Evaluation of the mydriatic effects of topical administration of rocuronium bromide in Hispaniolan Amazon parrots (*Amazona ventralis*)

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OBJECTIVE
To determine the mydriatic effects of topical rocuronium bromide administration in Hispaniolan Amazon parrots (*Amazona ventralis*) and to identify any adverse effects associated with treatment.

DESIGN
Randomized crossover study.

ANIMALS
8 healthy adult Hispaniolan Amazon parrots.

PROCEDURES
Rocuronium bromide (20 μL/eye; 10 mg/mL) or saline (20 μL/eye; 0.9% NaCl) solution was administered in both eyes of each bird with a 26-day washout period. The birds were manually restrained in lateral recumbency with the apex of the cornea positioned upward for 2 minutes following administration in each eye. Infrared pupillometry and direct pupillary light reflex measurements were used to evaluate the mydriatic effects. Pupillary measurements were recorded prior to administration and every 20 minutes for 2 hours after administration, then hourly for a total of 7 hours. A brief physical examination was performed, direct pupillary light reflex was tested, and fluorescein staining was performed on each eye of each bird 24 hours after administration.

RESULTS
A significant difference in pupillary diameter for the active versus control treatment group was noted from 20 to 360 minutes after drug administration, but not at 420 minutes. Minimal adverse effects were noted. Three birds had transient inferior eyelid paresis noted in both eyes after receiving rocuronium; 24 hours after the treatment, no differences in ocular measurements existed between the active and control treatments.

CONCLUSIONS AND CLINICAL RELEVANCE
Results suggested that topical rocuronium bromide administration may be safely used for pupillary dilation in Hispaniolan Amazon parrots and could be used for clinical evaluation, fundus imaging, and surgical interventions involving the lens and posterior segment in this species. (*J Am Vet Med Assoc* 2016;248:67–71)

The avian iris is composed predominantly of striated muscle fibers, with a small but varied component of smooth muscle fibers.[1–4] The predominance of striated muscle in the iris allows for very rapid pupillary excursions and makes parasympatholytic agents clinically ineffective. Induction of a deep plane of anesthesia has been reported to produce mydriasis in some avian species.[5] Intracameral injections of both depolarizing and nondepolarizing NMBAs have been used successfully for mydriasis in several avian species.[6,7] Intracameral injections typically require general anesthesia and can cause damage to intraocular structures if not performed appropriately.[8]

ABBREVIATIONS

NMBA	Neuromuscular blocking agent
PLR	Pupillary light reflex

Topical ocular use of depolarizing and nondepolarizing NMBAs has been evaluated in conscious birds, with varied outcomes. Consistent mydriasis has been achieved with vecuronium bromide in a range of avian species.[4,9,10] The use of surface-acting penetrating agents prior to topical NMBA administration did not improve the duration or reliability of mydriasis in several psittacine species in 1 study.[4] Topical ocular administration of NMBAs may eliminate the need for general anesthesia but is not completely without risk; alcuronium chloride caused temporary complete paralysis of 1 kestrel (*Falco tinnunculus*) and was not recommended for use by the authors of that study.[10]

Rocuronium bromide is a nondepolarizing NMBA with a rapid onset of action and minimal cardiovascular and histamine-releasing effects in mammals. The duration of action of rocuronium bromide is similar to that of vecuronium, but the onset of action is 2 to 3

times faster. It is also stable in aqueous solution, unlike other NMBAs such as vecuronium and atracurium.[6] Rocuronium bromide has been successfully and safely used topically for mydriasis in several species of birds of prey.[7,11,12] However, to our knowledge, its efficacy for producing mydriasis in psittacines has not been reported. The objective of the study reported here was to determine the efficacy, appropriate dose, and optimal method of administration of topical rocuronium bromide to induce reversible mydriasis in Hispaniolan Amazon parrots (*Amazona ventralis*). Our hypothesis was that topical rocuronium would be an effective mydriatic agent in this species, with minimal adverse systemic effects.

