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Research Article

Effects of Isoflurane and Midazolam on Tear Production and Intraocular Pressure in Capuchin Monkeys (*Sapajus libidinosus*)

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Abstract

Background: Anesthetics may be necessary for ophthalmic examination in capuchin monkeys and may affect the intraocular pressure (IOP) or aqueous tear production; therefore, these may be of concern when performing these evaluations.

Aim: To evaluate the influence of isoflurane alone, or combined with midazolam, on tear production by STT and on IOP by applanation and rebound tonometry in S. libidinosus.

Methods: Thirty-eight monkeys received isoflurane (n = 16), midazolam-isoflurane (n = 12), or physical restraint (control; n = 10) for evaluation of aqueous tear production (STT) and IOP (applanation or rebound tonometry).

Results: Anesthesia lowered STT values. Isoflurane was not significantly different compared to midazolam-isoflurane (P = 0.59) or control (P = 0.06); however, STT was significantly more reduced in midazolam-isoflurane compared to control (P = 0.01). IOP showed no significant differences among groups (P > 0.05), but rebound tonometry resulted in higher IOP values when compared to applanation tonometry values (P = 0.02). There were no significant differences in STT and IOP between right and left eyes or between males and females (P > 0.05).

Conclusion: Midazolam-isoflurane reduced aqueous tear production; however, IOP was not affected by either restraint protocol.

Keywords: *Anesthesia; Intraocular Pressure; Monkey; Primate; Schirmer Tear Test*

Introduction

Among New World primates, the capuchin monkey (*Sapajus libidinosus*) reproduces easily in captivity and is not considered an endangered species, except for its subspecies, the black-striped capuchin (*S. libidinosus flavius*) [1-3]. Primates kept in captivity are of great importance in research, increasing the demand for specialized medical care, including veterinary ophthalmology [4,5]. Capuchin monkeys have evolutionarily converged with humans and chimpanzees in several ways, including large brain size, omnivory, and extractive foraging, extensive cooperation and coalitionary behavior and a reliance on social learning [6]. Investigating capuchin monkeys have drawn increasing interest from researchers in comparative studies of the visual system, given their close behavioral, biological and phylogenetic relationship with humans [7].

Tear production and intraocular pressure (IOP) diagnostic tests are of the utmost importance in research of dry eye models like monkeys [8] and in animals with uveitis and glaucoma [9,10], respectively. These tests are important components of a complete ophthalmic examination and were previously evaluated in non-sedated [4] and sedated [11,12] Capuchin monkeys.

The precorneal tear film is considered to be the first defense barrier of the ocular system, and its aqueous portion can be measured, among other ways, by the Schirmer tear test (STT), which is performed by inserting an absorbent millimeter paper strip into the conjunctival fornix without topical anesthesia for one minute, with its value measured in mm/min [13-15]. The test measures basal and reflex tearing [16].

Applanation and rebound tonometry are the most commonly used techniques for measuring IOP in animals. Applanation tonometry measures IOP based on the principle that in an ideal sphere with a very thin wall, its internal pressure is determined by the force, in grams, necessary to flatten its surface divided by the area of applanation in millimeters [17]. Rebound tonometry measures IOP by assessing the acceleration and deceleration of its probe in contact with the cornea [18]. Knowledge of the normal IOP results when using these different tonometry techniques and the bias between them will facilitate the interpretation of IOP values obtained by these devices and the comparison between them. In this context, veterinary ophthalmologists need to be aware of the different tonometers used in animals, as well as the effects of anesthesia on IOP measurements.

Anesthetic and chemical restraint techniques play a very important role in veterinary medicine, especially when it comes to primates, due to their physical strength and sometimes aggressive temperament, which requires sedation or anesthesia to perform routine clinical procedures. Ophthalmic measurements can be impracticable without chemical restraint in animals, especially wild animals which are not used to be physically handled [11]. In this case, the understanding of the effects of anesthetic drugs on ocular physiology is of great importance for the interpretation of the results obtained, which has prompted studies in this area [19-23].

Isoflurane is safe and effective at concentrations of up to 3%, for anesthetic maintenance in capuchins [24]. Midazolam is a central benzodiazepine pre-anesthetic drug with sedative, anxiolytic, and hypnotic effects [25], that reduces excitation and facilitates immobilization [26] during the anesthetic induction period, thereby combining well with inhalation anesthetic drugs [27].

