Differential Intraocular Pressure Measurements by Tonometry and Direct Cannulation After Treatment with Soluble Adenylyl Cyclase Inhibitors

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Abstract

Purpose: To validate the increase in intraocular pressure (IOP) caused by soluble adenylyl cyclase (sAC) inhibitors and determine reasons behind variation in IOP measurements performed by tonometry.

Methods: C57BL/6J mice were administered DMSO solubilized sAC inhibitors (KH7 or LRE-1) by intraperitoneal injection. Two hours post-treatment, mice were anesthetized with avertin or ketamine/xylazine/acepromazine (KXA). IOP was measured by a rebound tonometer or direct cannulation of the anterior chamber. Spectral-domain optical coherence tomography was used to measure anterior chamber depth and corneal thickness in live mice. Outflow facility was measured in perfused, enucleated mouse eyes.

Results: Compared with DMSO controls, KH7 treatment caused an increased IOP in avertin- and KXA-anesthetized mice when measured by direct cannulation [avertin: 14.4 ± 2.1 mmHg vs. 11.1 ± 1.0 mmHg ($P=0.003$); KXA: 14.4 ± 1.0 mmHg vs. 11.3 ± 0.8 mmHg ($P<0.001$)] and tonometry [avertin: 10.8 ± 1.4 mmHg vs. 7.4 ± 0.6 mmHg ($P<0.001$); KXA: 11.9 ± 0.9 mmHg vs. 10.3 ± 1.7 mmHg ($P=0.283$)]. However, treatment with KH7 in non-anesthetized mice showed a significant decrease in IOP measured by tonometry and compared with DMSO-treated animals [13.1 ± 2.6 mmHg vs. 15.6 ± 0.5 mmHg ($P=0.003$)]. Both KH7- and DMSO-treated groups anesthetized with avertin showed increased corneal thickness, whereas KH7-treated mice anesthetized with KXA exhibited a shallower anterior chamber compared with untreated mice. KH7 decreased outflow facility by 85.1% in nonanesthetized, enucleated eyes ($P<0.003$).

Conclusions: Systemically administered DMSO and anesthesia have significant effects on anterior chamber characteristics, resulting in altered IOP readings measured by tonometry. In the presence of DMSO and anesthesia, tonometry IOP readings should be confirmed with direct cannulation.

Keywords: intraocular pressure, tonometry, cAMP, cornea

Introduction

TONOMETRY IS A WIDELY ACCEPTED method for measurement of intraocular pressure (IOP) because of its ease of use, cost effectiveness, reproducibility, and noninvasiveness. Rebound tonometry uses a physical probe to measure induction–impact on the cornea, with the speed of probe rebound used to calculate IOP indirectly.^{1,2} It is commonly used as an efficient way to test the effect of various drugs on IOP. Because of its minimal contact with the eye, tonometry is often used in conscious animals without topical or systemic anesthesia to avoid unknown and confounding effects of the drug with commonly used anesthetic agents.^{3–5}

Many reports have confirmed the accuracy of tonometry readings through simultaneous validation with direct cannulation methods, which have been traditionally accepted as the most accurate method of measuring IOP.^{6–8} Unlike tonometry, direct cannulation studies require the use of an anesthetic agent.

KH7 and LRE1, specific inhibitors of soluble adenylyl cyclase (sAC), have been proposed as therapeutic agents for ocular hypotony.^{9–11} sAC is found in the ciliary body and is known to catalyze the formation of cAMP in response to increasing intracellular bicarbonate [HCO_3^-].^{12,13} KH7 has previously been shown to increase IOP in mice through direct cannulation.¹⁰ During subsequent studies looking at

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the effect of KH7 on IOP, we were surprised to see an opposite (ocular hypotensive) effect of KH7 when IOP was measured by rebound tonometry in nonanesthetized mice.