Materials and Methods

Birds

Eight adult (10 to 26 years old) Hispaniolan Amazon parrots of unknown sex were enrolled in the study. Mean ± SD body weight was 301.9 ± 19 g. All parrots were considered healthy before and during the study as determined by physical examinations that were performed within a month of the start of the study. Prior to study enrollment, each bird underwent a complete ophthalmic examination including assessment of PLR, slit-lamp evaluation of the anterior segment, evaluation of the posterior segment by means of indirect ophthalmoscopy, and intraocular pressure measured with both rebound tonometry[a] (without topical anesthetic) and applanation tonometry[b] (after topical anesthetic, 1 drop/eye in both eyes applied once).[c] All ophthalmic examinations were performed within 2 months of the start of the study. A Schirmer tear test I[d] was performed on both eyes of each bird as described elsewhere.[13] Finally, fluorescein stain[e] was applied to the cornea of each eye of all birds to detect any corneal epithelial defects. Only birds determined to have bilaterally normal ocular examinations were included in the study (n = 8). For the duration of the study, the birds were housed individually in stainless steel cages (0.6 × 0.6 × 0.6 m), provided with multiple perches and toys, and maintained on a 12-hour light cycle. A commercial pelleted diet formulated for psittacine birds[f] and fresh water were provided ad libitum. The study protocol was approved by the University of California-Davis Institutional Animal Care and Use Committee.

Experimental design

A within-subjects, masked, complete crossover experimental design was used. At different times, each bird received both rocuronium bromide (10 mg/mL)[g] and saline (0.9% NaCl) solution topically in both eyes. The parrots were randomly assigned to 2 equal groups as determined by drawing numbered pieces of paper from a bag. On the basis of results of 4 pilot studies (data not shown), a drug dose and administration protocol was established. Each bird was manually restrained in a towel in right lateral recumbency with the apex of the cornea positioned upwards **(Figure 1)**, and 20 µL of either rocuronium bromide (10 mg/mL) or saline solution was administered via micropipette topically to the left eye. The bird was held in this position for 2 minutes following topical application of the test substance, and the procedure was then repeated with the same test substance for the right eye with the bird in left lateral recumbency. During the first study period, birds assigned to group 1 received rocuronium bromide (range, 1.19 to 1.46 mg/kg [0.54 to 0.66 mg/lb]; median, 1.32 mg/kg [0.6 mg/lb]; total dose administered to both eyes), and birds assigned to group 2 received an equivalent volume of saline solution. After a 26-day washout period, the procedure was repeated with birds assigned to each group receiving the other test substance under the same conditions. Birds were manually restrained in a towel for all treatments and subsequent data acquisition. Respiratory rates, posture, and overall mentation were monitored throughout.

Pupil measurement

Infrared pupillometry was used to assess pupil size without stimulating the PLR because the avian eye is not responsive to infrared light. Brief video clips of each pupil on each bird were obtained in ambient fluorescent lighting (430.6 lux) using a 60-mm lens[h] with a distance of 0.7 m from the lens to the patient and a relative aperture of *f*/2.8, fitted with an infrared videophotoretinoscopic attachment.[14] Birds were handled in random order with respect to treatment group assignment, and personnel performing pupillometry and subsequent image analysis were unaware of treatment group assignment. Each bird was manually restrained and the eye to be analyzed positioned to be centered in a 0.75" circular hole cut in the center of a piece of wood, which contained an offset millimeter ruler, so that both the pupil and

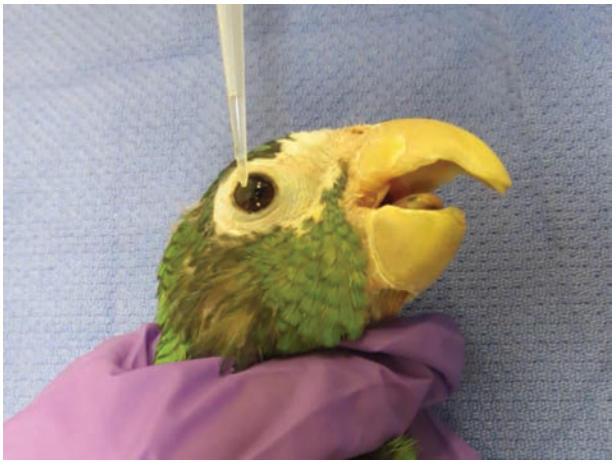

Figure 1—Photograph of a Hispaniolan Amazon parrot (*Amazona ventralis*) manually restrained in a towel and held horizontal to a table surface while 20 µL of either rocuronium bromide or saline (0.9% NaCl) solution is administered to the corneal surface with a micropipette. Each bird was held in this position for 2 minutes following topical administration of the test solution to each eye.

