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Timothy Olsen, Emory University
Hans Grossniklaus, Emory University
J Tran, Emory Eye Center
C Craven, Emory Eye Center
K Wabner, Emory Eye Center
J Schmit, Emory Eye Center
B Matter, University of Colorado Health Sciences Center
U Kompella, University of Colorado Health Sciences Center

Journal Title: Investigative Ophthalmology & Visual Science
Volume: Volume 58, Number 9
Publisher: (publisher) | 2017-07-01, Pages 3732-3740
Type of Work: Article
Publisher DOI: 10.1167/iovs.16-21230
Permanent URL: https://pid.emory.edu/ark:/25593/s49vf

Final published version: http://dx.doi.org/10.1167/iovs.16-21230

Accessed August 25, 2017 7:10 AM EDT
A Pharmacodynamic Analysis of Choroidal Neovascularization in a Porcine Model Using Three Targeted Drugs

Jeffrey Tran,1 Caroline Craven,1 Kathy Wabner,1,2 Jenn Schmit,1,2 Brock Matter,3 Uday Kompella,3 Hans E. Grossniklaus,1 and Timothy W. Olsen1

1Emory Eye Center, Emory University Department of Ophthalmology, Atlanta, Georgia, United States
2Department of Civil, Environmental, and Geo-Engineering, University of Colorado, Colorado, United States
3Department of Pharmaceutical Sciences, University of Colorado School of Pharmacy, Aurora, Colorado, United States

PURPOSE. To compare the efficacy of microneedle-delivered suprachoroidal (SC) pazopanib to intravitreal (Ivit) delivery of pazopanib, bevacizumab, or a fusion protein hI-con1 versus vehicle controls on choroidal neovascularization (CNV) growth in a pig model.

METHODS. Forty-one pigs were injected on the day of CNV induction (hI-con1 on postinduction day 14) with either 2.5 mg Ivit bevacizumab (n = 9), 1 mg Ivit pazopanib (n = 9), 300 Ivit µg hI-con1 (n = 4), or 1 mg SC pazopanib (n = 9), vs. 10 vehicle controls (3 SC + 7 Ivit = 10). Pigs were euthanized at week 2 (11), 3 (9), 4 (11), and 8 (11), and eyes were fixed for histology. The size of the CNV was determined from histology, and CNV height was the primary outcome measure. Immunostaining for cytotoxic T-cells was performed in the hI-con1 study.

RESULTS. In 39 of 41 (95%) eyes, type 2 CNV lesions were identified. One CNV lesion was lost during dissection. One animal was euthanized due to surgical complications. For mean CNV size comparisons, Ivit pazopanib had smaller mean height measurements (90 ± 20 µm) versus controls (180 ± 20 µm; P = 0.009), and Ivit pazopanib had smaller maximum CNV height (173 ± 43 µm) compared to SC pazopanib (478 ± 105 µm; P = 0.018). The mean lesion size in hI-con1-treated animals trended smaller than in controls (P = 0.11). Immunostaining did not detect cytotoxic T-cells.

CONCLUSIONS. Intravitreal pazopanib and to a lesser extent hI-con1 reduced the size of CNV lesions. The pig model has nearly a 100% rate of type 2 CNV induction and is a reliable preclinical model with pharmacodynamics similar to humans.

Keywords: choroidal neovascularization, intravitreal drug delivery, porcine
largely upon the underlying disease state, the agent being delivered, the target tissue, and the local pharmacokinetics. For example, SC drug delivery bypasses transscleral diffusion and may selectively target the choroidal tissue or the RPE.30,21 Gilger et al.22 demonstrated that a lower dose of corticosteroid delivered through the SCs was as effective as a higher Ivit dose at reducing inflammation in a porcine model.