To understand the reasons behind this discrepancy, we treated mice with KH7 or DMSO vehicle control along with 2 distinct anesthetic agents [avertin and ketamine/xylazine/acepromazine (KXA)] and measured IOP in these animals with both tonometry and direct cannulation. Corneal thickness and anterior chamber depth were measured by spectral-domain optical coherence tomography (SD-OCT) to give insight into possible explanations of the discrepancy. Finally, a second, structurally unrelated, sAC-specific inhibitor, LRE1, was tested to reaffirm that the discrepancy was independent of the KH7 compound.

Methods

Animal husbandry and treatment

All animal procedures were performed in accordance with the guidelines in the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research, and received prior approval from the Mayo Clinic Animal Care and Use Committee (IACUC). Wild-type, female C57BL/6J mice, aged 8–12 weeks, obtained from Jackson Laboratories were maintained in the Mayo Clinic Animal Care Facility under a 12-h light–12-h dark cycle. Mice received standard rodent chow and water *ad libitum*. After a minimum 5-day acclimation to the facility, mice were injected intraperitoneally with KH7 dissolved in DMSO (1.25 mM in 4 mL/kg), LRE1 dissolved in DMSO (1.25 mM in 4 mL/kg), DMSO alone (4 mL/kg), saline alone (4 mL/kg), or left untreated. With time of drug administration at 0 h, IOP readings were taken by tonometry at –1, 0, and 2 h.

Two hours after drug administration, mice were anesthetized by intraperitoneal injection with avertin (250–500 mg/kg) or ketamine (80 mg/kg)/xylazine (5 mg/kg)/acepromazine (1 mg/kg) (KXA). Studies were initiated between 10 am and 1 pm to remove the possibility of a circadian rhythm effect on IOP. Animals were sacrificed by CO₂ asphyxiation using a rodent euthanasia machine with 4-step automatic CO₂ inflow, as per IACUC guidelines.⁵

IOP measurements

The experiments were conducted within a precise sequence of time. At –1 h, nonanesthetized, untreated rebound tonometry readings were taken of all animals. At time zero, another tonometer reading was taken. Immediately after the time zero measurement, the drug was administered. At +2 h, another tonometer reading was taken followed immediately by anesthesia. Approximately 10 min after confirming unconsciousness, OCT imaging was performed. Following OCT (~25 min after unconsciousness), direct cannulation IOP was measured. Saline drops were administered before OCT imaging and direct cannulation to maintain hydration.

Tonometry. IOP was measured in conscious and anesthetized mice with a hand-held rebound tonometer (Icare TonoLab; Colonial Medical Supply, Franconia, NH) pre and post-treatment. Briefly, the tonometer was placed in front of the eye so that the probe would hit the cornea perpendicularly. The tonometer records 6 pressure readings at each

measurement, discards the highest and lowest values, and the average of the 4 remaining values is recorded as the IOP. Three measurements were taken at a given time point and the average of the values was recorded as the overall IOP. The tonometer does not measure IOPs below 7 mmHg. Therefore, individual trial values less than 7 mmHg on the rebound tonometer are reported as 7 mmHg.

IOP cannulation. Cannulation of the anterior chamber was performed as previously described,^{14,15} with the following modifications. Briefly, following systemic administration of anesthesia, the eye was anesthetized with topical 0.05% proparacaine (Bausch & Lomb, Rochester, NY). The anterior chamber was cannulated with a 33-gauge stainless steel needle (WPI, Sarasota, FL) entering the anterior chamber through the cornea anterior to the limbus. The location of the bevel in the up position was visually confirmed anterior to the iris before recording measurements. The cannula was connected to a pressure transducer (Deltran II; Utah Medical Products, Midvale, UT) using a teflon tube and calibrated to a water height equivalent of 0 mmHg. The transducer signal was amplified (Bridge 8; World Precision Instruments, Sarasota, FL) and converted to a digital signal (iWorx 118; iWorx, Dover, NH) and voltage was read on a computer using LabScribe 3 software (iWorx). IOP measurements were determined by comparing the change in voltage to a standard calibration curve generated at the end of each experiment using a water height column. Compliance of the setup, including the mouse eye, was assumed to fall within the standard deviation of each group.

