Fluorophotometric and tonometric evaluation of ocular effects following aqueocentesis performed with needles of various sizes in dogs

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Objective—To measure ocular effects (blood-aqueous barrier breakdown and intraocular pressure [IOP]) following aqueocentesis performed with needles of various sizes in dogs.

Animals—28 healthy adult dogs.

Procedures—24 dogs underwent unilateral aqueocentesis (24 treated eyes and 24 contralateral untreated eyes); 25-, 27-, or 30-gauge needles were used in 3 treatment groups (n = 8/group). Four dogs were untreated controls. Aqueocentesis was performed during sedation and topical anesthesia. Anterior chamber fluorophotometry was performed before and after aqueocentesis on day 1. On days 2 through 5, sedation and fluorophotometry were repeated. Intraocular pressure was measured with a rebound tonometer at multiple time points.

Results—Aqueocentesis resulted in blood-aqueous barrier breakdown detected via fluorophotometry in all treated eyes, with barrier reestablishment by day 5. On day 2, the contralateral untreated eyes of all 3 groups also had significantly increased fluorescence. Use of a 25-gauge needle resulted in a significant increase in treated eyes’ anterior chamber fluorescence on days 3 and 5 as well as a significant increase in IOP 20 minutes following aqueocentesis, compared with the other treatment groups.

Conclusions and Clinical Relevance—Aqueocentesis performed with a 25-gauge needle resulted in the greatest degree of blood-aqueous barrier breakdown and a brief state of intraocular hypertension. Use of a 27- or 30-gauge needle is recommended for aqueous paracentesis. A consensual ocular reaction appeared to occur in dogs following unilateral traumatic blood-aqueous barrier breakdown and may be of clinical importance. (Am J Vet Res 2011;72:556–561)

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Abbreviation

| IOP | Intraocular pressure |

- Ocular anterior chamber paracentesis (ie, aqueocentesis) is a commonly performed procedure in which aqueous humor fluid is removed from the eye. Aqueocentesis is used in clinical practice for diagnostic and therapeutic purposes. It may be performed in dogs with anterior chamber disease to collect a sample of material for diagnostic purposes.1–3 It is also used as adjunct therapy in the emergency management of glaucoma to protect the retina from the harmful effects of high IOP.4–7

- Aqueocentesis is accomplished by inserting a 25- to 30-gauge hypodermic needle through the limbal cornea into the anterior chamber, with the needle passing parallel to the iris while avoiding contact with the iris, lens, or corneal endothelium.8,9 With proper patient preparation and aqueocentesis technique, the risk of complications is exceedingly low, but could include keratitis, cataract, or even endophthalmitis.2,10 It is important to note that aqueocentesis inevitably causes intraocular inflammation by inducing breakdown of the blood-aqueous barrier. For this reason, anterior chamber paracentesis has been used as a model of intraocular inflammation for research investigations in a variety of species.11–17

- The ocular blood-aqueous barrier is formed by the endothelium of the iris blood vessels, the nonpigmented layer of the ciliary epithelium, and the posterior pigmented epithelium of the iris.18 These structures normally prevent substances in the blood from entering the eye. When the barrier is disrupted, the blood vessels dilate and plasma proteins leak into the aqueous humor.19 Common causes of blood-aqueous barrier breakdown are anterior uveitis, ocular surgery, trauma, aqueous paracentesis, and ocular irritants. Blood-aqueous barrier breakdown can be assessed by subjective ophthalmic examination by use of a time-honored semiquantitative grading scheme, with aqueous flare indicating protein-
logic examinations were performed, and all dogs were
sion in the study, individual physical and ophthalmo-
binformation in the literature. Therefore, the purposes of the study reported here were to use an-
terior chamber fluorophotometry to evaluate the degree
of blood-aqueous barrier breakdown and use tonom-
metry to determine the IOP response, following aqueo-
centesis performed by use of variable needle sizes in
clinically normal dogs.