In an earlier study, we described a novel system for SC drug delivery using a flexible cannula with a fiberoptic illumination system that localizes delivery in the pig model.20 This system was used to access the SCS and demonstrated sustained-release pharmacokinetics of triamcinolone with very low drug levels in the systemic circulation. However, studies in the pig model of SC bevacizumab delivery demonstrated rapid clearance from the SCS when compared to the Ivit route.23 Hollow microneedles represent another method to gain access to the SCS.24 Particles of different size can be delivered to the SCS by changing the microneedle length and the infusion pressure.25

Pazopanib is a drug that has been reported to inhibit both VEGF pathways and the platelet-derived growth factor receptor-B (PDGFR-B) in mice, thus could represent an excellent candidate antiangiogenic agent that also has a theoretic potential to induce vessel regression.26–28 Due to the relative solubility and molecular size, pazopanib likely has pharmacokinetics similar to triamcinolone acetonide and fluocinolone acetonide. Pazopanib is a small molecule (MWt = 437.5) with a very low solubility and may have a favorable pharmacokinetics similar to triamcinolone acetonide and fluocinolone acetonide. Pazopanib is a small molecule (MWt = 437.5) with a very low solubility and may have a favorable dissolution-controlled, sustained pharmacokinetic profile from both the vitreous and SCS. Oral pazopanib has been shown to support sustained delivery of pazopanib in human subjects did not demonstrate a meaningful therapeutic clinical result in the treatment of CNV.29,30

The hI-con1 is a human immune conjugate fusion protein that contains a protein composed of a mutated factor VII conjugated to the Fc domain of human IgG1 immunoglobulin that binds to tissue factor (TF) and has been studied in tumor biology.31 Currently in phase 1/2 clinical trials, hI-con1 is a chimeric antibody with a high affinity for TF and activates a potent immune response through recruitment of natural killer (NK) cells and complement leading to cytosis of CNV in mouse models.32–35 TF is expressed on endothelial cells and is located on CNV but not on normal vessels.34 A study published by Bora et al.36 demonstrated that mice injected with either the hI-con1 protein or an adenoviral vector expressing this protein significantly reduced CNV formation in a laser-induced mouse model.26 However, the topical delivery of pazopanib in human subjects did not demonstrate a meaningful therapeutic clinical result in the treatment of CNV.29,30

A total of 41 pigs were enrolled and 40 completed the study (one was euthanized for surgical complications). Pigs were treated on the day of induction of choroidal neovascularization. The hI-con1 first day of treatment was postoperative day 14. All groups were compared to vehicle-treated controls.

<table>
<thead>
<tr>
<th>Study</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
<th>Week 8</th>
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<tr>
<td>Pazopanib SC</td>
<td>N = 9</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Pazopanib Ivit</td>
<td>N = 8</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Bevacizumab Ivit</td>
<td>N = 9</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>hI-con1 Ivit</td>
<td>N = 4</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Controls</td>
<td>N = 10</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>

**TABLE 1. Study Design**

Induction of CNV and Drug Delivery

The right eyes of 41 pigs were operated, and the groups are summarized in Table 1. After general sedation, eyes were dilated using a combination of atropine (1%) and phenylephrine (2.5%). Animals were placed on nasal oxygen (4 L/min) and a warming blanket (37°C) and were carefully monitored with a pulse oximeter and rectal temperature probe throughout the procedure. Body temperature was maintained at 37°C. The pig head was positioned on a conforming pillow to optimize surgical exposure and stability.

CNV was induced using minimal modification of a published procedure in the pig model described by Lassota et al.4 and Kiilgaard et al.6 The surgical steps are summarized in Figures 1A to 1E. A 23-gauge pars plana vitrectomy and lensectomy was performed followed by elevation of the posterior hyaloid to a region just anterior to the vascular arcades, followed by a core vitrectomy. The retina was detached in the area centralis (macular equivalent in the pig) using an infusion of balanced salt solution (BSS; Alcon Laboratories, Fort Worth, TX, USA) through a 27-gauge cannula.
Black is yellow pazopanib suspension remaining at the needle hub (smooth, easy plunger advancement ensures that delivery is into the SCS tip of the microneedle to reach the suprachoroidal space (SCS) while posterior to the limbus. There is sufficient scleral indentation for the perpendicular to the sclera with the entry site measured 6 mm suspension being injected into the suprachoroidal space of an air-filled, SCS.