Spectral-domain optical coherence tomography

SD-OCT imaging was used to generate digital images of the cornea and the anterior chamber of anesthetized mice (Envisu R4110; Leica Microsystems, Buffalo Grove, IL) before cannulation. Briefly, anesthetized mice were placed on a 3-axis holding tunnel to achieve proper orientation of the cornea to the OCT probe. Following corneal alignment, 2 mm linear B-scans were performed 3 times on each cornea with 30 frames per B scan. To obtain the final image, data were averaged from all frames to eliminate background noise. Measurements were performed using the caliper function of the accompanying software (Envisu In Vivo Imaging Software). Three separate measurements were averaged for each animal's corneal thickness. The anterior chamber depth was measured as perpendicular distance from the apex of the cornea to the lens.

Outflow facility

Mice were sacrificed by CO₂ asphyxiation and cervical dislocation. Outflow facility was determined in *ex vivo* mouse eyes according to the method of Boussemier-Calleja et al.¹⁶ In brief, eyes were cannulated with a 33-gauge needle and perfused with Dulbecco's phosphate-buffered saline, including divalent cations and 5.5 mM glucose (DBG) containing 5 μ M KH7 prepared by diluting a 1.25 mM stock solution of KH7 dissolved in DMSO, or DBG containing DMSO, the approximate body mass dilution following systemic delivery. Eyes were perfused for 40 min at 8 mmHg and then pressure was clamped at 4 pressures (4, 8, 15, and 25 mmHg) in succession for 20 min at each pressure. Outflow facility was

determined from the slope of the line generated from the function of flow versus pressure.

Statistics

Statistical analysis was performed using JMP 10 software (JMP; SAS, Cary, NC). Comparisons of all drug-treated groups as per anesthetic, which included tonometry, corneal thickness, anterior chamber depth, and cannulation IOP, were assessed by 1-way analysis of variance. A Tukey's test was utilized to compare individual groups directly. For all experiments, significance was established with $P < 0.05$.

Results

Effect of KH7 treatment on IOP

We have previously reported that KH7 has an ocular hypertensive effect on anesthetized mice as measured by direct cannulation.¹⁰ These data were reconfirmed using direct cannulation on untreated mice or mice administered KH7, saline, or vehicle alone (DMSO), and anesthetized with avertin. KH7 caused an increase in IOP compared with both DMSO-treated and untreated mice (KH7, 14.5 ± 1.0 mmHg, $n=8$; DMSO, 11.1 ± 1.0 mmHg; saline, 12.8 ± 1.7 mmHg; untreated, 12.3 ± 1.6 mmHg; $n=6$, $P=0.003$) consistent with our previous observations.¹⁰

To validate this result in nonanesthetized animals, we treated C57BL/6J mice with KH7, saline, or vehicle only (DMSO) and measured IOP with a rebound tonometer. Interestingly, mice administered with KH7 showed a reduction in IOP compared with the DMSO, untreated, and saline-treated groups (KH7, 13.1 ± 2.6 mmHg; DMSO, 15.6 ± 0.5 mmHg; saline, 14.7 ± 0.3 mmHg; untreated 16.3 ± 1.3 ; $n=6$, $P < 0.001$). This contradicts the data obtained using direct cannulation.

Rebound tonometry was then repeated after anesthetizing the animals with avertin. This time, KH7-treated animals showed an increase in IOP compared with the DMSO control group (11.9 ± 0.7 mmHg vs. 7.4 ± 0.6 mmHg; $n=6$, $P < 0.001$). However, compared with the untreated and saline group, IOP was significantly decreased in both KH7- and DMSO-treated animals (saline, 13.0 ± 1.8 mmHg; untreated, 15.1 ± 1.0 mmHg; $n=6$, $P < 0.001$) (Fig. 1). Because the tonometer records IOP as 7 mmHg for any IOP below 7 mmHg, this may skew the average values of the DMSO and KH7 groups higher than the true reading. Nevertheless, a low reading of 7 mmHg will show the trend of IOP reduction obtained by tonometry. It is also important to note that, while the trends are similar, the IOP values measured by tonometry and cannulation are not the same, likely due to the different mechanism of calibration.

