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Mydriatic effect of topically applied rocuronium bromide in tawny owls (*Strix aluco*): comparison between two protocols

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Abstract

Objective To evaluate the mydriatic efficacy of a neuromuscular blocking agent (rocuronium bromide) applied topically to only one eye of nocturnal birds of prey and to assess for any general and/or local adverse effects due to its use.

Animal studied Twelve healthy adult tawny owls (*Strix aluco*) were randomly divided in two groups.

Procedures Six birds (Group 1) received a single dose of 0.35 mg of rocuronium bromide. The second group of subjects (Group 2) received two doses of 0.35 mg of rocuronium bromide (total 0.70 mg/eye). In both groups, the curariform agent was instilled topically. Pupil diameter was measured with a pupillary gauge in 10 min intervals for a total of 100 min and then every 20 min for a total of 240 min. The assessment of the pupillary light reflex was performed using a standard light source during pupillary size recording.

Results Maximal pupillary diameter was 11.5 ± 0.3 mm for Group 1 and 11.0 ± 0.6 mm for Group 2 and no statistically significant differences were detected among the two groups. The maximal pupillary diameter was achieved at T80 for Group 1, and at T60 for Group 2. A complete fundus examination was possible on all treated eyes of subjects of both groups. The drug did not cause any noticeable adverse effects in any of the examined birds.

Conclusion Results of the present study suggest that a single topical administration of 0.35 mg of rocuronium bromide to the eyes of healthy tawny owls results in sufficient mydriasis to allow for a complete examination of the fundus.

Key Words: bird of prey, mydriasis, neuromuscular blocking agent, raptor, rocuronium bromide, tawny owl

INTRODUCTION

In the avian species, the iris is mainly composed of striated muscle fibers, with varying amounts of nonstriated fibers.[1,2] The striated fiber component allows for a rapid pupillary response and can be considered an adaptation to the visual requirements of flight. Essentially, the presence of striated musculature permits voluntary control of the pupil size, as well as rapid constriction of the pupil.[3] Members of mammalian species in contrast possess pupillary dilator and sphincter muscles composed of smooth muscle fibers, which respond to autonomic sympathetic and parasympathetic stimuli. Consequently, as the primary constrictor is composed of striated muscle in birds, the parasympatholytic drugs used to dilate the pupils of mammals are ineffective and neuromuscular blocking agents (NMBAs), such as curariform drugs, are generally used instead to induce mydriasis in conscious birds.[3,4] Other methods used to achieve mydriasis in birds are more invasive and require either the administration of short acting anesthetics such as ketamine hydrochloride and xylazine,[5] or sac perfusion general anesthesia,[6] both procedures require well trained, experienced clinicians and special equipment.

A number of NMBAs have been used to date to induce mydriasis in raptors with varying results. In some species, a partial mydriasis or no mydriasis was achieved using 3% d-tubocurarine (0.01–0.03 mL) instilled 3–4 times over 20 min.[7] However, when administered intracamerally

d-tubocurarine was noted to be very efficacious in producing mydriasis. If the latter route of administration is chosen, the drug is injected directly into the anterior chamber by passing a fine needle (27–30 G) through the limbus;[1] but this procedure bears some risk for intraocular structure damage.[1,8,9]

In one study, conducted on *Falco tinnunculus*, the mydriatic effect of a topical administration of alcuronium chloride, pancuronium bromide, and vecuronium bromide was evaluated and the results suggest that only vecuronium bromide, administered at a dose of two drops, three times, in 15-min intervals, provides an effective mydriasis of the iris without side effects.[9] It is important to note that in this study the NMBAs were applied to only one eye, therefore the safety of the bilateral topical administration of these drugs was not tested.

Rocuronium bromide, a derivative of vecuronium bromide, has been introduced recently for use in veterinary medicine. It has been administered systemically to reptiles and mammals for different procedures and it demonstrated good efficacy, a rapid onset and the absence of side effects.[10–15]

The objective of this study was to evaluate the mydriatic effect of a single dose vs. two doses of rocuronium bromide administered topically to one eye of nocturnal birds of prey and to determine whether this NMBA would provide pupil dilatation sufficient to allow for a complete fundus examination, without causing any general and/or local adverse effects.

