MEASURING INTRAOCULAR PRESSURE IN WHITE'S TREE FROGS (*LITORIA CAERULEA*) BY REBOUND TONOMETRY: COMPARING DEVICE, TIME OF DAY, AND MANUAL VERSUS CHEMICAL RESTRAINT METHODS

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Abstract: Ocular diseases reported in frogs include uveitis and glaucoma, which are associated with changes in intraocular pressure (IOP). The objectives of this study were to characterize the normal IOP for White's tree frogs (*Litoria caerulea*) using two types of rebound tonometers, and to assess whether time of day or method of restraint affected IOP. Eighteen conscious, unrestrained, ophthalmologically normal frogs were used to measure IOP using TonoVet® and TonoLab® tonometers, at three time points during the day. In a subset of 12 frogs, IOP was measured while under manual restraint using the TonoVet. Anesthesia was induced in 9 frogs using two different concentrations of MS-222 (0.5 g/L and 2 g/L) in order to evaluate for changes in IOP with the TonoVet. Mean (± SD) IOP values for the TonoLab (16.8 ± 3.9 mm Hg) were significantly higher than TonoVet values (14.7 ± 1.6 mm Hg; $P < 0.01$). TonoVet IOP values did not significantly change with time of day. TonoLab values were significantly lower in the evening (1600–1800; 14.5 ± 3.1 mm Hg), compared with morning and midday measurements (0800–1000 and 1200–1400; 18.0 ± 3.8 mm Hg; $P < 0.01$). Manually restrained frogs had significantly lower IOP (13.4 ± 1.5 mm Hg) compared with unrestrained frogs (15.3 ± 1.2 mm Hg; $P < 0.01$). Chemical restraint did not cause significant changes in IOP. Intraocular pressure can be measured with both types of rebound tonometers in White's tree frogs, but time of day and manual restraint can affect IOP values.

Key words: Circadian rhythm, intraocular pressure, *Litoria caerulea*, MS-222, rebound tonometry, White's tree frogs.

INTRODUCTION

Intraocular pressure (IOP) is a result of a delicate balance between the production of aqueous humor and its outflow from the eye.[18] Intraocular disorders frequently disrupt this balance, resulting in increases or decreases in IOP over baseline values.[18] Therefore, measuring IOP with a tonometer is regarded as an essential component of a full ophthalmic examination in domestic mammals and an important tool in directing the therapy of ocular disorders.

The anatomy and physiology of the amphibian eye has been frequently studied, but little is known regarding the clinically relevant ocular abnormalities in amphibians.[10,12,23,25] The major ophthalmologic conditions reported in the literature for amphibians include trauma, lipid keratopathy, cataracts, uveitis, glaucoma, hypopyon, hyphema, chorioretinitis, conjunctivitis, ulcera-tive keratitis, parasite migration, and neoplasia.[10,12,23,25] However, very little is known about causal factors, and treatment options are primarily based on anecdotal reports and presumptions based on therapy for similar disorders in mammals.[23,25]

The production and flow of aqueous humor within the amphibian eye is very similar to other species, with the exception that aqueous humor flows out of the iridocorneal angle into two ciliary venous sinuses instead of an episcleral venous plexus.[25] This indicates that intraocular disorders in amphibians are also very likely to result in IOP abnormalities as in mammals. However, only recently have a limited number of studies begun to describe normal IOP values in amphibians and reptiles.[2,3,9,16,19–22,24,26]

Currently, there are only two published reports describing the normal range of IOP in any amphibian species.[9,17] One report used the Tono-Vet rebound tonometer to measure IOP in six amphibian species.[9] Establishing normal ranges and instrumentation for routine, noninvasive ocular diagnostics will aid in the identification of intraocular pressure abnormalities, such as glaucoma, which may severely or permanently impair vision, or ocular hypotension, which may indicate uveitis.