Effect of KH7 treatment on IOP of mice anesthetized with KXA

To determine if avertin use was one of the confounding factors behind the inconsistencies observed in the effect on IOP of KH7 in conscious versus anesthetized mice, we performed similar experiments in animals anesthetized with KXA. When IOP was measured by direct cannulation, the KH7-treated group exhibited a significantly higher IOP than the DMSO control group (14.4 ± 1.0 mmHg vs. 11.3 ± 0.8 mmHg; $n=6$, $P < 0.001$). By tonometry, treatment with KH7 resulted in an increase in IOP compared with the DMSO control group

(11.9 ± 0.9 mmHg vs. 10.3 ± 1.7 mmHg; $n=6$). However, this increase was not significant ($P=0.281$). As was observed with avertin anesthesia, IOP values after KH7 treatment, although higher than DMSO controls, were still significantly lower than saline and untreated controls (KH7, 11.9 ± 0.9 mmHg; saline, 14.2 ± 1.6 mmHg; untreated, 15.3 ± 0.9 mmHg; $n=6$, $P < 0.001$).

In the DMSO vehicle-treated group, nonanesthetized mice showed no change in IOP in comparison to saline and untreated controls (DMSO, 16.4 ± 1.1 mmHg; saline, 15.5 ± 0.8 mmHg; untreated, 16.0 ± 1.0 mmHg; $n=6$, $P=0.10$). However, when DMSO-treated animals were anesthetized with KXA, IOP dropped to 10.3 ± 1.6 mmHg ($n=6$, $P < 0.001$). Tonometry values were similar for IOP in untreated and saline-injected animals, before and after administration of KXA (untreated: 15.3 ± 1.2 vs. 16.9 ± 2.0 mmHg, $P=0.070$; saline: 15.5 ± 0.8 vs. 14.2 ± 1.6 mmHg, $P=0.051$; $n=6$) (Fig. 2). These trends are similar to those observed with avertin-anesthetized mice.

Corneal thickness and anterior chamber depth measurements using SD-OCT

Since tonometry measurements can be confounded by anterior chamber properties independent of IOP, we assessed corneal thickness and anterior chamber depth in KH7-treated and DMSO control mice in the presence of different anesthetics (Fig. 3A). In animals anesthetized with avertin, KH7-treated (92 ± 11 μ m) and DMSO control-treated corneas (92 ± 12 μ m) were both significantly thicker than saline-injected and untreated control animals anesthetized with avertin (saline, 71 ± 10 μ m; untreated, 71 ± 7 μ m; $n=6$, $P < 0.001$). Interestingly, no difference in corneal thickness was noted in mice anesthetized with KXA and treated with KH7 (100 ± 13 μ m) versus DMSO (101 ± 11 μ m), saline (77 ± 16 μ m), or left untreated (93 ± 19 μ m; $n=6$, $P=0.63$) (Fig. 3C). However, DMSO treatment did significantly increase corneal thickness compared with saline ($P=0.039$). Due to the inconsistency between untreated avertin and untreated KXA corneal thickness, a comparison of corneal thickness between avertin and KXA treatment showed a statistical difference ($P=0.006$).

Avertin-anesthetized mice showed no difference in anterior chamber depth between untreated (399 ± 12 μ m), DMSO-treated (390 ± 19 μ m), saline-treated (410 ± 16 μ m), and KH7-treated (394 ± 21 μ m) ($n=6$, $P=0.65$) groups. However, anterior chambers for KH7-treated groups anesthetized with KXA were significantly shallower than anterior chambers of untreated control mice (KH7, 365 ± 32 μ m; DMSO control, 393 ± 13 μ m; saline, 395 ± 17 μ m; untreated, 405 ± 18 μ m; $n=6$, $P=0.026$), (Fig. 3B).