MATERIALS AND METHODS

Twelve adult tawny owls (*Strix aluco*) of different sexes, weighing from 350 to 450 g were enrolled in the study. The birds were submitted to the Department of Veterinary Clinics of Pisa University for an ophthalmologic examination, to verify the absence of ocular lesions prior to their release into the wild.

Approval to conduct this study was obtained from the local Ethics Committee on Animal Experimentation (D.Lgs.vo 116/92).

The eyes and the periocular region were examined in ambient light for gross abnormalities. Schirmer tear test readings were obtained of both eyes (OU) using commercially available test strips (Dina strip Schirmer-Plus®, GECIS sarl, Neung sur Beuvron, France) and palpebral, corneal and direct pupillary light reflexes were also performed OU. The intraocular pressure was assessed by means of applanation tonometry (Tonopen-XL, Mentor, Norwell, MA, USA) following a topical administration of 0.4% oxibuprocaine chloridrate (Benoxinato chloridrate INTES®, ALFA INTES Industria Terapeutica Splendore S.r.l., Naples, Italy). The adnexa and the anterior segment of both eyes were examined with a slit-lamp (Kowa SL-14, Kowa Company, Tokyo, Japan). Mydriasis induction was performed in one randomly assigned eye of each bird and the fundus was examined using a binocular indirect ophthalmoscope (Omega 180, Heine, Berlin, Germany) with a 30 D lens. A fluorescein stain was performed OU as a final step to exclude corneal lesions.

Mydriasis was achieved using the NMBA, rocuronium bromide (Esmeron®, Organon Italia S.p.a., Rome, Italy), which was administered topically to only one eye with a mechanical pipette (Pipetman 0-999 mcL, Gilson, France). Esmeron® is an aqueous solution of rocuronium bromide with 10 mg/mL concentration and it was applied without dilution. For a period of 1 min, the third eyelid was retained with a lid retractor, to prevent its excursion and avoid the rapid elimination of rocuronium from the ocular surface. 0.9% Saline solution was instilled into the untreated eye (not dilated with the mydriatic agent) as a control (control group).

Birds were randomly divided into two groups of six tawny owls (Group 1 and Group 2). Group 1 received a single dose of 0.35 mg of rocuronium bromide (total 0.35 mg/eye) and Group 2 a total of two doses of 0.35 mg of rocuronium bromide 15 min apart (0.70 mg/eye).

Pupil diameter and direct pupillary light reflex were recorded prior to the administration of rocuronium bromide (Tbase) and every 10 min postadministration until the 100 min mark was reached. Thereafter, measurements were performed and recorded in 20 min intervals for a maximum of 240 min at which point measurements were stopped to avoid excessive handling of the birds and reduce the amount of related stress exposure.

The assessment of the direct pupillary light reflex was performed in a darkened room using a 15-W halogen lamp (SL-14, Kowa, Japan) as a light source (intensity selected: ¼, distance from the eye approximately 30 cm). The pupillary light reflex was evaluated based on a three point scale with a score of 2 indicating normal, 1 decreased and 0 absent light reflex.

The response of the pupil (pupil diameter) to the aforementioned light source was measured manually using a pupil gauge calibrated to the nearest 0.5 mm. Tawny owls (*S. aluco*) have dark brown irises and it is difficult to distinguish the pupil from the iris in ambient light conditions. Hence, the diameter of the pupil, in response to the pupillary light reflex, was measured in a darkened room.

The occurrence of ocular irritation signs (lacrimation, blepharospasm, conjunctival hyperemia and chemosis), eyelids, wings, hind limbs and neck muscle paralysis and any kind of respiratory distress were monitored and registered as local and/or general adverse effects.

Any subjects which presented with an ophthalmic abnormality were excluded from the study to minimize the possible influence of an ophthalmic disease on mydriasis.

Data were analyzed statistically using Graph Pad Prism4® (San Diego, CA, USA). Data was summarized as mean (X) ± standard deviation (SD) and evaluated for normal distribution using the Kolmogorov–Smirnov method and Wilcoxon Signed Rank Test was used to define Gaussian distribution. An unpaired Student's *t*-test was applied to

compare differences between the two groups in relation to pupil diameter and a one-way analysis of variance for repeated measures (RM ANOVA) was used to assess differences among values recorded at Tbase and subsequent time intervals within the observation timeframe. *Post hoc* evaluation was performed using Tukeys test. Differences were considered statistically significant for $P \leq 0.05$. A one-way ANOVA Friedmann test was employed to compare the trend of pupillary light reflex in Group 1 and Group 2 over time.

