Treatment of Acute Posterior Uveitis in a Porcine Model by Injection of Triamcinolone Acetonide Into the Suprachoroidal Space Using Microneedles

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PURPOSE. To evaluate the effect of triamcinolone acetonide (TA) administered into the suprachoroidal space (SCS) using a microneedle and compare it with intravitreal (IVT) TA injections in a porcine model of acute posterior segment inflammation.

MATERIALS. An IVT injection of balanced salt solution (BSS) or lipopolysaccharide (LPS) was followed 24 hours later with an injection of 0.2 mg or 2.0 mg of TA into the SCS or IVT. The SCS was accessed using microneedles in a minimally invasive procedure. Ocular inflammatory scores and IOP measurements were collected daily, whereas electroretinography, optical coherence tomography, and wide-field ocular fundus photography was performed on day 1, 3, and 5 days after treatment. Aqueous and vitreous humor cell counts and protein levels and histopathology were also compared.

RESULTS. Delivery of TA to the SCS using microneedles was simple, effective, and not associated with adverse effects or toxicity. SCS injection of low (0.2 mg) and high doses (2.0 mg) of TA was as effective in reducing acute inflammation in the ocular posterior segment as high-dose IVT injection. Low-dose SCS TA was also effective in reducing inflammation; however, low-dose IVT TA was not.

CONCLUSIONS. Results from this study suggest that 0.2 mg and 2.0 mg of SCS TA was as effective in reducing inflammation as 2.0 mg IVT TA injection in a model of acute posterior segment inflammation. There were no adverse effects, increased IOP, or evidence of procedural or drug toxicity following injection of TA into the SCS in porcine eyes.

Keywords: drug delivery, suprachoroidal space, microneedle, posterior segment, uveitis

Diseases of the ocular posterior segment are among the most common causes of blindness. More than 1.5 million US adults older than 50 suffer from neovascular (wet) age-related macular degeneration and more than 1 million people in the United States suffer from macular edema following retinal vein occlusion. Macular edema is also common and a result of retinal vascular occlusion caused by hypertension, atherosclerosis, diabetes (diabetic retinopathy), or glaucoma. Uveitis is the third leading cause of blindness worldwide and, according to a study on the incidence of uveitis in Northern California, uveitis affects 94 people per 100,000 who are 65 or older.

Treatment of these common diseases and delivery of medication to the ocular posterior segment is challenging due to inherent barriers of the eye and because ocular tissues are highly sensitive to toxicological effects of many drugs. Most eye drops applied topically do not penetrate to the ocular posterior segment in therapeutically relevant drug concentrations. Instead, these posterior segment diseases are currently treated by local injections or surgical placement of sustained-release drug delivery devices directly into the vitreous. However, these treatments are subject to complications, such as hemorrhage, postinjection endophthalmitis, cataract formation, elevated IOP, and local drug toxicity.

The suprachoroidal space (SCS) is a potential space that is located between the choroid and sclera and thus surrounds the entire ocular posterior segment. Use of the SCS as a potential site for ocular drug delivery is advantageous because this route avoids substantial drug barriers, such as the tear film, surface epithelium, conjunctival lymphatics, and the sclera. Because the SCS is adjacent to the entire choroid, drug delivery into the SCS has the potential to provide targeted drug delivery to tissues within the ocular posterior segment.

Use of hollow microneedles may greatly simplify access to the SCS by eliminating the need for a scleral incision. Microneedles were demonstrated to directly and effectively deliver nanoparticle and microparticle suspensions into the SCS of rabbit, pig, and human cadaver eyes. In rabbits, microneedle access to the SCS was consistent and without complication in vivo, resulting in concentrations of injected materials 10-fold or greater in the ocular posterior segment compared with the anterior segment. The lower anterior segment drug distribution after SCS injections may decrease complications commonly associated with intravitreal (IVT) injections, such as development of glaucoma and cataract. Despite the favorable results of these studies, several questions need to be answered before routine clinical use of microneedle SCS therapy. For example, can microneedles effectively and safely deliver in vivo a commonly used drug such as triamcinolone acetonide (TA) to the SCS of eyes similar in size and anatomy to humans?
Additionally, can delivery of TA to the SCS effectively control posterior segment inflammation as well as IVT TA injection? The purpose of this study was to evaluate the effect of TA administered into the SCS using a microneedle and compare it to IVT TA injections in a porcine model of acute posterior segment inflammation.