Aim of the Study

This study aimed to evaluate the influence of isoflurane alone, or combined with midazolam, on tear production by STT and on IOP by applanation and rebound tonometry in *S. libidinosus.*

Materials and Methods

Animals

Thirty-eight capuchin monkeys (*Sapajus libidinosus*) were evaluated, 15 males and 23 females, aged between 3 and 35 years and weighing between 1.55 and 5.00 kg. The animals were from the Primatology Center of the University of Brasília-DF, Brazil, an experimental nursery, licensed by the Brazilian Institute for the Environment and Renewable Natural Resources (IBAMA). Two to three animals were kept in each ecological cage (2.5m high x 2.0m wide x 4.0m deep), with water *ad libitum* and supply of balanced diet formulated by the facility for the species.

Prior to the start of this study, all animals received a physical and ophthalmologic examination, and those with signs of ophthalmic diseases were excluded. All animals evaluated were free of any ophthalmic problems.

The monkeys were initially restrained with a catch net and allocated into three groups before ophthalmic examination: isoflurane (IG; n = 16), midazolam-isoflurane (MIG; n = 12), and control (CG; n = 10). Isoflurane group received 3% isoflurane diluted in 100% O_2 by facemask until the loss of palpebral reflexes, whereas MIG received 0.5 mg/kg midazolam intramuscularly 10 minutes prior to isoflurane administration. The CG animals were subjected to handling and physical restraint by restricting movement of the head and arms. The STT and IOP were measured 10 minutes after the physical (CG) or chemical (IG, MIG) restraint.

Ophthalmic evaluation: Schirmer tear test (Ophthalmos Fórmulas, São Paulo, SP, Brazil) was performed in both eyes followed by rebound or applanation tonometry. Half of the animals in each group underwent to STT. IOP was measured in all animals, by applanation (Tonopen XL - USA) or rebound tonometry (TonoVet - Finland), randomly (half of the animals of each group). The tests were carried out in this sequence (STT, tonometry), by the same investigator.

Schirmer tear test: The STT strips (Ophthalmos Fórmulas, São Paulo, SP, Brazil) were inserted into the conjunctival fornix of the lower eyelid for 60 seconds and immediately read by the researcher after removal from the conjunctival sac.

Tonometry: Proxymetacaine hydrochloride (0.5%) ophthalmic solution (Anestalcon; Alcon Laboratories do Brasil Ltda., São Paulo - SP, Brazil) was administered topically before applanation tonometry by Tono-Pen XL®. Topical anesthesia was not required for the TonoVet® 'P' setting with internal calibration for 'other' (non-canine/feline or equine) species. During tonometry, the animals were carefully restrained to avoid excessive pressure in the neck region.

Statistical analyses: Descriptive statistical analyses and Shapiro-Wilk normality test were performed for the interpretation of data. Data not normally distributed were log10 transformed, as previously described (Delgado., *et al*. 2014) prior to subsequent statistical analyses using parametric tests for the inferential analysis. Descriptive statistics was reported using the original scale in mmHg. The JMP program (SAS Institute, Inc., Cary, NC, USA) was used for descriptive and inferential statistical analyses. IOP data were normally distributed. Data between groups were analyzed by ANOVA and Tukey-Kramer post-hoc statistical tests. T-tests were used to compare data obtained by rebound versus applanation tonometry as well as to compare data from right versus left eyes and between males and females. P values < 0.05 were deemed significant.

Ethical approval

This research was approved by the Ethics Committee for Animal Use of the University of Brasília (n.131088) and all procedures were carried out according to the Association for Research in Vision and Ophthalmology (ARVO).

Results

Tear production

Eight (42%) out of the 19 animals subjected to STT (GC, 5; GI, 8; and GMI, 6) had zero mm/min at least in one eye. The mean and standard deviations for STT results for each group were: CG 6.7 ± 6.0 mm/min, IG 3.5 ± 4.3 mm/min and MIG 2.12-2.68 mm/min. There were no significant differences between right and left eyes or between males and females (P > 0.05). There also were no significant differences between IG and MIG (P = 0.59), neither between IG and CG (P = 0.06); however, a significant difference was noted between MIG and CG (P = 0.01).