Materials and Methods

Animals—The use of dogs and all procedures in
this study were approved by the Institutional Animal
Care and Use Committee at Kansas State University.
Adult Beagles were obtained from the Kansas State Uni-
versity Department of Diagnostic Medicine/Pathobiol-
ogy following use in prior studies unrelated to ophthal-
mic research and, following completion of the study,
were returned for eventual adoption. Dogs were housed
individually in a temperature-controlled environment
illuminated by fluorescent lights that were automatical-
ly turned on (from 8 AM to 8 PM) and off. Prior to inclu-
sion in the study, individual physical and ophthalmo-
logic examinations were performed, and all dogs were
deemed healthy with no confounding conditions. Ocu-
lar examination included rebound tonometry, slit-lamp
biomicroscopy, and indirect ophthalmoscopy. Dogs
were adapted to human contact for a minimum of 3 to
6 weeks during previous research investigations. Three
dogs (2 sexually intact males and 1 sexually intact fe-
nal) were used for preliminary work to determine ide-
tal time points for study design. Twenty-eight dogs (13
sexually intact male dogs, 2 neutered male dogs, and
13 sexually intact female dogs) were used for the re-
search study, with 24 dogs in the treatment groups and
4 dogs in the control group. During the study, 1 control
dog developed a unilateral corneal ulcer; therefore, this
dog's data were removed from the control group for the
purposes of statistical analysis.

Aqueocentesis—Twenty-four dogs were allocat-
ed into 3 equal aqueocentesis treatment groups (25-, 27-,
or 30-gauge needle) via permuted block ran-
donimization. In each dog, the eye undergoing aqueo-
centesis was determined randomly by coin tossing and
the contralateral eye remained untreated. Four
dogs did not undergo aqueocentesis in either eye but
participated in all other aspects of the study and were
used as controls, with each eye treated as an indepen-
dent variable. Aqueous paracentesis, anterior cham-
ber fluorophotometry, and tonometric measurements
were all performed by a single investigator (RAA).
Dogs were sedated with ketamine (8.8 mg/kg, IM) and
xylazine (0.88 mg/kg, IM) prior to aqueocente-
sis and fluorophotometer scans for optimal patient
positioning and accurate measurements. Topical an-
esthetic (0.5% proparacaine) and povidone-iodine
ophthalmic solution were applied to the eye prior to
aqueocentesis. Bishop-Harmon forceps were used to
grasp the bulbar conjunctiva to stabilize the eye, and
a needle was inserted through the lateral perilimbal
cornea parallel to the iris. Care was taken to avoid
the iris, lens, and corneal endothelium. The needle
hub was allowed to fill halfway and then was rapidly
removed from the eye. No effort was made to prevent
regurgitation of aqueous humor through the corneal
puncture site. The aim of the study was to evaluate
the clinical practice of therapeutic aqueocentesis;
therefore, uncontrolled paracentesis was performed.

Fluorophotometry—A computerized scanning
ocular fluorophotometer with an anterior chamber
adapter was used to measure fluorescein concentra-
tions in the central anterior chamber of each eye following
administration of 10% fluorescein (20 mg/kg, IV). Each
dog was placed in sternal recumbency, the head was
stabilized, the eyelids were held open, and the eye was
positioned in front of the scanner. For consistency, the
left eye was always scanned first, followed immediately
by the right eye, with no more than 2 minutes elaps-
ing between measurements at each time point. Aque-
os humorr fluorescein values are reportedly maximal
and stable in dogs between approximately 30 and 90
minutes after IV injection of fluorescein. Results from
preliminary work with 3 dogs confirmed this finding,
and for the research study, all fluorophotometric read-
ings were scheduled during this appropriate postinjec-
tion period. Fluorophotometry was performed on se-
dated dogs prior to and following aqueocentesis on day
1, then daily through day 5 (and at equal time points in
control dogs). To minimize motion during fluorophoto-
metric readings, chemical restraint is commonly need-
ed in dogs, but administration of ketamine and xylazine
does not alter blood-aqueous barrier permeability.