FIGURE 2. Surgical photograph of the microneedle with a pazopanib suspension being injected into the suprachoroidal space of an air-filled, aphakic pig eye following induction of CNV. The syringe is oriented perpendicular to the sclera with the entry site measured 6 mm posterior to the limbus. There is sufficient scleral indentation for the tip of the microneedle to reach the suprachoroidal space (SCS) while smooth, easy plunger advancement ensures that delivery is into the SCS (resistance indicates intrastromal scleral injection). Note also that there is yellow pazopanib suspension remaining at the needle hub (black arrow). The majority of the suspension was visibly passing into the SCS.

connected by tubing to the variable infusion pressure-controlled viscous fluid injection system of the vitrectomy machine (Accurus, Alcon Laboratories). Intraocular pressure was increased to approximately 90 mm Hg; Bruch’s membrane was punctured using a 23-gauge micro-vitreoretinal (MVR) blade at approximately five to seven sites to approximate the size of the optic nerve. Thus, all CNV lesions were created with a similar two-dimensional area. Next, a soft-tip cannula was extended into the subretinal space, and the RPE cells along with any associated hemorrhage were gently aspirated from around each Bruch’s puncture site. The intraocular pressure was slowly returned to approximately 35 mm Hg. Small hemorrhages at the puncture site were common. An air-fluid exchange was completed, and the sclerotomies, conjunctiva, and sclera were sutured using 7-0 Vicryl suture (Ethicon, Cincinnati, OH, USA). All attempts were made to maintain an intraocular pressure of at least 20 mm Hg during globe closure in order to minimize the risk of subretinal hemorrhage and the secondary influences that such hemorrhages induce. Drug groups were randomly assigned and revealed to the surgeon only after CNV induction in order to minimize possible bias.

One pig was euthanized at the time of surgery due to a severe intraoperative hemorrhagic complication. Thus, on the day of surgery, pigs (n = 40) were each given a 100-µm injection of either 2.5 mg Ivit bevacizumab (n = 9), 1 mg Ivit pazopanib (n = 8), 1 mg SC pazopanib (n = 9), or a vehicle control (n = 10). 10 control animals had either a pars plana Ivit or transscleral SC injection of vehicle control using either a 30-gauge needle (Ivit) or microneedle (SC; n = 7 and 3, respectively, for 10 total controls; Table 1).

Pazopanib was purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA), and the material within the container was sterilized using gamma-irradiation. Pazopanib was suspended in sterile BSS and a viscoelastic material (ProVisc, Alcon Laboratories) to achieve a final concentration of 2 mg/mL. Pazopanib particles were crushed with a sterile 18-gauge needle in a microfuge container, essentially a mortar and pestle mechanical approach, and vortex mixed well over 5 minutes. Then, the suspension was subjected to forceful and aggressive turbulence using a back-and-forth mixing through a three-way stopcock connecting two dual syringes. Particle size was not quantified.

A 1100-µm (length) 30-gauge microneedle (donated by Clearside Biomedical, Inc., Alpharetta, GA, USA) was used to deliver either 1 mg pazopanib or vehicle control into the SCS. During the 100-µL SC injections (Fig. 2), the needle was carefully directed perpendicularly (90°) to the sclera 6 mm posterior to the limbus. Low resistance on the syringe plunger confirmed successful delivery into the SCS.

FIGURE 3. Gross dissection. (A) Transection of the anterior segment (black arrowhead at the edge of cut sclera) exposing the detached retina (outlined with a yellow dashed circle), old hemorrhage (red in color), and whitish proliferative vitreoretinopathy. (B) After dissection of the neurosensory retina, the bare retinal pigment epithelium, the optic nerve (right), and the choroidal neovascular lesion (white dashed circle) are visible.

Factor VII Conjugate Fusion Protein: hl-con1

We used the same pig model for CNV induction and identical methodology as described above. Importantly, the initial and only injection was delivered on postoperative day 14. The Ivit injection of either 500 µg/0.1 mL hl-con1 (n = 4) or vehicle (control, n = 4) was performed using a 30-gauge needle. The delayed injection was performed in order to compare CNV lesion size of treated eyes with controls and assess for possible CNV regression. Animals were examined with color fundus photos and fluorescein angiograms and then enucleated on postoperative week 3 (n = 4, 2 treated and 2 control) and week 4 (n = 4, 2 treated and 2 control). The eyes were studied with histopathology and fluorescence immunohistochemistry using anti-CD31, anti-CD56, anti-CD105, anti-CD3, and anti-CD68 antibodies directed to NK cells, T-cells, and macrophages.