Intraocular pressure

The means and standard deviations of IOP of capuchin monkeys subjected to applanation and rebound tonometry are listed in table 1. There were no significant differences in IOP between males and females, or right and left eyes. Regarding the influence of restraint protocol on IOP, there were no significant differences among groups with applanation tonometry (16.40 ± 3.24, 15.68 ± 3.32 and 16.16 ± 3.95 mmHg) or rebound tonometry (19.60 ± 4.30, 17.18 ± 4.29 and 18.08 ± 4.05 mmHg). There was a significant difference between the two methods of tonometry, whereas rebound tonometry showed higher values compared to applanation tonometry (P = 0.02).

	Experimental Groups		
Ophthalmic tests	**CG**	**IG**	**MIG**
STT (mm/min)	6.7 ± 6.0	3.5 ± 4.3[a,b]	2.2 ± 2.6[b]
Applanation (mmHg)	16.40 ± 3.20	15.68 ± 3.32[a]	16.16 ± 3.95[a]
Rebound (mmHg)	19.60 ± 4.30[a]	17.18 ± 4.29[a]	18.08 ± 4.05[a]

Table 1: *STT and IOP values from Capuchin monkeys submitted to different anesthetic protocols. *CG: Control Group; IG: Isofluorane Group; MIG: Midazolam-Isofluorane Group; STT: Schirmer Tear Test. STT, applanation and rebound tonometry results reported as mean ± standard deviation. For each ophthalmic test, means followed by different superscript letters in the same line differ statistically (P < 0.05).*

Discussion

Although IOP and STT were previously investigated in capuchin monkeys [4,11,12] this is the first report comparing animals without chemical restraint to animals submitted to two different protocols of anesthesia. In addition, we compared applanation and rebound tonometry in this species.

The reference value for tear production by STT was previously reported by Montiani-Ferreira., *et al*. [4] in *S. libidinosos* submitted to physical restraint, and in *S. xanthosternos* [12] and *S. libidinosos* [11] under chemical restraint, with a mean value of 14.9 mm/min [4,12] and 2.5 mm/min [11] respectively.

Comparing STT results only from animals submitted to physical restraint [4], the values were higher (14.9 mm/min) than the median values observed in the present study (CG: 6.7 mm/min). Bezerra., *et al*. [11] reported similar results (2.5 mm/min) in *S. libidinosos* anesthetized with detomidine and ketamine to those observed in the MIG animals (2.1 mm/min), and in animals submitted to isoflurane (4 mm/min). Tear production may be influenced by several factors such as age, natural habitat, captivity, environmental factors, stress level during physical restraint, and animal weight. Probably, the physical restraint of the animals, without a period of adaptation to the stress could be responsible for the difference found in those studies [11,12]. Perhaps, stress, was a factor in the 42% that had a zero result on STT for example than the type of anesthesia in all groups, including CG. In addition, STT and IOP were measured only 10 minutes after the physical (CG) or chemical (IG, MIG) restraint and this might have affected the results. Also, to be considered are climatic differences such as temperature and humidity between regions where these animals live [4]. In those reports, the monkeys were from South [4] and Northeast [11,12], whereas the animals of this study were from Midwest Region of Brazil.

Both basal and reflex tear productions may decrease during general anesthesia [28], reducing the measurement of tear production, as previously described in other species [29,30]. In this study, 42% of the animals had a STT result of zero. Since there were no evident clinical signs of deficiency of the precorneal tear film in any of the animals, this was probably a result of all anesthetic protocols used, as it was previously reported in other species [31]. Anesthesia and individual variation might also explain why the median STT results were lower than previously reported for this species. The decreased intra-anesthetic lacrimation observed in the present study may be attributable to vagolytic or sympathomimetic effects of inhalant anesthetics [32]. In dogs, it was also suggested that inhalant anesthesia blocks both afferent (sensory) and efferent (motor) pathways to the lacrimal gland [29]. Median values of STT were lower in both groups that received chemical restraint; however, a significant difference was only noted in the group pretreated with midazolam, in comparison to control group. As the effect of midazolam on STT was not previously described [32], the authors infer that this drug can reduce the lacrimal production in association with isoflurane. Although, a limitation of this study was the absence of a midazolam group, encouraging a future study to determine the effect of this drug on tear production.