**Materials and Methods**

Use of animals in this study adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Visual Research and was approved and monitored by the North Carolina State University Institutional Animal Care and Use Committee. Animals were acclimated to the study environment for 1 week before uveitis induction.

A total of 20 domestic weanling pigs (Sus scrofa domesticus), male or female (12–20 kg), were used in this study. Only the left eye was tested in this study; the right eye was not injected, examined, or treated. All injections were performed with the pigs anesthetized (intramuscular telazol-ketamine-xylazine and isoflurane in oxygen via mask) and the eye prepared aseptically (sterile 5% betadine solution followed by irrigation with sterile eyewash). Immediately following the injections, 1 drop of moxifloxacin ophthalmic solution (Vigamox; Alcon Laboratories Inc., Fort Worth, TX) was applied topically.

Twenty-four hours before SCS or IVT injection of TA or vehicle (day −1), 100 ng of lipopolysaccharide (LPS; Escherichia coli 055:B55; Sigma, Inc., St. Louis, MO) in 100 µL balanced salt solution (BSS) (Alcon Laboratories, Inc.) was injected using a 27-gauge needle into the posterior central vitreous.

Twenty-four hours after the LPS injection (day 0), 0.2 mg or 2.0 mg of commercially available TA (Triessence; Alcon Laboratories, Inc.) or vehicle was injected either intravitreally (27-gauge needle) or into the SCS (33-gauge, 850 µM microneedle; Clearside Biomedical, Alpharetta, GA) in eyes prepared aseptically (Table). The dose of TA was selected to represent a typical therapeutic dose (e.g., 2.0 mg) and a dose 10 times less to compare therapeutic effect. The authors are aware that a wide range of TA doses are used clinically.

All injections were made superiorly, approximately 5 to 6 mm posterior to the limbus. To help stabilize the eye for SCS injection, a sterile laboratory spatula (Corning sterile flat end spatula; Corning Life Sciences, Corning, NY) was placed in the inferior conjunctival fornix. To become proficient at the SCS injection technique with microneedles, approximately 10 to 15 SCS injections were made in cadaver porcine eyes before conducting this study. TA was diluted using vehicle to provide a low dose (0.2 mg/100 µL) or high dose (2.0 mg/100 µL). The vehicle (100 µL) was also used in the control groups, but without TA. Treatment groups are listed in the Table.

**Ocular Inflammatory Scores**

A Hackett-McDonald microscopic ocular inflammatory scoring system, modified for use in pigs (instead of New Zealand White rabbits) as described below, was used to evaluate the ocular anterior segment and anterior vitreous. Scores of the conjunctiva (congestion, swelling, discharge, 0–4), aqueous flare (0–5), pupillary light reflex (0–2), iris involvement (0–4), cornea (involvement and area, 0–4), pannus (vascularization, 0–2), and anterior vitreal cellular infiltrate (0–4) were summed to provide a single inflammatory score for each animal for each examination. Using a portable slit lamp biomicroscope (Zeiss HSO-10; Carl Zeiss Meditec Inc., Dublin, CA), ocular inflammatory scores were evaluated at day −1 (before LPS injection), at day 0 (before vehicle or TA injection), and then at 1, 2, and 3 days after injection.

**IOP**

IOP was measured at −6, −4, −1, 0, 1, 2, and 3 days using a TonoVet Tonometer (iCare, Helsinki, Finland). In addition, IOP was measured 1, 3, and 6 hours after SCS or IVT injections on day 0. The measurements were collected without use of topical anesthetic, per manufacturer recommendation. Conditioning of the pigs during acclimation permitted routine ocular examinations and IOP measurements to be done with minimal manual restraint. The tip of the tonometer (TonoVet; iCare) probe was directed to contact the central cornea and six measurements were made consecutively. After the six measurements, the mean IOP was shown on the display providing the IOP that was recorded.