ruler were in focus. The ruler provided a fixed point of reference that remained unchanged between both periods of data acquisition **(Figure 2)**. A short video clip was recorded for each eye, and then a direct PLR measurement was immediately obtained for both eyes in a dark room with a transilluminator on a direct ophthalmoscopic examination. Direct PLR measurements were scored as 0 (absent), 1 (partial), or 2 (normal, complete). Measurements were obtained every 20 minutes until 120 minutes, then every 60 minutes until 420 minutes. This procedure was repeated for the second study period 26 days later. A brief physical examination and direct PLR measurement were performed on both eyes of each bird 24 hours after each study period, twice in total for each bird. Fluorescein stain was also applied to the cornea of both eyes for all birds to evaluate the integrity of the corneal epithelium. A single image from the video clip was selected at the point of maximum mydriasis with the pupil and ruler in focus, and these were subsequently analyzed via image analysis software[i] to determine pupil diameter for each bird for all time points, every 20 minutes until 120 minutes, then every 60 minutes until 420 minutes.

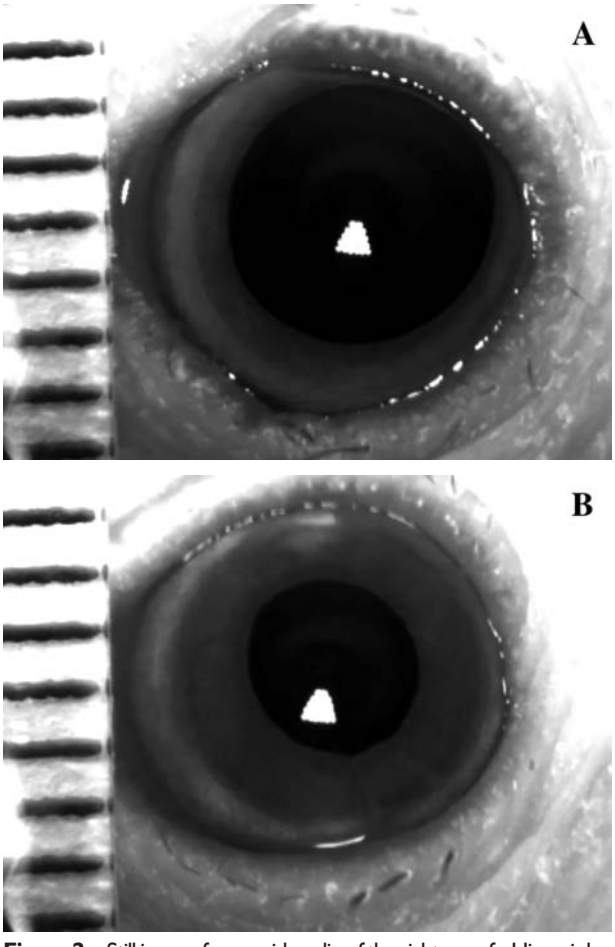

Figure 2—Still images from a video clip of the right eye of a Hispaniolan Amazon parrot obtained with a 60-mm lens fitted to a coaxial infrared videophotoretinoscopic attachment 100 minutes after topical administration of 20 µL of rocuronium bromide (A) or saline solution (B). A millimeter ruler is present on the left side of each image.

Statistical analysis

A mixed-effects ANOVA was used to evaluate differences between the control (saline) and treatment (rocuronium bromide [10 mg/mL]) groups over time. The individual bird was treated as a random effect because of sequential (dependent) measurements obtained for each bird. The model adjusted for eye, order of treatment, and period of treatment and tested the main effects of treatment and time as well as their interaction. Post hoc treatment comparisons at individual times were tested by means of a Bonferroni adjustment to preserve a nominal 5% type I error rate. Pupillary light reflex scores were initially compared across time within the treated group for each eye separately with exact Friedman tests; post hoc contrasts by means of exact Wilcoxon signed rank tests were adjusted for multiple comparisons with a Bonferroni-Holm procedure. For analyses of pupil diameter and PLR scores, commercial statistical software programs[j,k] were used. Values of $P \leq 0.05$ were considered significant.