KH7 treatment of nonanesthetized mice shows a decrease in outflow facility

We have previously shown that KH7 treatment of avertin-anesthetized mice decreases outflow facility without altering inflow. This supports the finding that KH7 increases IOP.¹⁰ The same work has also shown no difference in saline or DMSO infusion. As direct measurements of IOP are not possible in nonanesthetized mice, outflow facility was investigated using an *ex vivo* system¹⁶ with eyes obtained postmortem from mice treated with KH7 or vehicle control and euthanized by CO₂ and cervical dislocation. Mice were not

FIG. 1. IOP in avertin-anesthetized mice. KH7 treatment resulted in an increase in IOP for mice measured by direct cannulation compared with untreated, saline-injected, and vehicle-only (DMSO) controls (**A**, mean $\pm$ SD, $n=8$). In contrast, measurements of IOP made by tonometry before anesthesia suggested a decrease in IOP in mice treated with KH7 compared with both untreated, saline injected, and DMSO-treated controls (**B**, mean $\pm$ SD, $n=6$). After avertin anesthesia, IOP measured by tonometry was decreased in both KH7-treated and DMSO controls relative to untreated controls and saline-injected animals, although KH7 increased IOP relative to DMSO controls (**C**, mean $\pm$ SD, $n=6$). ** Indicates $P<0.01$ and *** indicates $P<0.001$. IOP, intraocular pressure; SD standard deviation.

anesthetized before sacrifice. Outflow facility for untreated mice using this technique was similar to mice receiving vehicle only and perfused with DPG containing DMSO (untreated: $0.0118 \pm 0.0048 \mu\text{L}/\text{min}$; DMSO: $0.0114 \pm 0.0019 \mu\text{L}/\text{min}$; $n=6$). Pretreatment with KH7 and addition of KH7 to the perfusate resulted in a significant reduction of 85.1% in outflow facility ($0.0017 \pm 0.0006 \mu\text{L}/\text{min}$; $n=6$, $P<0.003$) (Fig. 4).

LRE-1 treatment shows similar inconsistency in IOP between rebound tonometry and cannulation

To determine whether the inconsistency observed between tonometry and direct cannulation was due to KH7, we tested the effects of a second, structurally unrelated, sAC-specific inhibitor, LRE1 (Fig. 5A).^{17(p1)} The effect of LRE1 on IOP was previously unknown. Direct cannulation was performed to investigate if LRE1 elicits an increase in IOP in anesthetized mice similar to KH7. After LRE1 adminis-

tration, avertin-anesthetized animals exhibited an increase in IOP compared with DMSO-treated mice (LRE1-treated: $15.6 \pm 3.5 \text{ mmHg}$; DMSO-treated: $12.1 \pm 1.7 \text{ mmHg}$; $n=15$, $P=0.002$) (Fig. 5B). This result suggests that LRE1 causes an increase in IOP, similar to KH7 treatment.

Without anesthesia, tonometric IOP measurements of LRE1-treated animals showed a decrease compared with DMSO-treated animals, but were similar to IOPs measured in KH7-treated animals reported earlier (LRE1-treated: $12.1 \pm 0.8 \text{ mmHg}$; DMSO-treated: 16.1 ± 1.0 ; $n=12$, $P<0.001$) (Fig. 5B). Thus, the effects of LRE1 are similar to those of KH7.