Comparisons between rebound and applanation tonometry have been performed in dogs [18], owls [33], rabbits [34] and *Rhesus* macaques [35], but to our knowledge, this is the first study in capuchin monkeys subjected to the drugs described. As the visual function of monkeys is similar to humans, the non-primates are considered a good model for ocular studies [35]. Intraocular pressure in *Sapajus libidinosus* by the applanation method was reported earlier with a mean of 18.4 mmHg, somewhat higher than that observed in the present study (16.4 mmHg) [4]. As both applanation and rebound tonometers can reflect repeatable and accurate measurements in healthy and diseased eyes [35], these reference values can help the ocular evaluation of this species.

Considering that IOP can show an iatrogenic increase due to pressure exerted on the jugular vein and manipulation of the eyelids as well as age, this type of handling was avoided [21,34]. However, the age of the monkeys was not standardized, given the limited availability of these research animals. Another factor also related to IOP oscillations is circadian rhythm, as shown in cats, horses, rabbits and dogs [34-38]. The circadian cycle may have similar effect on capuchin monkeys, although this has not been reported yet. All evaluations here were performed in the morning.

Measurement of IOP by rebound tonometry resulted in higher values compared to the applanation method, corroborating the findings of Jeong., *et al*. [33] in Eurasion eagle owls (*Bubo bubo*) and of Ben-Shlomo and Muirhead [39] in dogs, but although the measures from both tonometers were different, they were highly correlated in *Rhesus* macaques [35]. A study in rabbits revealed that applanation tonometry is subject to imprecise estimation of IOP compared to rebound tonometry [34]. Because the globe itself is not a perfect sphere and the corneal curvature is not entirely regular, its thickness and elasticity may interfere with the strength required to perform applanation, underestimating IOP in thin corneas and overestimating it in thick corneas [40]. The IOP may be increased by 1 mmHg for applanation and 2 mmHg for rebound tonometry, for every 100-µm increase in corneal thickness [41]. Corneal pachymetry would be an essential examination to evaluate this variable [41]; therefore, its use in future investigations is suggested, as it was a limitation in this study. In this investigation, a first generation TonoVet® was used with the "P" setting. This setting was discontinued in latter models. This is an important detail to be considered before comparing the findings of this study with others.

Research dealing with data collection of healthy wild animals, especially non-human primates, are often limited to opportunistic sampling, meaning time and access restrictions to each of the animal. Thus, unfortunately, the authors were not able to repeat tests for aqueous tear production and intraocular pressure measurements using different techniques in the same animals. This option would have removed the variability due to the individual differences between subjects. The approach that was used (different tests in different animals) instead certainly constrained the types of statistical analyses that could have been used. Still, the groups of animals that were compared were homogeneous, meaning that the STT differences can be attributed to the type of chemical restraint protocol. Nevertheless, because of this experimental design the possibility of an effect of potential individual differences between subjects cannot be completely excluded. Even considering this possibility, the present work brings STT and IOP values for capuchin monkeys under two commonly used chemical restraint techniques, which may serve as parameters for both these tests in future investigations, if possible, with more robust group sizes. The variability introduced by a broad age range in the present investigation is also another factor to be considered. Regarding potential advantages, rebound tonometry was more practicable. The examiners also spent less time obtaining the measurements during investigation. This type of test also dispenses the use of topical anesthetics and it seemed not to be painful, as previously described in healthy mouse lemur [42] and in dogs [18].

The rebound tonometer was safer for the researcher in determining IOP in the animals without anesthesia, when compared to the need to keep the hand very close to the animal's mouth, but both tonometers were easy to handle and without operational risk to the researcher. We found that anesthesia with isoflurane (diluted in 100% O_2) and midazolam (0.5 mg/kg) did not alter IOP.

Conclusion

The combination of midazolam and isoflurane was shown to be a suitable protocol for chemical restraint of capuchins subjected to ophthalmic evaluation by keeping IOP unchanged. Aqueous tear production was significantly reduced in animals that received isoflurane

and midazolam, pointing to the need for lubrication of the ocular surface during anesthesia and monitoring of this parameter at the end of anesthesia. We also found that rebound tonometry showed higher values compared to applanation tonometry.

Conflict of Interest

The authors) declared no potential conflict of interest with respect to research, authorship, and/or publication of this article.

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