All animals were euthanized using a phenobarbitol solution (390 mg/mL). Right eyes were enucleated on postoperative week 2, 3, 4, and 8 following CNV induction and immediate treatment (Table 1). Eyes were immediately fixed in 10% formalin and processed for histopathology. The anterior segments were removed from each eye using a sharp blade, exposing the posterior segments (Fig. 3). The detached retina was carefully dissected with forceps and scissors to aid in visualizing the CNV induction site. The posterior pole was photographed. Eyes were then embedded into paraffin blocks and sectioned in a pupil–optic nerve (PO) manner to include the area centrals (macular equivalent) and optic nerve. Serial 7-µm step sections were prepared, numbered sequentially, and mounted on glass slides. For each eye, 50 to 250 slides were available. In the hl-con1 study, sections were also stained with antibody to CD31, CD56, CD105, CD3, and CD68 following a confirmatory positive control for T-cells, NK cells, and macrophages using sections from spleen tissue.

CNV Evaluation

During the first eye gross dissection, the CNV complex was inadvertently removed with the detached retina. Subsequently, we carefully separated the CNV and found that it was present in all of the remaining 40 globes. The CNV lesion size was assessed and quantified using previously described histopathologic measurements and mapping techniques and was done.
in a masked fashion.\textsuperscript{37} Lesion height, as described in the natural history of CNV in the pig model by Lassota et al.,\textsuperscript{3} was determined to be the primary measure of effect, and it should also be noted that this lesion height changed very little in measures taken more than 14 days post induction.\textsuperscript{3} We rely on lesion height at multiple postoperative time intervals as the key variable to assess treatment effect in this model (all enucleation time-point data within each group are pooled for the final analysis).

**Histopathology Mapping**

Slides were examined using light microscopy (Olympus BH2, Tokyo, Japan) equipped with a standard measuring reticule calibrated to the $\times 2$, $\times 4$, $\times 10$, and $\times 40$ objectives for reference lengths. Specimens were examined to localize the optic nerve, retinal detachment, and any visible breaks in Bruch’s membranes. Photographs were taken using the Olympus DP12 camera.

To assess the size and extent of the induced CNV, a reticule was used to measure cross-sectional CNV width ($X$). Most importantly, CNV height ($Z$) was measured at the thickest section. To capture the CNV length ($Y$), we recorded the slide numbers and number of consecutive serial sections. In the hI-con1 study, greatest dimensional pixel area from the histology images was quantified, three measures per globe, using a software image analysis program (Adobe Illustrator CS6v16; San Jose, CA, USA).

**Systemic Pazopanib Drug Levels**

The serum levels of pazopanib in each pig were measured from 5 mL blood obtained at the time of euthanasia. Samples were transferred on ice and centrifuged for 15 minutes at 2400g at 4°C in a refrigerated centrifuge (Beckman Coulter GS-6R with the GH3.8 rotor; Indianapolis, IN, USA). Next, 1.0-mL aliquots were drawn from each and frozen ($-80°C$).

**Statistical Analysis**

A 1-way ANOVA and unpaired Student’s $t$-test was used to assess for meaningful CNV lesion size from the observations and measurements (StataCorp LP, College Station, TX, USA). A $P$ value of $\leq 0.05$ was used to identify statistically significant groups. We constructed a mixed linear model of the measurements as a function of treatment with a random intercept per animal.

**RESULTS**

**CNV Induction**

In 39 of 41 pigs (95%), we confirmed the presence of type 2 CNV with histopathology (Fig. 4). We suspect that CNV was present in all 40 eyes, yet we cannot confirm one case. The other animal had a severe intraoperative massive hemorrhagic retinal detachment and was euthanized prior to recovery. Most animals had postoperative lid edema, mild corneal edema, and conjunctival injection that healed within 1 week. One animal in the Ivit pazopanib group required systemic antibiotics for a postoperative fever. Other complications are summarized in Table 2. In addition, subretinal hemorrhages were common in this model, yet difficult to quantify. Postoperatively, we tried to

**Table 2. Complications Observed During Gross Pathologic Review**

<table>
<thead>
<tr>
<th>Retinal Detachment</th>
<th>Vitreous Hemorrhage</th>
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<tbody>
<tr>
<td>Pazopanib SC</td>
<td>8</td>
</tr>
<tr>
<td>Pazopanib Ivv</td>
<td>5</td>
</tr>
<tr>
<td>Bevacizumab Ivv</td>
<td>9</td>
</tr>
<tr>
<td>Controls</td>
<td>4</td>
</tr>
</tbody>
</table>

Partial, funnel, and full retinal detachments were observed and are shown in the retinal detachment column.