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Rebound tonometers such as the TonoVet are commonly available in veterinary clinics for the measurement of IOP; however, the probe of the TonoLab tonometer was modified for the smaller cornea of rats and mice and hence may be more accurate in amphibian species with smaller eyes. Both instruments function very similarly and use an induction coil to impel a small plastic-tipped metal probe toward the cornea and then evaluate the voltage changes induced within the coil as the probe rebounds from the corneal surface. Internal algorithms optimized for certain species (dog and cat or horse for the TonoVet and rat or mouse for the TonoLab) are then used to convert these voltage changes to estimates of IOP.[3,18] Six measurements are acquired, the lowest and highest are discarded, and the mean value is displayed along with an indication of the range of standard deviation.

The objectives of this study were to characterize the normal IOP for White's tree frogs (*Litoria caerulea*) using the TonoVet and TonoLab rebound tonometers, and to assess whether time of day or method of restraint affected IOP. This information would be of value in developing more effective diagnostic and treatment strategies for ophthalmological disorders in the White's tree frog, and other amphibian species.

MATERIALS AND METHODS

Animals

A total of 19 subadult captive bred White's tree frogs (*Litoria caerulea*) with a mean ± SD body weight of 23.8 ± 2.6 g (range 19.6–28.4 g) were obtained from a commercial supplier and used for the different experiments of this study. The frogs were housed individually in 2-gal plastic enclosures equipped with a water bowl, plastic plants, and a plastic hide. The frogs were housed in an interior room at 26–30°C, with light provided 12 h/day (0600 to 1800) using an ultraviolet bulb (Exoterra Repti Glo 5.0 UVB 120 cm/48 in 40 W, Rolf C. Hagen Corp., Mansfield, Massachusetts 02048, USA). Frogs had free access to fresh, dechlorinated water, and were fed appropriately sized, gut-loaded crickets every other day. Crickets were also dusted with calcium carbonate powder (Repti Calcium without D3, Zoo Med Laboratories Inc., San Luis Obispo, California 93401, USA) once a week just prior to feeding to the frogs. The frogs were acclimatized to the housing conditions, and the room in which the experiments were performed in, for 4 wk prior to starting the experiments. The testing room was
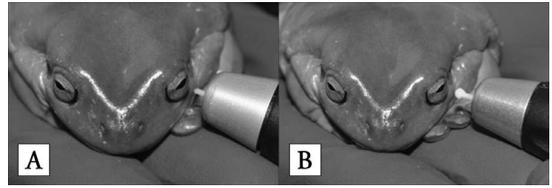
Figure 1. Photographs showing the size of the TonoLab (**A**) and the TonoVet (**B**) probe size as compared with the eye size of the White's tree frog (*Litoria caerulea*).

maintained at approximately 28°C. Baseline health testing included a full physical exam and fecal parasite testing. All frogs were dewormed with a prophylactic single dose of oral fenbendazole (Fenbendazole 10% suspension, Intervet Inc., Millsboro, Delaware 19966, USA; 30 mg/kg p.o. once). Sex of the frogs was not determined, as this species is not sexually dimorphic. All procedures were approved by the Animal Care and Use Committee at the University of Wisconsin, Madison, School of Veterinary Medicine.