Tonometry—All IOP measurements were per-
formed by use of a rebound tonometer as described.
Three consecutive IOP readings were obtained on each
eye according to manufacturer specifications, and IOP
was determined as the mean of these readings. Given
that the cornea would be anesthetized for IOP readings
immediately following aqueocentesis, initial tonom-
metric readings were taken both prior to and after appli-
cation of topical anesthetic to determine whether this
causedsignificant variation. To maintain consistent and
comparable IOP values throughout the study, topical
anesthesia was used for every tonometric measurement.

Study time points—The experimental sched-
ule was based on a report from a previous study and
results of preliminary testing on 3 dogs. Time points (hours:minutes) for the 24 treated dogs
were as follows on day 1, after initial examination and IOP determination: time 0:00, IV administration of fluorescein; 0:25, IM administration of sedative; 0:30, preaqueocentesis fluorophotometer scan (baseline); 0:33, preaqueocentesis IOP determination; 0:35, aqueocentesis; 0:36, 1 minute postaqueocentesis IOP determination; 0:55, 20 minutes postaqueocentesis IOP determination; 1:05, postaqueocentesis fluorophotometer scan; 1:15, 40 minutes postaqueocentesis IOP determination; 1:35, 60 minutes postaqueocentesis IOP determination; and IOP measurements continued every 60 minutes until 8 hours following aqueocentesis. Follow-up ocular examinations were performed 6 hours after aqueocentesis. Time points on days 2 to 5 were as follows, after examination and IOP determination: IV administration of fluorescein 1 hour prior to fluorophotometry; IM administration of sedative 10 minutes prior to fluorophotometry; and fluorophotometer scans every 24 hours after aqueocentesis. Control dogs were studied similarly in regard to data collection and timing; however, aqueocentesis was not performed, and only povidone-iodine solution and ophthalmic anesthetic were applied topically to the eyes at 0:30 on day 1.

Statistical analysis—Intracocular pressure values obtained prior to or following application of topical anesthetic were compared by use of a paired t test. Anterior chamber fluorescein values in the aqueocentesis treated versus the contralateral untreated eyes at each time period were compared within each treatment group by use of a paired t test. Aqueocentesis treatment groups were compared by use of repeated-measures ANOVA followed by a Newman-Keuls post hoc multiple comparisons test to discern individual differences. Anterior chamber fluorescein values in treated or contralateral untreated eyes were compared over time via repeated-measures ANOVA followed by a Newman-Keuls post hoc multiple comparisons test to discern individual differences. Multiple linear regression was used to evaluate the effect of treatment group on IOP over time, and ANOVA was performed to determine whether there were significant differences in IOP measurements at specific time points among treatment groups. Results are reported as mean anterior chamber fluorescence (ng/mL), as has been used in previous investigations.13-19 A commercial software program was used for all statistical analyses. Values of P < 0.05 were considered significant.

Table 1—Mean ± SD anterior chamber fluorescein concentrations (ng/mL) for treated, contralateral untreated, and control eyes at various time points in dogs that underwent aqueocentesis with needles of different gauges.