**FIGURE 4.** Histopathology of type 2 choroidal neovascularization (CNV). (A) A low-power, $\times 10$ hematoxylin and eosin (H&E) micrograph with a partially detached retina and an underlying type 2 CNV (black double arrow) sitting on the choroid (white double arrow). (B) A $\times 10$ H&E micrograph of CNV (black double arrow) extending through the surgically induced break in Bruch’s membrane (black arrows), from the underlying choroid (white double arrow) and secondary reactive retinal pigment epithelial (RPE) hyperplasia (yellow arrowheads) growing on the undersurface of the CNV. (C) A higher-power $\times 25$ H&E micrograph of subretinal CNV (black double arrow), endothelial lined vascular channels (white arrows), a defect in Bruch’s membrane (black arrows), and subretinal hemorrhage (black asterisk).
maintain a constant intraocular pressure and avoid hypotony, a condition that would lead to more subretinal bleeding. Due to the need for repeat anesthesia, assessment of postoperative subretinal blood was not performed.

**Suprachoroidal Delivery Using Microneedles**

Delivering pazopanib using the microneedle (Fig. 2) was efficient and simple. Small, unquantifiable, yet visible particulate elements of the poorly soluble drug clustered at a turbulent flow portion at the connection of the syringe and needle shaft, so the delivered dose was probably less than the calculated dose.

**Histopathology for CNV Lesion Size Evaluation**

Following removal of the anterior segments (Fig. 3), retinal detachments, vitreous hemorrhage, and proliferative fibrosis were commonly observed. The key histologic features (Fig. 4) demonstrate type 2 CNV lesion growing through breaks in Bruch’s membrane, under the neurosensory retina, and with the presence of reactive RPE proliferation over the surface of the CNV. The maximum heights (shown graphically) reached significance, while the mean height (average of multiple measurements taken at 5 to 10 intervals along the course of the histology slide that had the greatest horizontal diameter), surface area, and calculated volumes did not reach statistical significance (Table 3; Fig. 5). In the Ivt hl-con1 group as compared to vehicle control, the average pixel area size of the hl-con1-treated CNV lesions (58,018 ± 37,884 pixels²) was smaller than that of control CNV lesions (192,740 ± 65,888 pixels², P = 0.11). The vehicle-treated control CNV lesions were visibly thicker than in the hl-con1-treated eyes (Fig. 6). The color fundus photograph of an animal at postsurgical week 3, postinjection week 1 along

<table>
<thead>
<tr>
<th></th>
<th>Maximum Height, Z max</th>
<th>Mean Height, Z avg</th>
<th>Surface Area, mm², X × Y</th>
<th>Volume, mm³, X × Y × Z</th>
<th>Area in Pixels², X × Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pazopanib SC</td>
<td>478 ± 105 µm</td>
<td>147 ± 33 µm</td>
<td>0.28 ± 0.04</td>
<td>0.15 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Pazopanib Ivt</td>
<td>173 ± 43 µm</td>
<td>90 ± 20 µm</td>
<td>0.24 ± 0.11</td>
<td>0.15 ± 0.08</td>
<td></td>
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<tr>
<td>Bevacizumab Ivt</td>
<td>348 ± 123 µm</td>
<td>146 ± 37 µm</td>
<td>0.24 ± 0.07</td>
<td>0.11 ± 0.05</td>
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</tr>
<tr>
<td>hl-con1</td>
<td>650 ± 267 µm</td>
<td>180 ± 20 µm</td>
<td>0.36 ± 0.15</td>
<td>0.15 ± 0.03</td>
<td>58,018 ± 37,884</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>192,740 ± 65,888</td>
</tr>
</tbody>
</table>

Z max = lesion height, measured from Bruch’s membrane to the maximum measured height of the choroidal neovascular membrane (CNV). The Z avg is the average height taken from multiple measurements of lesion height along the length of the histology section of greatest horizontal diameter (5–10). These measurements were derived from differing time points following CNV induction. Thus, the data are consolidated as our goal was to determine the maximum lesion height at any postoperative point.