**ERG**

With the pigs anesthetized on days −1, 0, and 3, and pups dilated with 1% tropicamide HCl and corneas anesthetized with 0.5% proparacaine HCl, whole field ERGs were recorded from the left eye before injections. All animals were dark adapted for 15 minutes before ERG. A monopolar contact lens electrode (ERGJet; Universo SA, La Chaux-de-Fonds, Switzerland) was placed on the cornea to serve as an active electrode. A subdermal electrode at the lateral canthus served as the indifferent electrode. A Barraquer eyelid speculum was placed to maintain open eyelids and a subdermal needle electrode was inserted dorsally as the ground electrode. ERGs were elicited by brief flashes at 0.33 Hz delivered with a mini-Ganzfeld photostimulator (Roland Instruments, Wiesbaden, Germany) at maximal intensity. Twenty responses were amplified, filtered, and averaged (Retiport Electrophysiologic Diagnostic Systems; Roland Instruments). B-wave amplitudes were recorded from each pig at the designated times.

**Wide-Field Ocular Fundus Digital Photography**

On study days −1, 0, and 3, with the animals anesthetized and pupils dilated with tropicamide 1%, the ocular fundus was photographed using standardized illumination and focus by a wide-field digital imaging system (Retcam II; Clarity Medical Systems, Pleasanton, CA).

**Table. Treatment Groups and Study Design**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment 100 µL, Day −1/Day 0</th>
<th>No. of Animals</th>
<th>Examinations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BSS IVT/vehicle SCS</td>
<td>2</td>
<td>Ocular inflammatory scores: days −1, 0, 1, 2, and 3</td>
</tr>
<tr>
<td>2</td>
<td>100 ng LPS IVT/vehicle SCS</td>
<td>2</td>
<td>IOP: days −6, −4, −1, 0, 1, 2, and 3*</td>
</tr>
<tr>
<td>3</td>
<td>100 ng LPS IVT/0.2 mg TA SCS</td>
<td>4</td>
<td>ERG, OCT, photo: days −1, 0, and 3</td>
</tr>
<tr>
<td>4</td>
<td>100 ng LPS IVT/2.0 mg TA SCS</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>100 ng LPS IVT/0.2 mg TA IVT</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>100 ng LPS IVT/2.0 mg TA IVT</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

Photo, ocular fundus photography.

* Plus 1, 3, and 6 hours after treatment injections.
Optical Coherence Tomography

Following wide-field ocular fundus photography on days −1, 0, and 3, the central retina was imaged with spectral-domain optical coherence tomography (SD-OCT) (SD-OCT EnVisu Ophthalmic Imaging System; Bioptigen, Inc., Durham, NC) using six imaging protocols (including rectangular volume scans of 6, 8, and 12 mm, and Doppler rectangular volume). The SD-OCT allowed in vivo assessment of retinal pathology and retinal thickness was measured, using internal calipers, of three representative areas one disc diameter superior to the optic disc, then averaged to provide a mean value retinal thickness per eye per time period.

Ocular Histopathology

Pigs were euthanized on study day 3 after clinical scoring, OCT, ERG, and wide-field ocular fundus photography was completed. After euthanasia with an overdose of intravenous barbiturate, both eyes were removed. Aqueous humor (AH) was aspirated and a 1-mL sample of vitreous humor (VH) was collected from each eye immediately after euthanasia. The globe was then fixed in Davidson’s solution for 24 hours, followed by alcohol. Central, sagittal sections of each globe, including the optic nerve, were stained with hematoxylin and eosin and examined by light microscopy. Two pathology-trained observers, masked to the study groups, graded degree of inflammatory infiltrate of the ocular anterior and posterior segments. The grading scale for the anterior and posterior ocular segment used was as follows: 0 = no evidence of cellular infiltrate; 1 = a few cells infiltrated (mild) focal; 2 = a few cells infiltrated (mild) diffuse; 3 = moderate number of cells infiltrated; and 4 = high amount of cellular infiltrate.

Aqueous and Vitreous Humor Inflammatory Cell Counts and Protein Concentration

After aspiration from the eyes, AH and VH were immediately placed on ice, transferred to the laboratory, then frozen at −80°C until processing. Samples were thawed at room temperature, vortexed, and total cell counts were performed with a hemocytometer (Hausser Scientific, Horsham, PA). Total protein concentration was measured using the Bradford Assay (Pierce BCA Protein Assay Kit; Thermo Scientific Pierce, Rockford, IL).