RESULTS

Of nineteen tawny owls admitted to the Department only 12 were included in the study, because 7 birds presented with ophthalmic disease.

Throughout the study, control eyes of both groups maintained an average pupil diameter of 4.0–4.5 mm and a normal pupillary direct light reflex (Fig. 1). A statistically significant difference was found compared to the treated eyes from T20 to T160 for Group 1 and from T20 to T240 for Group 2.

In treated eyes, maximal pupillary diameter was 11.5 ± 0.3 mm for Group 1 and 11.0 ± 0.6 mm for Group 2 and no statistically significant differences were detected among both treated groups (Fig. 2). The resultant degree of pupil dilatation was sufficient for a complete fundus evaluation in all the birds of each group (Fig. 3). The maximal pupillary diameter was achieved at T80 for Group 1 and at T60 for Group 2.

A normal pupillary light reflex was re-obtained at around T240 for Group 1, whereas the pupillary diameter was still superior to the pre-administration values at T240 for Group 2 (Fig. 4).

The pupillary light reflex completely disappeared at T90 in Group 1, while it was strongly reduced but still present in Group 2 even though no statistical differences were evident between the 2 groups (Fig. 4). No local and/or general adverse effects occurred in any of the examined birds.

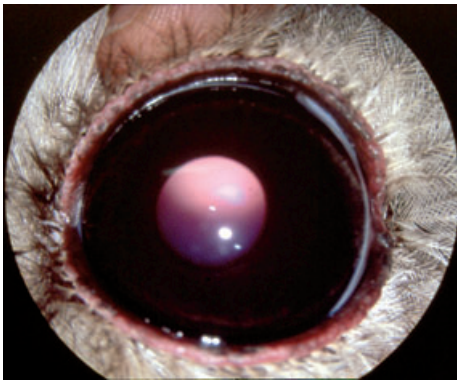

Figure 1. Left normal eye of an adult tawny owl. Note the dark brown iris and the diameter of the pupil at around 4 mm, under direct light stimulation.

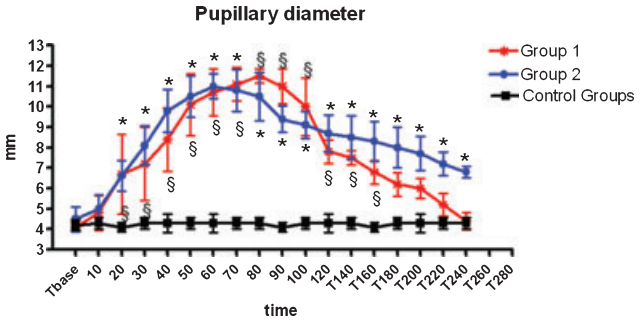

Figure 2. Pupillary diameter of treated eyes (Groups 1 and 2) and nontreated eyes (control groups); Tbase: pre-treatment diameter. §Significant difference of Group 1 compared to control group. *Significant difference of Group 2 compared to control group.

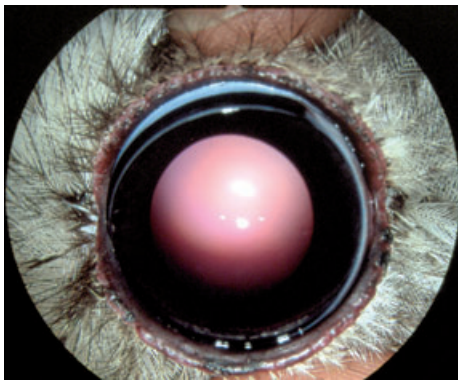

Figure 3. Iris dilatation of the left eye in a tawny owl belonging to the Group 1. A single topical dose of 0.35 mg of rocuronium bromide determines a consistent mydriasis.