**FIGURE 5.** Choroidal neovascular size (‘statistically significant, P < 0.05). (A) Comparison of maximum lesion height with standard error (SE) bars (n = number of animals per group). (B) Mean lesion height as measured by taking the average of multiple measures across the greatest diameter of each CNV lesion.
with the corresponding fluorescein angiogram, is shown in Figure 7.

The maximum CNV height of the Ivit pazopanib-treated eyes (173 ± 43 μm) was significantly smaller than in the SC pazopanib group (478 ± 105 μm; \( P = 0.018 \)) and smaller than in the controls (650 ± 268 μm; \( P = 0.06 \)) (Fig. 5A). The maximum CNV height in the Ivit bevacizumab group (348 ± 123 μm) was only marginally less than in the SC pazopanib group and controls (\( P = 0.44 \) and \( P = 0.27 \), respectively). The mean Ivit pazopanib CNV height (90 ± 20 μm) was smaller than in the SC pazopanib-treated eyes (148 ± 35 μm; \( P = 0.15 \)) and significantly smaller than in the controls (180 ± 20 μm; \( P = 0.009 \)). There was only a marginal decrease in the mean height of lesions for the Ivit bevacizumab group (146 ± 37 μm) compared to controls (180 ± 20 μm; \( P = 0.49 \); see Fig. 5B).

There were no meaningful differences in CNV surface area measurements in any group. The CNV surface area measures from the Ivit bevacizumab (0.24 ± 0.07 mm²), the SC pazopanib (0.28 ± 0.04 mm²), and Ivit pazopanib (0.24 ± 0.11 mm²) groups did not reach statistical significance (\( P = 0.41 \), \( P = 0.57 \), and \( P = 0.52 \), respectively) when compared to controls. Similarly, in 13 of 32 CNV volume calculations, there were no significant differences between any groups when compared to the relevant control group.

In the subgroup comparing IVit injection of hlon1 to vehicle control, the average pixel area size of the hlon1–treated CNV lesions (58,018 ± 37,884 pixels²) was smaller than for control CNV lesions (19,2740 ± 65,888 pixels², \( P = 0.11 \), Fig. 5C). The vehicle-treated control CNV lesions were consistently thicker than in the hlon1–treated eyes (Fig. 6). This approached yet did not reach statistical significance.

**Serum Pazopanib**

The calculated and expected pazopanib concentrations of 12 different spiked serum samples were compared. The calculated and expected pazopanib concentrations are similar, suggesting the suitability of the analytic method. The lower limit of quantitation for the assay was 600 pg/mL. All serum samples analyzed were below these levels. Also, background levels could be detected in control samples. Serum concentration measured was the highest in the SC pazopanib group (239 ± 65 pg/mL), followed by Ivit pazopanib (142 ± 26 pg/mL), Ivit bevacizumab (126 ± 27 pg/mL), and then vehicle controls (124 ± 40 pg/mL). However, the values were well below the effective lower limit of quantitation (600 pg/mL), and there was an interfering background signal in animals not treated with pazopanib.