Measurement of intraocular pressure

In all frogs, both eyes were determined to be clinically normal on the basis of slit-lamp biomicroscopy (KOWA SL-14, Optimed Inc, Torrance, California 90502, USA) and indirect ophthalmoscopy (Heine Omega 180; Heine USA Ltd, Dover, New Hampshire 03820, USA) performed by a board certified veterinary ophthalmologist (PEM). For the experiments, both the TonoVet (Icare Oy, Helsinki, Vantaa 01510, Finland) and the TonoLab (Icare Oy, Helsinki, Vantaa 01510, Finland) rebound tonometers were used according to manufacturer's instructions (Fig. 1) and all measurements were acquired by a single individual (JCH) to minimize interindividual variation. The TonoVet was used in the "d" setting (calibration setting for dogs and cats) and the TonoLab was used on the "r" setting (calibration setting for rats). For all experiments, the order of measurements of the right (OD) and the left (OS) eye was randomly assigned. For the initial experiment comparing TonoVet and TonoLab, measurements were taken at three time points throughout the day and only measurements indicated with no bar (a standard deviation of <1.0), bar "down" (a standard deviation of 1.8 to 2.5), or bar "middle" (a standard deviation of 2.5 to 3.5) on the instrument display were recorded. For all following experiments, involving comparing restraint method or anesthesia, only measurements with no
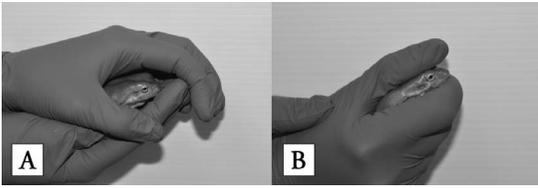
Figure 2. Photographs showing the hand positions of the restrainer for the unrestrained (**A**) compared with the restrained (**B**) position for the White's tree frogs (*Litoria caerulea*).

bar or bar "down" on the instrument display were recorded.

To compare the two different rebound tonometry devices, triplicate IOP measurements were acquired from both eyes with both tonometers in 19 conscious, unrestrained subadult frogs (38 eyes). Triplicate measurements were obtained from both tonometers from each frog during three time periods throughout the day to assess for changes due to circadian rhythm. Measurements were taken between 0800 and 1000 (morning period), 1200 and 1400 (midday period), and 1600 and 1800 (evening period). All frogs were held in a flat open palm with another hand cupped over the frog without touching the head or body of the frog for the unrestrained measurements (Fig. 2). The sequence of measurement of left and right eye IOP as well as the order of tonometer used was randomized.

To evaluate the effects of manual restraint on IOP, measurements were performed under manual restraint and without restraint, with at least 20 min between measurements, using the TonoVet device on 12 frogs. Manual restraint was performed by digital pressure along the entire length of the body and head or with no restraint as described previously (Fig. 2). The order of manual or no restraint used during IOP measurements was randomized. IOP measurements were taken only with the TonoVet in triplicate as described above.

The effect of two different concentrations of MS-222 (Tricaine-S, Tricaine Methanesulfonate, Western Chemical Inc. Ferndale, Washington 98248, USA) as a topical bath on IOP was evaluated using the TonoVet device. The concentrations of MS-222 used were 0.5 g/L and 2 g/L in distilled water, buffered with sodium bicarbonate until the pH was within the range of 7–7.4. The effect of the MS-222 on IOP was evaluated in 9 frogs in a randomized complete cross-over study design. The anesthetized IOP measurements were taken once withdrawal reflex, righting reflex, and

spontaneous movement were lost or after 25 min following placement of the frogs in the anesthetic bath, if loss of the mentioned reflexes and spontaneous movement did not occur within 25 min. IOP measurements under chemical restraint were compared with IOP measurements from the same individuals just prior to placement within the induction bath and were obtained under no restraint.

Statistical analysis

Triplicate data obtained from each eye was analyzed with commercial software (SigmaPlot 13.0, Systat Software, Inc. San Jose, CA 95131, USA). Normal distribution of the data was evaluated using the Shapiro-Wilk test. Comparison of the right versus the left eye for all experiments was performed using a paired two-tailed Student's t-test. The mean of triplicate IOP measurements for both the right and left eyes of each frog were averaged and used in the subsequent statistical analysis as no significant difference in IOP between the right and left eyes was found in any study comparison. Equal variance was assessed by Brown-Forsythe test. Repeated measures two-way ANOVA were used to analyze the data for differences in IOP measured by the two different tonometer devices during different time periods and effects of two different concentrations of MS-222. A paired t-test was used to analyze the effect of manual restraint on IOP. The Holm-Sidak method was used for post hoc corrections. Differences were considered significant if $P < 0.05$. Data were reported as mean ± SD unless otherwise indicated.