Discussion

In our efforts to test the effect of sAC inhibitors on IOP, we discovered a discrepancy in readings through tonometry with and without anesthetic. Our data suggest that this

FIG. 2. IOP in KXA-administered mice. KH7 treatment resulted in an increase in IOP for mice measured by direct cannulation compared with both untreated, saline-injected, and DMSO groups (**A**, mean $\pm$ SD, $n=6$). In contrast, measurements of IOP made by tonometry before anesthesia suggested a decrease in IOP in mice treated with KH7 compared with both untreated, saline-injected, and DMSO-treated controls (**B**, mean $\pm$ SD, $n=6$). After KXA anesthesia, IOP measured by tonometry was decreased in both KH7-treated and DMSO controls relative to untreated and saline controls, but KH7 increased IOP relative to DMSO controls (**C**, mean $\pm$ SD, $n=6$). These trends are the same as with the avertin data. ** Indicates $P<0.01$ and *** indicates $P<0.001$. KXA, ketamine/xylazine/acepromazine.

discrepancy appears to be due to effects of DMSO and anesthesia on anterior chamber properties, *viz.* corneal thickness and anterior chamber depth, parameters that can affect IOP readings as measured by tonometry. Therefore, use of tonometry to assess the effects of experimental drugs, par-

ticularly those dissolved in DMSO and related solvents should proceed with caution and be validated by direct cannulation and/or outflow facility measurements.

Tonometry is an indirect measure of IOP, which assumes that corneal hoop stress reflects IOP. Rebound tonometry

FIG. 4. *Ex vivo* outflow facility measurements. Outflow facility was determined by pressure clamping enucleated eyes obtained from nonanesthetized animals sacrificed by CO₂ asphyxiation. By plotting the relationship of flow to pressure in various drug-treated eyes, outflow facility (C_1) is calculated as the slope of a line drawn through the points (A, mean $\pm$ SD, $n=6$). KH7 treatment resulted in a significant reduction in C_1 compared with control (untreated) and DMSO groups (B, mean $\pm$ SD, $n=6$). ** Indicates $P<0.01$.

FIG. 5. Effect of sAC inhibitor LRE1 on IOP. Chemical structures of the 2 sAC inhibitors, KH7 and LRE1 (**A**). Note that these compounds are distinctly different. LRE1 dissolved in DMSO increased IOP compared with DMSO alone as measured by direct cannulation (**B**, mean $\pm$ SD, $n = 15$). In contrast, when measured in nonanesthetized mice by tonometry, LRE1 appeared to decrease IOP in comparison to DMSO alone (**C**, mean $\pm$ SD, $n = 12$). ** Indicates $P < 0.01$ and *** indicates $P < 0.001$. sAC, soluble adenylyl cyclase.

assumes that the rebound force is proportional to the IOP, and considers that the proportionality is constant. That is, the influence of the cornea is unlikely to change between readings or across experimental and control groups. However, this proportionality can be affected by the mechanical properties of the cornea. A previous report has shown that the Goldmann applanation tonometer is sensitive to corneal biomechanics.¹⁸ This was seen as an artificial drop in IOP with confounding decrease in corneal thickness in the same patients before and after LASIK surgery.¹⁹ Pascal dynamic contour tonometry was used to show that there was no drop in IOP when measured independently of corneal biomechanics.¹⁹

Corneal thickness, the most commonly studied parameter of corneal biomechanics, has been known to have a large effect on IOP.^{20–22} It is important to note that this variation is intrapersonal, likely due to the variation in corneal composition between patients. In general, an increase in corneal thickness was noted with increased IOP.^{20,21} However, no correlation between variable corneal thickness and IOP was found across numerous knockout mouse strains when IOP was measured by tonometry with KXA.²² Interestingly, corneal thickness values reported with KXA were similar to the corneal thickness measurements reported within our study. However, this study did not account for acute variation in thickness.

Our study used genetically similar mice and identified changes in corneal thickness correlating with treatment. C57BL/6J mice have previously been shown to have low variation of corneal thickness, under KXA anesthesia.²³ The increase in corneal thickness due to DMSO and avertin is likely due to changes of hydration within the tissue. As swelling increases the thickness of the cornea, a softer cornea would result in a reduction in tonometric IOP. This may explain why the IOP reading is lower after DMSO and avertin treatment in our study since the tonometer probe would rebound at a slower speed after hitting a flaccid cornea. The hypothesis that DMSO and avertin-dependent swelling is causing the discrepancy is supported by the OCT data, which shows the increase in corneal thickness. Whether this increase in thickness is caused by swelling or edema alone is difficult to determine from OCT images. It is also important to note that DMSO has previously been reported with systemic toxic effects,²⁴ including in the cornea.

