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A Pharmacodynamic Analysis of Choroidal Neovascularization in a Porcine Model Using Three Targeted Drugs

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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 (8), 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.

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Subretinal choroidal neovascularization (CNV) in the macula is a known complication of end-stage age-related macular degeneration (AMD). The goal of this in vivo preclinical study was to optimize pharmacodelivery to the macula using a well-documented pig model of CNV. AMD is a leading cause of irreversible blindness in those over age 60 from developed countries. Furthermore, neovascular AMD (nAMD) has been effectively managed in many patients by using agents that target the vascular endothelial growth factor (VEGF) protein. Using a preclinical large animal study, we sought to determine the best and most effective pharmacologic management of pathologic CNV by examining strong candidate drugs. Drug delivery was either by intravitreal (Ivit) injection or into the suprachoroidal space (SCS) using a microneedle delivery system.

Surgically induced CNV in the pig is a reliable and reproducible model that has been extensively studied and is well characterized in the literature. In general, fibrovascular growth extends through surgically induced defects in Bruch’s membrane followed by debridement of the retinal pigment epithelium (RPE) that leads to subretinal CNV, subretinal fluid accumulation, hemorrhage, scaring, and fibrosis. While not specifically an aging model, the pig model has relevance for preclinical pharmacologic studies due to the anatomic similarities between a pig and a human eye. Key parallels include eye size, choroidal blood flow, retinal pigment epithelial characteristics, and scleral thickness.

Ranibizumab, aflibercept, and the off-label use of bevacizumab are commonly used VEGF inhibitors, each demonstrating therapeutic efficacy in treating nAMD. Lassota et al. demonstrated that Ivit bevacizumab treatments led to a reduction in vascular endothelial cells as well as fluorescein leakage in the pig model of CNV. In humans, treatment demands of Ivit delivery require frequent injections, thus creating a tremendous burden for patients and their care providers, and also creating an added burden to the health care system. Endophthalmitis, cataract, retinal detachment, and retinal pigment epithelial rips are all uncommon, yet represent meaningful, potential risks with each injection.

There are several drug delivery systems and sustained-release formulations that are currently in various stages of investigation. Delivery routes include Ivit, transscleral, and suprachoroidal (SC). The ideal drug and route depends
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.20,21
Gilger et al.22 demonstrated that a lower dose of corticosteroid
delivered through the SCs was as effective as a higher IVT 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 IVT route.23 Hollow micronee-
dles 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 acetone and
flucinolone acetone. Pazopanib is a small molecule (MW =
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
suppress CNV in a mouse model.28 However, the topical
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 combines a protein composed of a mutated factor VII
coujugated 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 cytolysis of CNV in
mouse models.31–33 TF is expressed on endothelial cells and
is located on CNV but not on normal vessels.34 A study published
by Bora et al.33 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 of CNV. Tezel et al.35 have shown that hi-con1 binds to
the endothelium of CNV in a pig model.

In the present study, we used the pig CNV model to
compare the efficacy of several novel anti-VEGF agents in
combination with selected drug delivery routes. We used
microneedles to deliver pazopanib into the SCs and compared
this to IVT delivery of either pazopanib, bevacizumab, or hi-
con1 with appropriate vehicle controls.

**Materials and Methods**

All animal studies were performed with permission from the
Institutional Animal Care and Use Committee (IACUC) at
Emory University and in accordance with the guidelines on
conduct of animal experiments and experimental design in
keeping with the ARVO Statement for the Use of Animals in
Ophthalmic and Vision Research.

**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 phenyleph-
rine (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 through-
out 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 Kilgaard et al.5 The surgical steps are summarized in
Figures 1A to 1E. A 23-gauge pars plana lensectomy
and vitrectomy 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 23-gauge cannula

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**Table 1. Study Design**

<table>
<thead>
<tr>
<th>Group</th>
<th>Study</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
<th>Week 8</th>
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<td>Pazopanib IVT</td>
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<tr>
<td>Bevacizumab IVT</td>
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<td>3</td>
<td>3</td>
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<tr>
<td>hi-con1 IVT</td>
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<td>4</td>
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</tr>
<tr>
<td>Controls</td>
<td>N = 10</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>2</td>
</tr>
</tbody>
</table>

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.
Surgically Induced CNV Pharmacodynamics in the Pig

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 tip of the microneedle to reach the suprachoroidal space (SCS) while posterior to the limbus. There is sufficient scleral indentation for the aphakic pig eye following induction of CNV. The syringe is oriented suspension being injected into the suprachoroidal space of an air-filled, 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 epithelium, the optic nerve (right), and the choroidal neovascular lesion (white dashed circle) are visible.

delivered 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.