RESULTS

There was no statistically significant difference in the IOP measurements between the right and the left eyes. The IOP values measured with the TonoVet ranged from 10.8 to 18.7 mm Hg with a mean ± SD 14.7 ± 1.6 mm Hg during the three time periods (Table 1). There was no statistically significant difference in IOP between the three times points measured with the TonoVet. The IOP values measured with the TonoLab ranged from 10.3 to 25.5 mm Hg throughout the day with a mean ± SD 16.8 ± 3.9 mm Hg. TonoLab values were significantly lower in the evening period (mean ± SD 14.5 ± 3.1) compared with the morning (18.2 ± 3.7) and midday (17.8 ± 3.9) time periods ($P < 0.01$). In general, TonoLab IOP measurements were significantly higher than TonoVet measurements ($P < 0.001$) at all time

Table 1. Intraocular pressures (IOP) of White's tree frogs (*Litoria caerulea*) (*n* = 18) measured with two different tonometers (TonoVet and TonoLab) at three different time periods throughout the day.

Time of day	IOP with TonoVet		IOP with TonoLab	
	Mean ± SD	Range	Mean ± SD	Range
Morning (0800–1000)	14.9 ± 1.6[a]	12.3–18.7	18.2 ± 3.7[a,b]	11.5–24.2
Midday (1200–1400)	14.7 ± 1.3[a]	12.0–17.3	17.8 ± 3.9[a,b]	12.2–25.5
Evening (1600–1800)	14.4 ± 1.8	10.8–17.7	14.5 ± 3.1	10.3–21.2
All time periods	14.7 ± 1.6[a]	10.8–18.7	16.8 ± 3.9[a]	10.3–25.5

[a] Significant difference between devices ($P < 0.001$) at morning and midday time period.
[b] Statistically significant difference in IOP compared with evening time period ($P < 0.01$) within device.

points during the day, except within the evening time period where no statistical difference between TonoLab and TonoVet ($P = 0.875$) was found.

In all further experiments the IOP was measured using the TonoVet only, as this tonometer is much more widely available to veterinarians than the TonoLab.

Manually restrained animals had a significantly lower mean IOP (mean ± SD 13.4 ± 1.5 mm Hg) compared with unrestrained animals (mean ± SD 15.3 ± 1.2 mm Hg; $P < 0.001$; Table 2).

Chemical restraint induced with either concentration of MS-222 had no significant effect on IOP compared with pre-anesthetic values (Table 2). There was a trend toward a decrease in IOP values with both MS-222 concentrations, but this was not statistically or clinically significant. The difference in the mean IOP for both MS-222 concentrations to pre-anesthesia IOP was ≤1 mm Hg.

No procedure-associated adverse effects attributable to either tonometer were observed.

Table 2. Effect of manual (*n* = 12) and chemical (*n* = 9) restraint on intraocular pressure (IOP) in White's tree frogs (*Litoria caerulea*) measured with the TonoVet tonometer.

	Intraocular pressure (mm Hg)	
	Mean ± SD	Range
Manual restraint (*n* = 12)		
Unrestrained	15.3 ± 1.2[a]	13.7–17.8
Restrained	13.4 ± 1.5[a]	10.3–15.5
Chemical restraint (*n* = 9)		
0.5 g/L MS-222		
Pre-anesthesia	15.3 ± 1.3	13.5–17.2
Anesthetized	14.3 ± 1.5	12.3–16.8
2 g/L MS-222		
Pre-anesthesia	14.8 ± 1.6	12.5–17.2
Anesthetized	14.2 ± 1.0	12.8–16.2

[a] Significant difference within restraint ($P < 0.001$).