Data and Statistical Analysis

For histologic grading, two independent observers masked to the treatment group evaluated each eye at each time point and the average resulting scores for each animal were used for analysis. Parametric normally distributed data (i.e., IOP, ERG, retinal thickness, cell counts, protein concentration) were...
compared by time point for each group using one-way ANOVA models with Tukey-Kramer post hoc analysis. For nonparametric data (i.e., clinical scores, histologic grades), Wilcoxon tests were conducted per animal by time point. Differences were considered significant at $P < 0.05$. Results and probabilities were calculated using computerized statistical software (JMP 10; SAS Inc., Cary, NC).

**RESULTS**

Injections of TA or vehicle into the SCS were accomplished using microneedles without difficulty or adverse effect. Eyes were examined via slit lamp biomicroscopy and indirect ophthalmoscopy following each injection. No evidence of back-leakage of treatment materials through the microneedle scleral perforation or leakage of the white drug suspension into the vitreous was observed following SCS injection. Intravitreal TA injections were visible as central vitreal white depots on indirect ophthalmoscopy. Furthermore, there was no evidence of injection site or vitreal hemorrhage following any injections (SCS or IVT).

**Ocular Inflammatory Scores**

Following IVT injection of LPS on day $-1$, cumulative inflammatory scores elevated to between 6 and 10 in all groups (Fig. 1). Scores in eyes injected with LPS were significantly higher than in eyes injected with BSS ($P < 0.02$). Following treatment injections on day 0, inflammatory scores generally decreased, although 24 hours after treatment, eyes treated with vehicle (group 2) had mean scores significantly higher than the other treatment groups ($P < 0.02$) and eyes treated with IVT 0.2 mg TA (group 5) had mean scores that were significantly higher ($P < 0.03$) than groups 1, 3, 4, and 6. At 48 and 72 hours after treatment, eyes treated with 0.2 mg IVT TA (group 4) had significantly higher mean scores than eyes treated with SCS TA (0.2 and 2.0 mg; groups 3 and 4) and vehicle (group 1). Eyes treated with SCS TA (0.2 and 2.0 mg; groups 3 and 4) and IVT TA (2.0 mg; group 6) had mean inflammatory scores not significantly different from eyes treated with vehicle at each examination day (i.e., days 1, 2, and 3) after treatment (Fig. 1).

**IOP**

Intraocular pressure ranged from 19 to 24 mmHg during acclimation and decreased slightly over time as pigs became accustomed to being handled. On induction of uveitis, the IOP decreased by time 0 to between 12 and 16 mmHg in groups receiving LPS. Following treatment injections, IOP remained low in all groups through 6 hours post injection, then returned to baseline. Group 1 eyes, which did not receive LPS, had significantly higher IOP 1 and 3 hours after treatment injections ($P = 0.01; 0.04$). Otherwise, there were no significant differences between the groups (Fig. 2) and there were no acute elevations in IOP noted immediately (i.e., 1, 3, and 6 hours) after injections.

**ERG**

Scotopic B-wave amplitudes were not significantly different between any of the groups evaluated at each time point (i.e., days $-1$, 0, and $3$), except for group 4 at day $-1$, which was significantly higher than groups 1, 3, 5, and 6 ($P < 0.007$). This pretreatment result was likely a result of biologic variation and is not clinically significant; however, no evidence of retinal

**Figure 2.** Mean (± SD) IOP in porcine eyes before uveitis induction (day $-1$), before treatment (day 0) with SCS or IVT injections of 0.2 mg (low dose) or 2.0 mg (high dose) of TA, and 1, 2, and 3 days after treatment. (a) IOP in group 1 eyes was significantly higher than group 2 eyes at 1 and 3 hours after treatment injections ($P = 0.01; 0.04$).

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dysfunction (i.e., decrease in B-wave amplitude) was noted after injections.

Wide-Field Ocular Fundus Digital Photography

Wide-field ocular fundus images revealed substantial cloudiness of the ocular posterior segment 24 hours after LPS injection, except in group 1, which was injected with BSS and remained normal in appearance. Substantial cloudiness of the ocular posterior segment developed 24 hours after LPS injection in all eyes except in group 1 eyes. Treatment with low- and high-dose TA into the SCS and high-dose TA IVT resulted in fundus images near pretreatment appearance, whereas treatment with low-dose TA IVT resulted in images only slightly improved over vehicle-treated eyes. Eyes with high-dose IVT injections had a solid large depot of TA (arrow) visible in the central vitreous.