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 performed 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 phenobarbital 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. 5). 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 centralis (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. Lesion height, as described in the natural history of CNV in the pig model by Lassota et al., 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. 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 ×2, ×4, ×10, and ×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 2,400g 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 ≤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>
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<tr>
<th>Table 2. Complications Observed During Gross Pathologic Review</th>
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<td>Retinal Detachment</td>
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<tr>
<td>Pazopanib SC</td>
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<td>Pazopanib IvI</td>
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<td>Bevacizumab IvI</td>
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<td>Controls</td>
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Partial, funnel, and full retinal detachments were observed and are shown in the retinal detachment column.
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 histopathology slide that had the greatest horizontal diameter), surface area, and calculated volumes did not reach statistical significance (Table 3; Fig. 5). In the Ivit hI-con1 group as compared to vehicle control, the average pixel area size of the hI-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 hI-con1–treated eyes (Fig. 6). The color fundus photograph of an animal at postsurgical week 3, postinjection week 1 along

<table>
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<th>Table 3. Choroidal Neovascular Membrane Lesion Measurements</th>
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<tr>
<td><strong>Maximum Height, ( Z_{\text{max}} )</strong></td>
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<tr>
<td>Pazopanib SC</td>
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<td>Pazopanib Ivit</td>
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<td>Bevacizumab Ivit</td>
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<tr>
<td>hI-con1</td>
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<tr>
<td>Controls</td>
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\( Z_{\text{max}} \) = lesion height, measured from Bruch’s membrane to the maximum measured height of the choroidal neovascular membrane (CNV). The \( Z_{\text{avg}} \) 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 \pm 64 \text{ mm}) was significantly smaller than in the SC pazopanib group (478 \pm 105 \text{ mm}; P = 0.018) and smaller than in the controls (650 \pm 268 \text{ mm}; P = 0.06) (Fig. 5A). The maximum CNV height in the Ivit bevacizumab group (348 \pm 123 \text{ mm}) 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 \pm 20 \text{ mm}) was smaller than in the SC pazopanib-treated eyes (148 \pm 35 \text{ mm}; P = 0.15) and significantly smaller than in the controls (180 \pm 20 \text{ mm}; P = 0.009). There was only a marginal decrease in the mean height of lesions for the Ivit bevacizumab group (146 \pm 37 \text{ mm}) compared to controls (180 \pm 20 \text{ mm}; 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 \pm 0.07 \text{ mm}^2), the SC pazopanib (0.28 \pm 0.04 \text{ mm}^2), and Ivit pazopanib (0.24 \pm 0.11 \text{ mm}^2) 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 hI-con1 to vehicle control, the average pixel area size of the hI-con1–treated CNV lesions (58,018 \pm 37,884 pixels^2) was smaller than for control CNV lesions (19,274 \pm 65,888 pixels^2, P = 0.11, Fig. 5C). The vehicle-treated control CNV lesions were consistently thicker than in the hI-con1–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 \pm 65 pg/mL), followed by Ivit pazopanib (142 \pm 26 pg/mL), Ivit bevacizumab (126 \pm 27 pg/mL), and then vehicle controls (124 \pm 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,\textsuperscript{4,6} 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

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**Figure 6.** Histopathology of type 2 choroidal neovascularization (CNV). (A) A 25× toluidine blue stain of a hI-con1–treated, partially regressed CNV (orange arrow) on underlying choroidal tissue. (B) A 25× toluidine blue stain of the vehicle CNV (orange double arrow) on underlying choroidal tissue (red double arrow). The vehicle-treated control CNV lesions are consistently thicker than in the hI-con1–treated eyes. (C) A 100× hI-con1–treated CNV with negative staining of anti-CD56 for NK cells. (D) A 100× vehicle CNV with positive staining of anti-CD56 (brown cells marked by yellow bracket).

**Figure 7.** Clinical images. Left: color fundus photograph of the postoperative appearance on postoperative week 3 of the subretinal choroidal neovascularization (CNV) in the pig model with reactive retinal pigment epithelium (RPE). Right: midphase fluorescein angiogram of the CNV demonstrating hyperfluorescence, especially at the puncture sites and some blocking defect from overlying reactive hyperplasia of the overlying RPE.
Surgically Induced CNV Pharmacodynamics in the Pig

JOVS | July 2017 | Vol. 58 | No. 9 | 3738

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 haphazardly if CNV involvement 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 polypoidal 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 PDGFR-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, trimacinolone 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 choroidal blood flow, Ivit pazopanib may have had a more sustained presence in the eye and especially at the area centralis. Fourth, it is possible that the outer choroidal blood flow, transcellular 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 underdosaging 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 postvitreectomy 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 mechanistic 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...
Surgically Induced CNV Pharmacodynamics in the Pig  

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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 debridement. The number of animals studied, combined with masked random assignments, should minimize bias and optimize the reliability of this model. The histopathologic 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 hC-1 (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 formulations, and identifying new and novel anti-aging agents all remain worthy goals for further alleviating the current burden of treating age-related macular disease.

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