<table>
<thead>
<tr>
<th>Group</th>
<th>Preaqueocentesis</th>
<th>Postaqueocentesis</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>25-gauge treated</td>
<td>298.31 ± 140.94</td>
<td>2,174.96 ± 942.66*</td>
<td>1,953.65 ± 1,360.54* 1,419.71 ± 778.85* 800.50 ± 309.61 723.71 ± 278.82</td>
<td></td>
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<tr>
<td>25-gauge contralateral</td>
<td>282.64 ± 116.72</td>
<td>310.53 ± 128.66</td>
<td>760.01 ± 518.49 631.70 ± 313.80 504.18 ± 250.46 509.15 ± 221.69</td>
<td></td>
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<tr>
<td>27-gauge treated</td>
<td>355.11 ± 113.24</td>
<td>1,087.4 ± 421.49*</td>
<td>1,219.06 ± 348.89* 758.02 ± 190.34* 862.31 ± 192.47* 546.18 ± 126.72</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>27-gauge contralateral</td>
<td>338.15 ± 127.35</td>
<td>950.34 ± 111.89</td>
<td>567.73 ± 190.72 433.90 ± 101.22 459.29 ± 137.72 466.44 ± 110.06</td>
<td></td>
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<tr>
<td>30-gauge treated</td>
<td>290.29 ± 95.18</td>
<td>761.20 ± 418.01*</td>
<td>1,395.36 ± 1,064.39* 706.45 ± 338.09* 594.86 ± 238.46 483.13 ± 117.62</td>
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<tr>
<td>30-gauge contralateral</td>
<td>285.44 ± 80.74</td>
<td>315.71 ± 96.10</td>
<td>651.28 ± 414.23 540.54 ± 243.23 521.43 ± 252.23 442.15 ± 157.54</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All treated eyes</td>
<td>314.57 ± 110.51</td>
<td>1,041.19 ± 951.70*</td>
<td>1,565.83 ± 1,014.18* 964.40 ± 593.51* 685.83 ± 257.72* 584.00 ± 207.75</td>
<td></td>
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</tr>
<tr>
<td>All contralateral eyes</td>
<td>295.41 ± 112.22</td>
<td>327.53 ± 110.20</td>
<td>659.67 ± 389.33 533.54 ± 240.68 494.96 ± 212.00 465.91 ± 164.85</td>
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<td></td>
</tr>
<tr>
<td>Control eyes</td>
<td>289.57 ± 122.74</td>
<td>323.72 ± 119.42</td>
<td>372.83 ± 113.06 400.77 ± 125.82 349.65 ± 135.59 442.90 ± 129.31</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For each treated and contralateral untreated group, n = 8; for control eyes, n = 6. *Significant (P < 0.05) difference at that time point between treated and contralateral untreated eyes.
points. The day 4 fluorescence value was also significantly greater than prior to aqueocentesis. In the treated eyes of the 30-gauge treatment group, day 2 fluorescence was significantly greater than at all other time points. In the contralateral untreated eyes of the 30-gauge treatment group, the fluorescence value on day 2 was significantly greater than preaqueocentesis, postaqueocentesis, and day 5 values. Days 3 and 4 fluorescence values were significantly greater than prior to and after aqueocentesis. The day 5 fluorescein value was significantly greater than prior to aqueocentesis.

Although fluorescence changes were detected over time in the contralateral untreated eyes of all groups, no significant difference in anterior chamber fluorescence was found at any time point, compared with the control group. An unexpected finding was detected in the 1 control dog that developed a unilateral corneal ulcer. For the purposes of statistical analysis, this dog’s data were not included in the control eyes group; however, data values were still collected over the 5 days. On day 2 of the study, the anterior chamber fluorescence value in the ulcerated eye was 1,385 ng/mL, whereas the value in the contralateral healthy eye was 661 ng/mL. The 95% confidence interval for the day 2 control eyes was 282.4 to 463.3 ng/mL (n = 6; mean ± SD fluorescein concentration, 372.83 ± 113.06 ng/mL), which indicated that values from both the ulcerated and contralateral eyes of the dog in question were outliers.

Tonometry—Initial tonometric readings taken in each dog were obtained prior to and after application of topical anesthetic, and a significant (P = 0.001) difference in IOP was observed following topical anesthesia (no topical anesthesia, mean ± SD IOP, 21.20 ± 4.36 mm Hg; after topical anesthesia, 19.17 ± 3.60 mm Hg). This result was relevant for design of the study but did not have clinical importance. To maintain consistent and comparable IOP values throughout the study, topical anesthetic was used for every tonometric measurement.

When IOP was compared among treatment groups, a significant difference was present at 20 minutes after aqueocentesis; IOP in the 25-gauge treatment group (32.96 ± 13.03 mm Hg) was significantly (P = 0.03) higher than that in the 27-gauge (20.15 ± 8.07 mm Hg) or 30-gauge (19.54 ± 9.77) treatment groups (Figure 1). Aside from transient ocular hypertension in the 25-gauge treatment group, IOP rapidly returned to baseline values in treated eyes, and the IOP of contralateral untreated eyes had no clinically important changes.