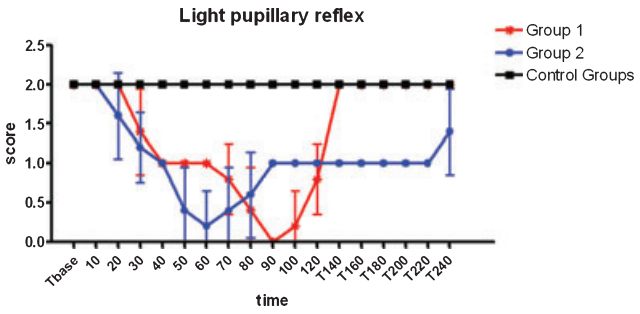

Figure 4. Light pupillary reflex score of treated eyes (Groups 1 and 2) and of nontreated eyes (control groups).

DISCUSSION

Results of the present study suggest that a single topical administration of 0.35 mg of rocuronium bromide to the eyes of healthy tawny owls results in good mydriasis, sufficient to allow for a complete fundus evaluation. A second topical administration of 0.35 mg of rocuronium bromide 15 min following the first administration is unnecessary. In fact, no significant differences in mydriasis onset, time for

maximal effect or maximal pupillary diameter could be discerned when effects of both doses of rocuronium bromide used in this study were compared. Instead mydriasis duration was longer in Group 2 probably due to the repeated dose of rocuronium, which may have caused an accumulation of the drug and consequently its slow release into the eye.

Moreover, rocuronium bromide seems to be safe at the doses used by the authors of the present study, because no general or local adverse effects developed in the birds during both protocols.

Following correct procedure of the drug's topical administration is essential for achieving good mydriasis. During preliminary trials, some birds did not show pupil dilatation because the third eyelid was not retained or was insufficiently retained and its excursion caused a rapid elimination of rocuronium bromide from the ocular surface.

The pupillary light reflex never completely disappeared in some birds of both groups although the achieved mydriasis was consistent (≥11 mm) and sufficient for an entire indirect ophthalmic examination. The incomplete disappearance of the pupillary light reflex is possibly due to the heavy pigmentation of the iris of the examined birds. Iris pigmentation could influence the efficacy of a NMBA, as reported by Loerzel *et al*. These authors discovered that topical vecuronium provided only mild to moderate mydriasis in juvenile cormorants with brown iris. In adult cormorants, the iris becomes bright blue and vecuronium, used as topical mydriatic, was effective. Loerzel *et al.* speculated that a pigment binding effect might influence the availability of the NMBA. This theory seems to be confirmed by our experience on species of birds of prey with yellow irides (*Asio otus*). In these birds, a single topical administration of rocuronium, even at dosages lower than 0.35 mg, provided a maximal mydriatic effect (Barsotti G, unpublished data).

The mydriatic efficacy of topically administered pancuronium bromide, alcuronium chloride and vecuronium bromide has been studied in diurnal birds of prey (*F. tinnunculus*). Pancuronium bromide determined transitory and inconsistent mydriasis, while alcuronium chloride produced a complete mydriasis but general adverse effects developed. Only vecuronium bromide was found to be effective without side effects. This drug, applied to one eye only, provided an effective mydriasis of the pupil, but only after repeated administrations (three times) at 15-min intervals.[9]

To the authors' knowledge, the safety of the concurrent bilateral topical administration of any curariform agent to the eyes of birds has not been evaluated to date, possibly because the cumulative dose required to induce pupil dilatation in both eyes could be dangerous and might result in severe side effects. The adverse effects related to the topical use of NMBAs include eyelid, neck and hind limb muscle paralysis and death.[3,4,9] The side effects severity is dependent on the dose of the employed NMBA and the repeated topical administrations suggested in all the protocols reported could be responsible of a higher systemic absorption of the drugs.

It is important to note that the results of the present study demonstrate that the single application of rocuronium to the eyes of treated birds was sufficient to obtain good mydriasis. Third eyelid retention in birds during the topical application of the mydriatic drugs is not mentioned in veterinary literature, but according to our results the addition of the eyelid retractor in the instillation technique could be partially responsible of the good mydriasis obtained with a single drug administration. The absence of statistically significant differences between the two employed protocols and the lack of any side effects may also suggest that the administration of rocuronium simultaneously in both eyes may be safe. Considering the results of this study in healthy tawny owls, the authors of the present article are observing positive results in many species of birds, using rocuromiun as topical mydriatic drug, bilaterally applied at the same time.

In conclusion, the topical use of rocuronium bromide provides consistent mydriasis in nocturnal birds of prey with a single administration and seems to be a good alternative to the instillation of vecuronium bromide or to the intracameral injection of d-tubocurarine.

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