DISCUSSION

Both tonometers functioned well in White's tree frogs and resulted in useful and repeatable IOP estimates. The mean (± SD) IOP in White's tree frog measured with the TonoVet was 14.7 ± 1.6 mm Hg and the mean with the TonoLab was 16.8 ± 3.9 mm Hg throughout the day. These values are higher than the ones reported in a previously published IOP study in six amphibian species.[9] In that report, the mean (± SD) IOP values taken with TonoVet were 7.3 ± 1.2 mm Hg for American toads (*Anaxyrus americanus*); 5.1 ± 1.4 mm Hg for American bullfrogs (*Lithobates catesbeianus*); 6.3 ± 1.1 mm Hg for Great Plains toads (*Anaxyrus cognatus*); 6.3 ± 1.1 mm Hg for Plains leopard frogs (*Lithobates blairi*); 6.3 ± 1.4 mm Hg cane toads (*Rhinella marina*); 6.5 ± 1.5 mm Hg for spadefoot toads (*Spea bombifrons*); and 5.8 ± 1.5 mm Hg for Woodhouse's toads (*Anaxyrus woodhousii*).[9] The current study's IOP values are similar to the measurements taken from the amphibian species, the fringe leaf frog (*Cruziohyla craspedopus*) and the splendid leaf frog (*Cruziohyla calcarifer*), but once again were higher than the values from the coronated tree frog (*Anotheca spinosa*) as found in a separate study.[17] The differences in the values are likely due to species variation of normal IOP ranges.

Intraocular pressure research in reptile species has shown variable IOP ranges for different species as well. Red-eared sliders had a mean IOP of 11.32 ± 1.57 mm Hg with the canine setting[2] and in another study the mean IOP was 6.1 ± 2.3 OD and 5.6 ± 1.3 OS mm Hg with an undefined calibration setting.[16] Bearded dragons (*Pogona vitticeps*) had a median IOP of 6.16 mm Hg with a range of 5.61–6.44 mm Hg.[19] Hermann's tortoises (*Testudo hermanni*),[20] yellow-footed tortoises (*Geochelone denticulata*),[21] Andros Island iguanas (*Cyclura cychlura cychlura*),[26] and American alligators (*Alligator mississippiensis*),[24] all have had IOP studies performed with values

ranging from as low as 2.9 mm Hg to as high as 25.8 mm Hg. These variations in IOP ranges among reptile species likely also occur among amphibian species.

However, the variation could relate to the fact that the measurements were taken by different individuals, under different forms of manual restraint, and different internal calibration algorithms were used for the TonoVet. This study used the "d" setting (calibration setting for dogs), while the previously mentioned study in amphibians used the "p" setting (undefined species).[9] A study examining IOP in red-eared sliders did not show a statistically significant difference in the IOP values obtained with the three settings: equine, canine, and undefined, in the same individual.[16] As there is no setting for amphibians or many exotic species, one should select one setting and be consistent when using rebound tonometry on a new species and caution may be needed when trying to compare different sampling techniques.

Manually restrained frogs had a significantly lower IOP. This was an unexpected result, as manual restraint or pressure in the region of the neck or chest in domestic mammal species will cause an increase in IOP.[18] Restraint, by holding the sides of the neck of red-eared sliders, also significantly increased their IOP in one study.[3] In addition, this finding was different from that of a study in free-ranging anuran species that did not find a change in IOP with method of restraint.[9] However, in that study only a limited number of animals ($n = 4$) was used to evaluate the effect of manual restraint, which may have been insufficient to determine a statistical difference, a type 2 statistical error.[9] If other amphibian species tend to have a decrease in IOP with manual restraint, then this may contribute to the lower IOP values found in the Oklahoma anuran species as compared with this study. However, the Lewin study assessing South and Central American tree frogs did not find a significant difference in IOP with manual restraint[17] when the same restraint techniques were used as in the current study, so this change may be species specific. The mechanism for the potential reduction in IOP is unclear, but could be related to physiologic alterations associated with tonic immobility or "death feigning" in which heart rate and perhaps other physiologic parameters decline in a wide variety of species including mammals, reptiles, and birds.[5–8] However, this phenomenon has not been extensively examined in amphibians or in relation to IOP.[5–8]

Not only manual restraint, but body position can cause changes in IOP as well.[18] Loggerhead sea turtles (Caretta caretta) held in a head-down suspended position had an dramatic increase in IOP as compared with dorsoventral or ventrodorsal positions.[2] Humans, placed in a supine verses prone position also show different IOP values.[11] Similar trends of IOP values changing in relation to body or head position for dogs and horses have also been verified.[1,14] Therefore, the position of the animal in studies measuring IOP is very important and should be consistent. Frogs in this study, whether restrained or unrestrained, were always held in similar body position and orientation.