**DISCUSSION**

Our modified porcine model of surgically induced CNV, as originally described, represents a reliable and reproducible model to study type 2 CNV in vivo. Our results demonstrate consistent type 2 CNV induction, similar to the lesion type...
found in some cases of human AMD. However, all animal models of human disease have limitations. There are documented advantages of this methodology over the induction of CNV with laser. In fact, our attempts to replicate laser-induced CNV in the pig resulted in a very low rate of CNV induction and very haphazard formation of CNV lesions when compared to the nearly 100% CNV induction using the surgical model. Additionally, the surgery is thought to involve less retinal injury than the laser models. Most CNV lesion types in human AMD are classified as either type 1 CNV, located between Bruch’s membrane and the RPE, or polyoidal choroidal vasculopathy. The frequency of occurrence varies with genetic background. The CNV lesion type frequencies found in the clinical setting, based on fluorescein angiographic definitions of classic, occult, or predominantly classic, suggest that the majority of lesions found in human AMD cases are subfoveal and occult, or mixed lesions, most consistent with type 1 CNV. Despite these differences in lesion type, we feel that the pig model allows effective analysis of the pharmacodynamics of exudative AMD. A key advantage is the reproducibility of the CNV complex that allows for direct size comparisons, especially lesion height. However, a key limitation is the scarring and inflammatory nature of our induced lesions that may create an altered state from the lesions that result from aging. Also, type 1 CNV is likely due to aging and a more gradual deterioration in the integrity of Bruch’s membrane. Aging is difficult to reproduce in an animal model. Most of the measured differences in CNV lesion size in the pig model depend upon lesion height. This model relies on mechanical breaks in Bruch’s membrane, and the subsequent CNV lesion diameter (X and Y dimensions) can vary based on the number and distribution of punctures during surgical induction. For this reason, we attempted to match the size of the punctures with the optic nerve of the animal. Also, prior studies have documented the time course of CNV progression, beginning with enucleation 30 minutes (essentially time zero), post induction, to 3, 7, 14, 28, and 42 days. In our study, drug delivery was masked and randomly assigned after induction of the CNV, thus minimizing the chances for bias. In fact, there were no significant differences in lesion diameters or surface area in any of the control or treatment groups. Thus, we believe that the consistency of horizontal lesion size is important to allow for a reliable comparison between groups. For treatment effect, we rely more on lesion height as a primary indicator of antiangiogenesis.

In our study, we found that Ivit pazopanib had a statistically significant reduction of CNV height with a trend, yet not statistically significant, of thinner lesions in eyes treated with Ivit bevacizumab and hI-con1. Since each of these agent’s mechanism of action differs, translating the results back to humans may also require further analysis. We suspect that the humanized antibody to VEGF may not perform as well in porcine CNV as it does in humans. Also, the number of cases for comparison (especially with hI-con1, n = 4) may be too small to determine a meaningful difference. In fact, while Lassota et al. found less fluorescein leakage of surgically induced CNV treated with bevacizumab, the lesion sizes were no different from controls. Landiev et al. studied the effect of bevacizumab in the live pig model and measured a decrease in the transcriptional expression of VEGF-A. However, their model did not include induction of CNV. Pazopanib’s mechanism of action to inhibit both VEGF pathways and PDGF-B, along with its low solubility, combine to make pazopanib an excellent candidate for sustained delivery. We propose that this drug’s multifactorial mechanism of action makes it a potent inhibitor of CNV. Also, the low solubility may enable a prolonged treatment effect, and such an effect may differ from biologic antibodies that target these same pathways and are currently in human clinical trials. Thus, pazopanib has ideal physical properties conducive to studying this agent in the SCS. In fact, triamcinolone acetonide has similar physical properties and was found to be an excellent compound for effective delivery in the SCS (long-term action, low systemic levels). The Ivit route of pazopanib delivery demonstrated a meaningful inhibition of CNV lesion height in our model while the SC delivery did not. There may be several reasons for this difference. First, the location of the CNV is posterior, and the SC pazopanib was delivered 6 mm posterior to the limbus. While drugs may spread throughout the SCS, we did not measure this distribution, and perhaps the diffusional kinetics limit the amount of drug reaching the area centralis of the pig eye. Second, the slow dissolution of the drug may have been insufficient to allow adequate therapeutic levels to diffuse effectively from the location of delivery to the area centralis. Third, by avoiding the choroid blood flow, Ivit pazopanib may have a more sustained presence in the eye and especially at the area centralis. Fourth, it is possible that the outer choroid blood flow, transscleral diffusion, or uveoscleral outflow channels may act as a diffusional barrier to pazopanib reaching the inner choroid from the SCS. However, we do not believe that the total SC dose was insufficient, as the Ivit route appeared to be efficacious. Nevertheless, the low solubility of the pazopanib suspension could have led to underdosing as the larger, particulate material was retained in the eddy currents of the syringe, thus leading to an unexpected dosing variation. Following each SC injection, we were unable to confirm the amount of pazopanib in the resultant SC bleb or how much of the drug diffused posteriorly toward the area centralis. Also, we were unable to directly compare the systemic levels between the two delivery routes due to the limit of quantitation. Finally, the role of postvitrectomy pharmacokinetics should be considered and may be substantially different in this model.