Results

There was a significant interaction between treatment and measurement time points ($P < 0.001$), with significant differences between control (saline) and treatment (rocuronium bromide) for 20 to 420 minutes (adjusted P, 0.001 to 0.015). There were no significant effects of order ($P = 0.667$) or study period ($P = 0.057$). There was a significant ($P = 0.010$) difference between eyes, but the difference was small: the predicted mean measurement in the left eye was 4.22 mm (SE, 0.075 mm) and in the right eye was 4.28 mm (SE, 0.075 mm). Within the control treatment, there was no significant difference with baseline at any time point (adjusted P, 0.16 to 1.00), whereas within the treatment group, there was a significant difference from baseline from minute 20 until minute 360 (adjusted P values ranged from < 0.001 to 0.005).

In the rocuronium-treated birds, there were significant differences between PLR scores at time 0 and times 20 minutes through 180 minutes in both eyes ($P < 0.05$; **Figure 3**). All birds remained bright, alert, and responsive during both periods, and there was no evidence of systemic adverse effects, such as respiratory depression. Three birds (1 from the first study period and 2 from the second study period) had mild lower eyelid paresis after topical administration of rocuronium bromide, manifest as transient lower eyelid elevation in both eyes. Their ability to blink and nictitating membrane movement were unaffected during this time. All affected birds displayed normal eyelid position 24 hours after treatment, and no evidence of corneal defects was noted.

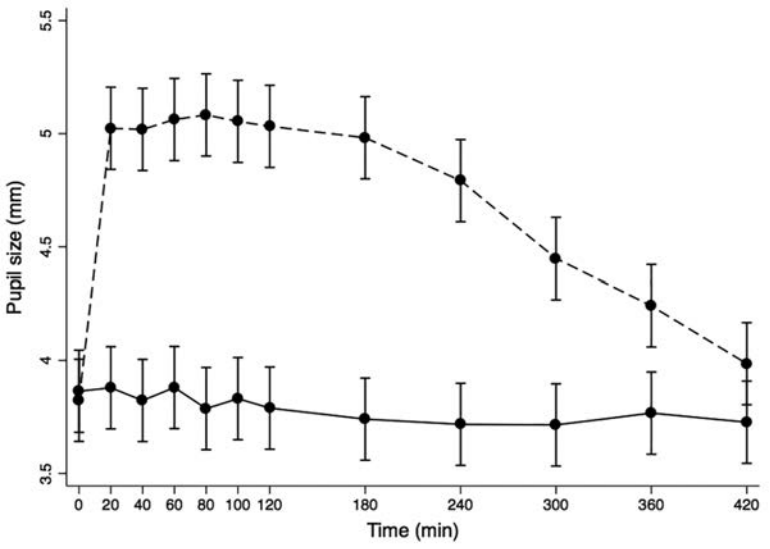

Figure 3—Model-predicted mean ± SE pupil diameter (mm) in Hispaniolan Amazon parrots after topical administration of 20 µL of either rocuronium bromide (dashed line; n = 8) or saline solution (continuous line; 8).

Discussion

In the present study, topical administration of rocuronium bromide (10 mg/mL; 20 µL/eye in both eyes) effectively induced mydriasis in healthy Hispaniolan Amazon parrots starting 20 minutes after application, with a duration of action of 360 minutes. The combined dose administered to both eyes in the present study (range, 1.19 to 1.46 mg/kg; median, 1.32 mg/kg) was slightly higher than the effective dose reported for tawny owls (0.78 to 1.0 mg/kg [0.35 to 0.45 mg/lb] topically),[7] a species of similar weight to Hispaniolan Amazon parrots. In previous studies[7,11,12] evaluating topical rocuronium bromide in birds, a third eyelid retractor was held in place for 1 minute in each eye to facilitate corneal absorption of the drug. In pilot studies, we found that retracting the third eyelid was technically challenging and potentially traumatic in this psittacine species and was not necessary if the bird was maintained in lateral recumbency for 2 minutes following administration of the drug.

In previous studies,[7,11,12] pupil diameter was measured directly with a pupillary gauge. Because of the small ocular size of the parrots in the present study, we utilized infrared pupillometry to obtain a calibrated, magnified image of the pupil, which was subsequently analyzed with image-processing software to determine the exact pupil diameter. This method has been used in a previous study of topical mydriatic agents in birds[4] as well as other studies.[14,15] An advantage of infrared imaging was a lack of pupillary stimulation with additional light intrinsic to the use of either flash photography or light supplied by a transilluminator.