Changes in IOP over the course of a day was not detected with the TonoVet in White's tree frogs, but were suggested to vary with time of day with the TonoLab in a laboratory setting with a 12-hr-on–12-hr-off light cycle. Changes in IOP due to circadian rhythm are common and have been noted in most species, and are attributable to physiologic alterations associated with periods of greater or lesser activity.[4,15,18] For example, bearded dragons had higher IOP values in the morning in one study.[19] The White's tree frog had a decrease in IOP from the 1600 to 1800 compared with the 0800 to 1000 and the 1200 to 1400 time periods with the TonoLab, suggesting a diurnal variation in IOP occurs in this species as well. In a manometric study of a small-eyed reptile, the red-eared slider, the TonoLab results more closely approximated true IOP values than the TonoVet.[3] This may be due to the fact that the TonoLab was developed for use in mice and rats, which have a smaller globe size, similar to the size of the red-eared slider's globe. Manometry was not performed in this study, so it is unclear which device more closely approximates true IOP for White's tree frogs, but both the TonoVet and TonoLab rebound tonometers yielded clinically useful values in this species and have been demonstrated to yield linear estimates of IOP over the physiologically relevant range of IOP in all other species evaluated to date.[3,13] However, the detection of a statistically significant change due to time of day with one device (the TonoLab) and not the other (the TonoVet), may support the hypothesis that the Tonolab may be more accurate and better able to detect subtle changes in IOP in small globes. However, the TonoLab is not widely available outside of specialized glaucoma research laboratories and therefore most evaluations in this study were performed with the TonoVet as this device is far more accessible to the clinician.

One potential limitation of this study was that the temperature was not recorded in the testing room. Frogs are poikilotherms and therefore ambient temperature can potentially affect their physiology and possibly IOP. Animals were housed and tested in interior rooms in a climate-controlled facility that lacked windows or other structures that could have resulted in wide temperature fluctuations. Additionally, the temperature of the testing room was maintained by thermostat set to 28°C, which was not altered during the course of the study and IOP measurements were acquired over a 10-hr window during the daytime when changes in room temperature were likely to be minimal. However, the room temperature was not continuously recorded in this study and as such it was not possible to correlate IOP with potential changes in temperature. The effect of temperature on IOP has not be previously evaluated in any poikilothermic species. This would be an interesting and novel factor to assess in future studies.

No significant difference was present in IOP values with anesthesia in White's tree frogs, but a mild trend of the IOP to decrease was present with both doses of MS-222. Anesthesia and sedation tends to decrease IOP in domestic mammals due to reduced extraocular and adnexal muscle tone, although ketamine has been shown to cause a slight elevation in IOP in dogs.[18] Red-eared sliders given dexmedetomidine and ketamine with or without midazolam had a significant decrease in IOP as compared with conscious individuals.[3] MS-222 may in fact cause a decrease in IOP in White's tree frogs, but the number of individuals tested may have been too low in this study to determine if there was a very mild statistically significant effect. However, the change in the IOP with both MS-222 concentrations was ≤1 mm Hg, which is not clinically significant, as this would not change the diagnosis or treatment plan.

In conclusion, rebound tonometry is feasible in amphibian species, such as the White's tree frog and both instruments were well tolerated. Measurement of IOP in more amphibian species will improve our ability to diagnose and treat ophthalmological disorders in these species. However, consistency in technique is important as are time of day and method of restraint, as these can affect IOP values in White's tree frogs. Future studies validating rebound tonometry using manometry are needed to determine the most accurate method of measurement IOP measurement in this frog species.

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