Discussion

Consistent with previous reports, aqueocentesis caused blood-aqueous barrier disruption in all treated eyes of this study. Anterior chamber fluorophotometry allowed noninvasive, repeated daily assessment of the breakdown and reestablishment of the blood-aqueous barrier. Maximal fluorescence was detected in the treated eyes of all groups on day 2 (24 hours after aqueocentesis), with declining values thereafter. By day 5 in all treatment groups, the mean anterior chamber fluorescein value of the treated eyes was not significantly different than the contralateral untreated eyes.

It was of interest that the contralateral untreated eyes of all groups had maximal anterior chamber fluorescence on day 2 (Table 1). Values declined subsequently, but no significant difference was found between the contralateral untreated eyes and control eyes at any point in time. There are 2 possible explanations for this finding. The first supports the statistical analysis and suggests that aqueocentesis did not affect the contralateral eye and that the value disparities over time were attributable to random variation. The second explanation is that aqueocentesis did affect the contralateral untreated eye by causing a subtle degree of blood-aqueous barrier disruption as measured via fluorophotometry, but because of limited sample size and high variability, a significant difference was not detected. This latter theory is supported by an inadvertent finding in the control dog that developed a unilateral corneal ulcer. In that dog, a corneal ulcer was present in the left eye on day 2, and at that time, increased anterior chamber fluorescein concentration was measured. In addition, increased fluorescence was present on day 2 in the same dog’s healthy right eye. Over days 3 through 5, anterior chamber fluorescence declined in both eyes as the ulcer healed. The increased fluorescence in the ulcerated eye of this dog was not surprising and can be attributed to axonal reflex causing blood-aqueous barrier breakdown.29,30 It was the increased fluorescence in this dog’s contralateral healthy eye that paralleled the mean response seen in the contralateral eyes of treated dogs in this study; further supporting the possibility of a consensual ocular reaction in dog eyes.

Consensual ocular reactions have been reported in humans and rabbits,31–36 but have not been documented in dogs. Scanning electron microscopic examination of rabbit eyes treated with paracentesis and contralateral control eyes reveals changes in ciliary body processes consistent with both a direct and consensual reaction.37 The mechanism for this reaction is hypothesized to be a neural reflex arc,32,33,37 but others suggest it is attributable to a transfer of prostaglandins via systemic circulation.31,35 The consensual ocular reaction is an important biological finding and is clinically noteworthy because it was documented immediately following and then up to 1 month after cataract surgery in humans.32 Although it is commonly recognized that drugs applied topically to 1 eye can result in effects in the opposite eye, likely...
because of systemic absorption of the medication,38-43 the present study is the first to suggest a consensual blood-aqueous barrier breakdown reaction in dogs.

Results of the present study indicated that aqueocentesis performed with a 25-gauge needle caused greater blood-aqueous barrier breakdown than aqueocentesis performed with a 27- or 30-gauge needle. Significant differences were documented by use of anterior chamber fluorophotometry on days 3 and 5. The large individual variances observed at other time points were likely caused by the uncontrolled aqueous paracentesis. This method causes larger variance than does controlled, timed paracentesis,44 but is the technique commonly used during therapeutic aqueocentesis. The aim of the study was to evaluate the clinical practice of aqueocentesis and its effect on blood-aqueous barrier breakdown, so uncontrolled paracentesis with various needle sizes was performed.