Optical Coherence Tomography

There was no significant difference in retinal thickness in any of the groups before or after the injections. Overt retinal pathology was not observed after the induction of uveitis or treatments; however, cells were observed emanating from retinal vasculature.
Figure 4. Ocular histopathology of eyes 3 days after IVT injection of BSS or 100 ng of LPS and 72 hours after SCS or IVT injection of vehicle, 0.2 mg TA (low-dose TA), or 2.0 mg of TA (high-dose TA). Hematoxylin and eosin stain. (A) Anterior segment of eyes injected with BSS IVT and vehicle in SCS (group 1). (B) Posterior segment of eyes injected with BSS IVT and vehicle in SCS (group 1). (C) Anterior segment of eyes injected with LPS IVT and vehicle in SCS (group 2). (D) Posterior segment of eyes injected with LPS IVT and vehicle in SCS (group 2). (E) Anterior segment of eyes injected with LPS IVT and low-dose TA in SCS (group 3). (F) Posterior segment of eyes injected with LPS IVT and low-dose TA in SCS (group 3). (G) Anterior segment of eyes injected with LPS IVT and high-dose TA in SCS (group 4). (H) Posterior segment of eyes injected with LPS IVT and high-dose TA in SCS (group 4).
Ocular Histopathology

None of the eyes examined in any group had evidence of substantial tissue structural or toxicologic changes on histopathology. However, all eyes, except group 1 (BSS IVT/vehicle SCS), had cellular infiltrate in the anterior uvea, vitreous, and retina. The cellular infiltrate was predominantly neutrophils. Group 2 eyes (LPS IVT/vehicle SCS) had moderate to severe neutrophilic infiltrate in the iris, iris root, and iridocorneal angles. Additionally, there was moderate to severe neutrophilic infiltrate in the vitreous body, inner retinal layers, and retinal perivascular cuffing of inflammatory cells (Fig. 4). In group 5 eyes (LPS IVT/low-dose TA SCS), there was mild neutrophilic infiltrate in the iris, and moderate infiltrate of neutrophils in the inner retinal layers and vitreous. The anterior segment of group 4 eyes (LPS IVT/high-dose TA SCS) was normal, with only an occasionally observed inflammation cell. The vitreous had very mild neutrophilic infiltration and very mild inner retinal cellular infiltrate. TA was visible in the SCS space on each eye in group 4 (Fig. 4), indicating that the injection technique indeed delivered TA to the SCS. There was no inflammation or histologic evidence of toxicity in the SCS as a result of the TA or injection. In group 5 eyes (LPS IVT/low dose TA IVT), there was mild neutrophilic infiltrate in the anterior uvea and moderate to severe cellular infiltrate in the vitreous and moderate infiltrate in the inner retina, including moderate perivascular infiltrate. In group 6 eyes (LPS IVT/high-dose TA SCS), there was mild neutrophilic infiltrate in the anterior uvea, and moderate vitreal infiltrates, including mild perivascular infiltrate (Fig. 4).

Review of ocular histopathologic inflammatory scores (Fig. 5) of the anterior and posterior segment revealed that group 1 eyes (BSS IVT/vehicle SCS) had mean histologic inflammatory scores that were significantly lower than the other groups (P < 0.04). Eyes in group 5 (LPS IVT/low-dose TA IVT) had mean histologic inflammatory scores in the anterior segment that were significantly higher than eyes receiving high-dose TA either in the SCS (group 4) or intravitreally (group 6) (P < 0.04). Eyes of group 4 (LPS IVT/high-dose TA SCS) had mean histologic inflammatory scores in the ocular posterior segment that were significantly lower than vehicle-treated eyes (group 2) and eyes treated with IVT TA (groups 5 and 6) (P < 0.04). Eyes treated with high-dose IVT TA had mean histologic inflammatory scores that were significantly lower than vehicle-treated eyes (group 2) (P = 0.018) (Fig. 5).

AH and VH Inflammatory Cell Counts and Protein Concentration

Mean AH cell counts ranged from 2000 cells/mL in group 1 (BSS IVT/vehicle SCS) eyes to 27,800 ± SD 530 cells/mL in group 2 (LPS IVT/vehicle SCS) eyes, which were significantly higher than each other group (P < 0.0023). Mean AH cell
counts of groups 5 (LPS IVT/low-dose TA IVT) and 6 (LPS IVT/high-dose TA IVT) were significantly higher than group 1 (BSS IVT/vehicle SCS) ($P = 0.022; P = 0.021$). Mean AH cell counts of groups 3 (LPS IVT/low-dose TA SCS) and 4 (LPS IVT/high-dose TA SCS) were not significantly different from AH cell counts of group 1 (BSS IVT/vehicle SCS), group 5 (LPS IVT/low-dose TA IVT), or 6 (LPS IVT/high-dose TA IVT) (Fig. 6).