For most clinical applications, an ideal topical mydriatic would have a short onset and duration of action to facilitate complete lens and fundic examination as an outpatient procedure or prior to intraocular surgery (eg, cataract extraction). A significant difference in pupil size was noted 20 minutes after topical rocuronium administration in this study, but the effects persisted for approximately 6 hours. In kestrels, mydriasis was maximal 90 minutes after administration and persisted for a mean of 4.5 hours.[10] This discrepancy may be a result of the greater delivered drug dose utilized in the present study or species differences in anatomy or physiology. If rocuronium is utilized for clinical examination, we suggest that it may be prudent to ensure the bird is not exposed to bright ambient light for at least 6 hours after administration.

Safety of NMBAs is also a concern, especially when used in a nonanesthetized patient without airway protection. A wide range of adverse effects has been reported in previous studies of topical NMBAs in avian species. Superficial corneal ulceration and ocular irritation were noted in cockatoos treated with topical atropine sulfate and phenylephrine mixed with 1% saponin.[4] Topical administration of pancuronium in a psittacine species caused systemic effects ranging from mild tachypnea and ataxia to acute collapse 5 minutes after administration that required immediate IV treatment with the reversal agent neostigmine.[4] Acute collapse and temporary paralysis have also been reported with topical application of alcuronium chloride in a kestrel.[10] There has been 1 acute death reported in a cockatoo treated topically with vecuronium mixed with 1% saponin.[4] Previous studies of topical rocuronium administration in avian species have not reported any adverse effects. The parrots in the present study were monitored for any change in mentation, posture, or respiratory effort. Certain NMBAs, such as pancuronium, can cause tachycardia in mammals.[6] Heart rates were not monitored in the present study, because tachycardia is also caused by manual restraint for cardiac auscultation in many avian species.

In this study, all birds were examined 24 hours after rocuronium or saline solution application, and fluorescein stain was applied to both eyes at that time to ensure no corneal lesions had developed. The only noted adverse effect in the present study was transient lower eyelid paresis as demonstrated by lower eyelid elevation in both eyes. This has not been reported in other avian species with topical administration of this drug and was likely due to local absorption of rocuronium and subsequent paresis of lower eyelid depressor muscles. Affected birds retained full function of their nictitating membrane and their ability to blink, which reduced the possibility of corneal epithelial defects developing. Despite the lack of systemic adverse effects in this and other studies on topical rocuronium

bromide, close monitoring of birds after topical application and availability of neostigmine are still strongly recommended.

Topical administration of NMBAs for mydriasis in avian species is minimally invasive and does not require general anesthesia. Topical application of rocuronium bromide successfully produced mydriasis for approximately 6 hours in Hispaniolan Amazon parrots with minimal adverse effects. Additional studies are necessary to determine the appropriate dose of topical rocuronium bromide in other avian species, as differences in drug metabolism, corneal uptake, and globe size may cause dosage variability.

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Footnotes

a. TonoVET, Jorgensen Labs, Loveland, Colo.
b. Tono-pen VET, Reichert Inc, Depew, NY.
c. 0.5% Proparacaine hydrochloride ophthalmic solution, Akorn Inc, Buffalo Grove, Ill.
d. ColorBar Schirmer tear test, Eagle Vision Inc, Memphis, Tenn.
e. Flu-Glo fluorescein sodium ophthalmic strips, Akorn Inc, Lake Forest, Ill.
f. Zupreem fruit blend, Premium Nutritional Products Inc, Mission, Kan.
g. Rocuronium bromide, Sagent Pharmaceuticals Inc, Schaumburg, Ill.
h. Nikkor lens, Nikkon Inc, Melville, NY.
i. ImageJ, National Institutes of Health, Bethesda, Md. Available at: rsb.info.nih.gov/nih-image. Accessed Jul 15, 2013.
j. Stata/IC, version 12.1, StataCorp LP, College Station, Tex.
k. StatXact, version 10, Cytel Software Corp, Cambridge, Mass.

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15. Mutti DO, Zadnik K, Johnson CA, et al. Retinoscopic measurement of the refractive state of the rat. *Vision Res* 1992;32:583–586.