In vivo, it is difficult to alter corneal swelling alone without affecting IOP. Drugs, such as ouabain, do induce corneal swelling, but also directly affect IOP.²⁵ Thus, experiments with artificial anterior chambers have been conducted to isolate corneal contributions. For example, a previous study showed the inaccurate reporting of IOP by multiple methods

of tonometry, including rebound tonometry (the least variable of the group), on human *ex vivo* corneas with edema.²⁶ Unfortunately, nonedematous corneas were not used as a control. Additionally, the *ex vivo* postmortem tissue source makes it difficult to study acute edema.

Anterior chamber depth measurements are a common tool in glaucoma prevention. Typically, shallow anterior chamber depth is associated with an increase in IOP.²⁷ However, it is not understood if this is a cause or an effect of the drug interaction on anterior chamber depth. While the KXA-KH7 group showed a decrease in anterior chamber depth, no changes were detected in the avertin groups. This result is perplexing, as a similar effect on tonometric IOP is still yielded. Other contributors, such as corneal extracellular matrix properties, may also have a role and warrant future studies.

While avertin is an anesthetic with an unknown mechanism of action, KXA is an anesthetic dissociative agent with analgesic and sedative properties resulting from *N*-Methyl-D-aspartate receptor and dopamine inhibition, and alpha-2 receptor activation.^{28,29} KXA-anesthetized animals did not appear to show a change in corneal thickness after DMSO or KH7 treatment. However, comparing untreated KXA and untreated avertin groups suggests that KXA may independently cause an increase in corneal thickness compared with avertin. This effect may disguise the effect DMSO would have on corneal thickness in the KXA group. Previous literature has shown that ketamine may be involved in pulmonary edema,^{30,31} lending support to this hypothesis.

Inhibitors of sAC represent a potential class of therapeutic compounds that could be used to treat ocular hypotony. Our efforts to better understand the effects of these compounds on IOP led us to observe a discrepancy between IOPs determined using rebound tonometry in the presence and absence of anesthetics. Direct cannulation and outflow facility measurements confirmed that tonometry in the presence of anesthetics accurately depicted IOP. In contrast, tonometry used on nonanesthetized animals suggested an IOP-lowering effect of the drug. By monitoring the confounding effects of anesthetics and vehicle (DMSO), our work suggests that significant changes to corneal thickness and anterior chamber properties may account for this discrepancy in tonometry readings. This discrepancy is not explained by the drug used, as a chemically different sAC inhibitor, LRE1, showed a similar discrepancy: an increase in IOP using direct cannulation but decrease in tonometric IOP without anesthetic. This discrepancy is also not explained by handling-related variables, as saline-treated animals showed similar values to untreated animals. As such, there appears to be a confounding effect of using DMSO in the presence of anesthesia that likely explains the difference in IOP readings with tonometry done in conscious and anesthetized animals. Despite this, some drugs that lower IOP, for diseases such as glaucoma, have been shown through both tonometry and direct cannulation to decrease IOP. For example, timolol has been shown to decrease IOP using direct cannulation as well as tonometry with and without anesthesia.³² It is important to note that timolol is water soluble, and does not require DMSO, providing support to the finding.

Conclusions

Based on these data, we suggest that indirect measures of IOP, such as rebound tonometry, should be used with cau-

tion, particularly in the presence of solvents such as DMSO. We suggest that direct cannulation and parallel validation with outflow facility measurements are necessary to confirm the IOP-altering ability of investigational compounds, especially those requiring nonaqueous solvents like DMSO.

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Author Disclosure Statement

No competing financial interests exist.

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