one swine prior to IAA administration (top) and 5 weeks post-IAA 10 mg/kg IAA. IAA administration causes optic disc pallor and thinning of the retinal vessels in swine administered ≥ 10 mg/kg IAA. (Stars indicate equivalent retinal areas.)

Figure 4. Glycemic control enhances the light-adapted ERG response

The ffERG (A & B) and mfERG (C) responses in control swine (Control) with and without glycemic levels adjusted to normal range. Control of hypoglycemia has no effect on the dark-adapted b-wave amplitude (A) but is related to increased ff ERG b-wave amplitudes (B) under light-adapted conditions and to increased mfERG N1-P1 amplitudes (C). (** $p < 0.01$; *** $p < 0.001$)

Figure 5. IAA affects dark-adapted function more than light-adapted function

Dark-(0.03 cd s m⁻² flash) and light-adapted (20 cd s m⁻² flash) ERG b-waves and mfERG N1-P1 responses from swine administered either 7.5 or 12 mg/kg IAA are normalized to their baseline response and plotted as a function of time post-injection. To estimate the normalized response variability in controls, we used responses from untreated swine recorded at similar time intervals (e.g., 2 and 5–6 weeks) after an initial ERG evaluation. The normalized responses were similar across controls at all time point and their data are pooled. (A) Dark-adapted ffERG responses are stable over time and there is a concentration dependent decrease in visual function between 7.5 and 12 mg/kg. (B) Light-adapted ffERG responses also are stable over time and show a concentration dependent decrease. (C) To

compare changes in rod vs cone function, the dark-adapted response from each swine at 2 and 5–6 weeks post-IAA (A) is plotted against its light-adapted response (B). All of the data points fall to the right of a line with a slope of 1 defining a function where rod and cone function would be similarly affected. This indicates that the dark-adapted response is consistently reduced more than the light-adapted response. (D) Using the same normalization as in A & B, mfERG responses are shown for swine administered 7.5 mg/kg IAA at all post-injection times. A significant increase in the response was found in swine administered 7.5 mg/kg between 2 and 5–6 week post-injection and responses were reduced from control at all post-injection times.

Table 1

Numbers of treated eyes for each parameter and age

IAA Concentration (mg/kg)	Weeks P.I.	Numbers of Eyes		
		ffERG	mfERG	Histology
5	2	6	4	3
	5-6	4	4	4
7.5	2	22	14	4
	5-6	12	12	6
	8	0	8	0
10	2	20	6	6
	5-6	10	4	4
12	2	21	0	6
	5-6	11	0	0

Table 2

Percent of IAA treated swine functionally affected

IAA Concentration (mg/kg)	N	Percent Affected
7.5	22	55
10.0	20	90
12.0	21	100

Table 3

ONL nuclear counts at all retinal locations and IAA concentrations greater than 5 mg/kg are similar and reduced from 5 mg/kg IAA and baseline.

Concentration (mg/kg)	Dorsal 8mm	2mm	Ventral 2mm	8mm
Baseline	5.0±0.3	4.6±0.2	5.2±0.3	4.2±0.3
5.0	3.8±0.4	4.1±0.3	4.1±0.3	3.9±0.2
7.5	1.6±0.4	1.7±0.5	1.6±0.4	1.8±0.5
10.0	1.9±0.3	1.9±0.3	1.8±0.4	1.8±0.4
12.0	1.0±0.2	0.9±0.3	1.2±0.1	1.1±0.01