Mean VH cell counts ranged from 6300 cells/mL in group 1 (BSS IVT/vehicle SCS) eyes to 55,000 ± SD 1620 cells/mL in group 2 (LPS IVT/vehicle SCS) eyes, which was significantly higher than each other group ($P < 0.018$). Mean VH cell count of group 3 (LPS IVT/low-dose TA SCS) was significantly higher than group 1 (BSS IVT/vehicle SCS) ($P = 0.031$) and group 4 (LPS IVT/high-dose TA SCS) ($P = 0.048$). Mean VH cell count of group 5 (LPS IVT/low-dose TA IVT) also was significantly higher than VH cell counts in group 1 (BSS IVT/vehicle SCS) ($P = 0.023$) and group 4 (LPS IVT/high-dose TA SCS) ($P = 0.032$). Mean VH cell count of group 3 (LPS IVT/low-dose TA SCS) was not significantly different from VH cell counts in groups 5 (LPS IVT/low-dose TA IVT) and 6 (LPS IVT/high-dose TA IVT).

Furthermore, mean VH cell counts of group 4 (LPS IVT/high-dose TA SCS) were not significantly different from the VH cell counts of group 1, the untreated control (Fig. 6).

Mean AH protein concentration ranged from 0.0 mg/mL in group 1 (BSS IVT/vehicle SCS) eyes to 3.0 ± SD 3.5 mg/mL in group 6 eyes. There were no significant differences in mean AH protein concentration among the groups. Mean VH protein concentration ranged from 0.0 mg/mL in group 1 (BSS IVT/vehicle SCS) eyes to 4.0 ± SD 0.8 mg/mL in group 6 (LPS IVT/high-dose TA IVT) eyes. Group 1 (BSS IVT/vehicle SCS) and group 4 (LPS IVT/high-dose TA SCS) had significantly lower mean VH protein concentrations than groups 3 (LPS IVT/low-dose TA SCS), 5 (LPS IVT/low-dose TA IVT), and 6 (LPS IVT/high-dose TA IVT) ($P < 0.035$).

**DISCUSSION**

Based on this study, we have concluded that delivery of TA to the SCS using microneedles was effective and tolerated with an acceptable safety profile for up to 3 days after injection in porcine eyes. Furthermore, SCS injection of 0.2 mg and 2.0 mg of TA was as effective in reducing inflammation in this model as 2.0 mg TA IVT injection. Mean inflammatory scores, vitreal cellular infiltrate OCT scores, and histologic grades of eyes receiving 0.2 mg and 2.0 mg of TA in the SCS were not significantly different from 2.0 TA injected IVT.

Lipopolysaccharide, or endotoxin, has been used in numerous animals to model acute uveitis. A single IVT injection of LPS has been used to induce uveitis in rabbits to evaluate both uveitis pathogenesis and effect of therapeutic. The time course and clinicopathologic features of inflammation in the LPS rabbit model were similar to that observed in this porcine model. The goal of this model, however, was not to define pathogenesis of uveitis, but instead to provide a consistent and reproducible model of inflamma-

![Figure 6](https://example.com/figure6.png)

**Figure 6.** Mean AH and VH cell counts 3 days after IVT injection of BSS or 100 ng of LPS and 72 hours after SCS or IVT injection of vehicle, 0.2 mg TA (low-dose TA), or 2.0 mg of TA (high-dose TA). (a) Group 2 mean cell counts were significantly higher than groups 1, 3, 4, 5, and 6 ($P < 0.002$). (b) Group 5 mean cell counts were significantly higher than group 1 ($P < 0.002$). (c) Group 6 mean cell counts were significantly higher than group 1 ($P < 0.002$). (d) Group 5 mean cell counts were significantly higher than groups 1 and 4 ($P < 0.048$). (e) Group 5 mean cell counts were significantly higher than groups 1 and 4 ($P < 0.054$).
tion in an eye that, unlike the rabbit, is more similar in anatomy, size, and retinal vascular pattern to the human eye. Therefore, this model of posterior uveitis has implications for patients with regard to drug delivery, pharmacology, and treatment of ocular inflammation. This study was conducted to determine whether the suprachoroidal route of TA delivery would reduce intraocular inflammation as effectively as IVTTA. Further studies will be required to determine if therapeutic duration is sufficient to effectively manage chronic uveitis.