The hI-con1 delivery was via the Ivit route, and the proposed mechanism of action is to induce regression of CNV. Thus, in our model, we delayed the Ivit injection of this agent until 2 weeks after CNV induction. We found a reduction in lesion area in the CNV using this agent, yet were unable to confirm the proposed NK-mediated regression of CNV with Ivit hI-con1. It is possible that our immune markers were not specific for porcine NK cells. Tezel et al. previously demonstrated a dramatic reduction in CNV lesion size in laser-induced CNV in the pig model. However, when we attempted the laser-induced CNV in our model, we found rapid involution of CNV in the controls as well as the treated eyes. In fact, we had difficulties finding histologic evidence of CNV at week 2 post induction and felt that the healthy RPE of the pig eye induces rapid, spontaneous involution of CNV. Therefore, we had a much more robust CNV induction using the surgical model in our studies.

We were not able to confirm the proposed mechanism of action of hI-con1. A possible explanation for not identifying NK cells is that the mechanism of CNV regression occurred prior to the time of enucleation and our specimens were obtained after the involvement of the NK cells. We intentionally delayed delivery of hI-con1 until 14 days post CNV induction. Thus, by examining the CNV lesions 1 to 2 weeks after drug delivery, we may have missed the presence of NK cell–mediated mechanism window. Another possible explanation is that the humanized antibody with a human Fc domain may have less affinity for NK cells in our pig CNV model. Nevertheless, there was a reduction of the lesion size of hI-con1–treated animals as compared to controls.

We found that SC drug delivery using the microneedles was very simple to administer and had technical requirements...
equivalent to a standard Ivit injection. Our study was not
designed as a pharmacokinetic analysis of tissue drug levels.
We believe that the location of the drug depot in the SCS is
likely to be an important variable. SC drug deposition is
expected to persist longer with greater doses, similar to the Ivit
route, due to dissolution.46

We have been successful in generating type 2 CNV in pigs
that is highly reproducible in nearly all cases. Additionally, the
globe size, blood flow patterns, RPE, and other anatomic and
physiological similarities between the pig and human eyes
make the pig model an excellent pharmacodynamic, preclinical
system to study angiogenesis. The CNV size may vary,
based on wound construction and the healing response. In
using this model, all attempts should be made to create similar,
reproducible lesions. Obviously, there are other inevitable
variations in surgical procedures, such as the amount and
degree of RPE debrideinent. The number of animals studied,
combined with masked random assignments, should minimize
bias and optimize the reliability of this model. The histopath-
ologic analysis of the CNV was also performed in a masked
fashion to further minimize possible bias. Finally, the consistent
and reproducible induction of CNV in the porcine model
creates reliable horizontal dimensions of the CNV. Thus, the
height measure is the most relevant measure of treatment
efficacy.

In summary, we have found that Ivit pazopanib inhibits
surgically induced CNV lesion size in the pig model when
compared to vehicle controls and warrants further studying.
CNV inhibition using either Ivit hI-con1 (strong trend) or SC
pazopanib did not reach statistical significance. The SC route
remains a viable route for further study. Optimizing the drug
depot locations, developing better sustained-release formul-
ations, and identifying new and novel anti-aging agents all
remain worthy goals for further alleviating the current burden
of treating age-related macular disease.

Acknowledgments

Supported in part by National Institutes of Health/National Eye
Institute RO1 EY022097, P30 EY006360 (Department Core Grant),
the Georgia Research Alliance, and an unrestricted grant from
Institute RO1 EY022097, P30 EY006360 (Department Core Grant),
Supported in part by National Institutes of Health/National Eye
Institutes.

Disclosure: J. Tran, None; C. Craven, None; K. Wabner, None; J.
Schmit, None; B. Matter, None; U. Kompella, None; H.E.
Grossniklaus, None; T.W. Olsen, iMacular Regeneration LLC (S)

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