In this study, the cause of increased blood-aqueous barrier breakdown was not clear, but appeared to be needle size. It is logical that a larger needle stimulates more corneal sensory nerves or results in greater corneal tissue trauma, ultimately releasing additional inflammatory mediators, compared with a smaller needle; however, consistent topical anesthesia should have minimized these responses. Although the degree of ocular hypotony does affect blood-aqueous barrier breakdown and causes increased protein content in the reformed aqueous,44 in the present study the mean IOP immediately after aqueocentesis was not significantly different between treatment groups (Figure 1). The speed of fluid flow into the needle could also be considered as a cause. A 1940 study44 revealed that speed of aspiration during aqueous paracentesis in humans had no effect on protein content of the reformed aqueous humor; however, aqueous protein content was measured subjectively via turbidity following addition of sulphosalicylic acid. Quantitative evaluation of aqueous humor protein concentration may reveal that outflow speed does affect blood-aqueous barrier breakdown and therefore may be the cause of differences in our study. In addition, there is considerable species variation in the responsiveness of the eye to damage.19,36-46 so prior studies on humans or other species may not be appropriate for extrapolation to dogs.

Not only did 25-gauge needle aqueocentesis induce greater blood-aqueous barrier breakdown, it also resulted in transient ocular hypertension 20 minutes following treatment. This point of increased IOP was an unexpected finding given that in all treatment group eyes, the IOP immediately after aqueocentesis was a mean value of 2 mm Hg. Increased IOP in the 25-gauge treatment group was consistent with a greater degree of blood-aqueous barrier breakdown because initial ocular hypertension is found in uveitis caused by prostaglandin release.15-47 Paracentesis-induced ocular hypertension is likely attributable to a sudden increase in anterior uveal blood volume with a subsequent increase in ultrafiltration and plasma extravasation.19,46 Paracentesis-induced blood-aqueous barrier breakdown in dogs is primarily mediated by prostaglandins.48 Although topically administered flurbiprofen significantly reduces blood-aqueous barrier breakdown as measured via anterior chamber fluorophotometry,48 the inability of flurbiprofen and proparacaine to completely abolish the response suggests that additional nonprostaglandin, non–sensorineurally derived mediators may be involved or that the rapid reduction in IOP causes physical damage to the blood-aqueous barrier.11,12,48

Although dogs with glaucoma were not evaluated in the present study, the rapid resolution of ocular hypotony in all groups confirmed the assumption that aqueocentesis alone is not sufficient treatment for increased IOP in dogs. This is consistent with a human study7 in which cataract surgery patients with postoperative ocular hypertension treated with paracentesis had an immediate reduction in IOP followed by rebounding pressures to near initial values 1 hour after treatment. Conversely, aqueous paracentesis combined with medical treatment provides rapid relief of acutely increased IOP and could be considered as adjunctive therapy.6,7

Aqueocentesis performed with a 25-gauge needle resulted in the greatest degree of blood-aqueous barrier breakdown and a brief state of ocular hypertension. Use of a 27- or 30-gauge needle is therefore recommended for aqueous paracentesis because there was no significant difference in fluorescein concentration or IOP between those treatment groups. Peak anterior chamber fluorescence was detected in the contralateral untreated eyes of all treatment groups on day 2, suggesting a consensual ocular reaction in dogs; however, values were not significantly greater than those in control eyes. Substantial variability common in biological systems complicates research studies, and as in this investigation, high variability and large SDs are a problem with statistical analysis. Given the known risks and possible complications, aqueocentesis should be performed only in select cases in which the diagnostic or therapeutic benefit outweighs the potential consequences.

References

a. TonoVet, Tiolat Ltd, Helsinki, Finland.
b. SL-14 Biomicroscope, Kowa Co Ltd, Tokyo, Japan.
c. HEINE Omega 180 Ophthalmoscope, HEINE Optotechnik, Herrsching, Germany.
e. AnaSed, Ben Venue Laboratories, Bedford, Ohio.
f. 0.5% proparacaine hydrochloride ophthalmic solution, Akorn Inc, Buffalo Grove, Ill.
g. Betadine 5% sterile ophthalmic prep solution, Catalent Pharma Solutions LLC, Woodstock, Ill.
h. FM-2 Fluorotron Master, OcuMetrics Inc, Mountain View, Calif.
i. AK-FLUOR, Akorn Inc, Buffalo Grove, Ill.

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