In this study, there was evidence that 0.2 mg TA injected in the SCS was as effective in reducing acute ocular inflammation as was 2.0 mg TA IVT, whereas 0.2 mg TA IVT was less effective. A 10-fold decrease in effective dose when the drug is delivered to the SCS may have occurred because of more targeted delivery of the TA to the choroid and retina. There was no evidence of injection site complications, acute elevated IOP, or retinal toxicity after SCS injections. The uveoscleral outflow pathway of aqueous humor runs from the anterior chamber, through the interstitial tissue of the ciliary muscle, and into the SCS.29 There is a positive pressure difference between the anterior chamber and the SCS, resulting in posterior flow of AH into the SCS.30 A concern of the use of SCS drug delivery is whether the presence of drugs or other materials in the SCS may block this flow of AH and result in acute or chronic elevated IOP. However, there has not been any evidence to suggest that is occurring in animal studies. Acutely elevated IOP was not observed in this study after SCS injections and was not observed chronically after placement of a drug implant into the SCS in another study.16,17

However, further studies are needed to determine the acute and long-term effects of SCS injections on IOP. In primates, and other animal models, chronic use of ocular steroids has been associated with elevated IOP.8,10,14,15 Because of the short-term follow-up and use of an animal model not known to develop steroid-induced hypertension, this study was not designed to evaluate or compare the chronic effects of SCS ocular TA injection on IOP; thus further studies are needed to specifically address this concern.

This porcine model of ocular inflammation is a very acute model, but many of the diseases of the ocular posterior segment are chronic, requiring prolonged therapy. As observed on this acute uveitis model, a vitreous depot of TA may not be as immediately effective as an injection of TA in the SCS; however, the vitreous depot may allow slow release and long-term disease control in chronic inflammatory models and clinical patients. Whether or not SCS TA can provide sustained anti-inflammatory effect was not evaluated and warrants further study. In fact, a recent study of IVT injection of bevacizumab demonstrated more sustained tissue levels compared with tissue levels obtained from similar doses delivered into the SCS in normal porcine eyes.19 Because the flow of AH is anterior to posterior in the SCS, this flow may carry drugs injected into the anterior SCS farther in to the ocular posterior segment.21,30 However, the predominant egress of fluids from the SCS is via the posterior trans-scleral route.30 Therefore, due to potential rapid trans-scleral outflow, especially with hydrophobic drugs, sustained release formulations or devices placed in or near the SCS may be required to provide chronic therapy for posterior segment diseases. Previous studies, which have demonstrated success in the control of disease using solid implants in the SCS of an animal model of naturally occurring uveitis, suggest that the SCS route of drug delivery is highly feasible and effective to treat chronic disease.16,17

There are several important limitations to this study. As mentioned previously, this model is acute, whereas most disease states of the ocular posterior segment are chronic. Therefore, the long-term therapeutic effect of SCS TA, or other drugs injected into the SCS, needs to be demonstrated in chronic disease models. The effect of SCS injections and medications in the SCS (e.g., IOP, choroid and scleral toxicity, and ocular blood flow) also needs to be evaluated in long-term studies. Another limitation was the modest number of animals in treatment and control groups. Minimizing the use of animals and the high cost of maintaining large animals, such as pigs, in a laboratory setting contributed to low animal group numbers in this initial study. Finally, although commonly performed in inflammatory ocular models, subjective scoring was used in several aspects of this study. When possible, standardized scoring schemes (e.g., Hackett-McDonald microscopic scores) were used and the observers were masked to the treatment groups to minimize bias and to increase objectivity in the observations.

Despite these study limitations, the results suggest that delivery of TA to the SCS provides effective therapy to reduce acute posterior uveitis in a model that is similar in anatomy, size, and retinal vascular pattern to the human eye. There were no adverse effects, increased IOP, or evidence of procedural or acute drug toxicity following injection of TA into the SCS in porcine eyes. Results of this study suggest that further evaluation of the use microneedles for the delivery of sustained release medications to the SCS is warranted.

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