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Bilateral mydriasis in common buzzards (*Buteo buteo*) and little owls (*Athene noctua*) induced by concurrent topical administration of rocuronium bromide

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Abstract

Objective To evaluate the achievement of a bilateral mydriasis in raptors induced by a concurrent topical application of rocuronium bromide and to assess any side effects that might result from its use.

Animals studied Ten healthy adult common buzzards (*Buteo buteo*) and 10 healthy adult little owls (*Athene noctua*).

Procedures Common buzzards (Group 1) received a single dose of 0.40 mg of rocuronium bromide in each eye (total dose 0.80 mg/bird), whereas the little owls (Group 2) received a single dose of 0.20 mg in each eye (total dose 0.40 mg/bird). The drug was topically instilled in all the birds of both groups. The pupil diameter was measured with a pupillary gauge and the assessment of the pupillary light reflexes was performed using a standard light source.

Results Maximal pupillary diameter was 8.10 ± 0.56 mm in the right eye and 8.05 ± 0.59 mm in the left eye for Group 1 and 10.0 ± 0.75 mm in both eyes for Group 2. No statistical differences were evidenced between the achieved pupillary diameters of both eyes in each group. The maximal pupillary diameter was achieved at T110 min and T40 min for Groups 1 and 2, respectively. The drug did not cause noticeable adverse effects in the examined birds.

Conclusions A single concurrent topical administration of rocuronium bromide to the eyes of the examined birds induced a complete bilateral mydriasis in both eyes without causing any adverse effect.

Key Words: common buzzard, little owl, mydriasis, neuromuscular blocking agent, raptor, rocuronium bromide

INTRODUCTION

The achievement of mydriasis in birds is only possible using neuromuscular blocking agents (NMBAs), such as curariform drugs (d-tubocurarine and its derivatives), since the avian iris is mainly composed of striated muscle fibers and the topical parasympatholytic drugs used in mammals are ineffective.[1–3] It is also possible to induce pupillary dilation in birds through general anesthesia.[4,5] D-tubocurarine was noted to be very efficacious in producing mydriasis when administered intracamerally.[1,6,7] A partial pupillary dilation or no pupillary dilation was achieved using topically instilled d-tubocurarine in pigeons.[7] The mydriatic effect of other topically applied curariform drugs, such as alcuronium chloride, pancuronium bromide, vecuronium bromide alone and their association with surface-acting agents (saponin or benzalkonium chloride), or autonomic drugs (atropine, phenylnephrine) have been investigated in different protocols with varying results.[8–10]

Results of these studies suggest that only vecuronium bromide topically applied alone or in association with autonomic drugs might be a good mydriatic agent in birds because it induces a good pupillary dilation without side effects but only after repeated administrations.

Recently, the mydriatic effect of topically applied rocuronium bromide (a derivative of vecuronium bromide) in

tawny owls has been reported.[11] The authors compared the mydriatic effect of a single dose vs. two doses (15 min-interval) of 0.35 mg of rocuronium bromide administered topically to one eye and the results demonstrated that a single topical administration to the eyes of the examined birds was sufficient to produce a good pupillary dilation, and a second topical administration was unnecessary. In the two protocols studied, the drug did not cause any adverse effect.

It is important to note that in all of the studies investigating mydriasis in birds using topical administration of d-tubocurarine derivatives, the drug was applied to one eye only. The safety of concurrent bilateral topical administration of any of these curariform agents has not been evaluated to date.

The objective of this study was to induce a bilateral mydriasis with concurrent administration of a single dose of rocuronium bromide to both eyes of two species of raptors and to assess any general and/or local side effects which might result from the use of this drug.

MATERIALS AND METHODS

Ten healthy, adult common buzzards (*Buteo buteo*), weighing 500–600 g, of different sexes, and ten healthy, adult little owls (*Athene noctua*), weighing 130–170 g, of different sexes, were enrolled in the study. The birds were presented to the Veterinary Clinic of the University of Pisa for an ophthalmic examination to evaluate the presence of any 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 periocular region were examined in normal light for gross abnormalities. Schirmer tear test readings were obtained for 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 topical administration of 0.4% oxibuprocaine chloridrate (Benoxinato chloridrate INTES®; ALFA INTES Industria Terapeutica Splendore S.r.l., Naples, Italy). The adnexa and anterior segment of both eyes were examined with a slit-lamp (Kowa SL-14; Kowa Company, Tokyo, Japan). Following pupillary dilation in both eyes of each bird, the fundus was examined using a binocular indirect ophthalmoscope (Omega 180; Heine, Berlin, Germany) with a 30–40D lens. A fluorescein stain was performed OU as a final step to exclude corneal lesions.

Mydriasis was achieved using the neuromuscular blocking agent, rocuronium bromide (Esmeron®; Organon Italia S.p.a., Rome, Italy), which was administered topically to both eyes 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. During each application, the third eyelid was retained with a lid retractor for a 1-min period, to prevent its excursion and avoid the rapid elimination of rocuronium from the ocular surface.

Pupil diameter and direct pupillary light reflex were recorded prior to the administration of rocuronium bromide (Tbase) and every 10 min post-administration until 100 min post-administration. Thereafter, measurements were performed and recorded in 20 min intervals for a maximum of 240 min in common buzzards and 320 min in little owls. At these points, measurements were stopped to avoid excessive handling of the birds and reduce the amount of related stress exposure. At the end of the monitoring of the pupillary diameter, all the animals were kept into single cages and they were controlled for any side effects for a period of 24 h. At the time of the study, all the examined birds had been living in captivity for a period of 2–6 months, were tolerant of handling during the procedures and required minimal restraint.

The assessment of the direct pupillary light reflex was performed in a darkened room using a 15-W halogen lamp (SL-14, Kowa, Tokyo, 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 and the same pupil gauge was used to evaluate the pupil diameter in ambient light conditions.

Birds were monitored for the occurrence of signs of ocular irritation (lacrimation, blepharospasm, conjunctival hyperemia, and chemosis), eyelid, wing, hind limb and neck muscle paralysis and any kind of respiratory distress and these were recorded 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 was analyzed statistically using Graph Pad Prism4® (San Diego, CA, USA). Data was summarized as mean (X) ± SD and evaluated for normal distribution using the Kolmogorov–Smirnov method and Wilcoxon Signed Rank Test was used to define Gaussian distribution. 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 whereas a one-way analysis of variance was used to verify differences between pupil diameters in ambient light conditions and in response to the direct artificial light for each eye. *Post hoc* evaluation was performed using Dunnett and Tukeys test, respectively. A one-way ANOVA Friedman test with a Dunn's *post hoc* test was used to assess differences between Tbase and subsequent time intervals within the observation timeframe and between the two eyes for the pupillary light reflex. Differences were considered statistically significant for $P \leq 0.05$.

RESULTS

Of 17 common buzzards and 20 little owls admitted to the clinic, 10 birds of each species were included in the study.

In the common buzzards (Group 1), the maximal mydriasis was achieved at T 110 min and measured 8.10 ± 0.56 mm in the right eye and 8.05 ± 0.59 mm in the left eye with direct light and 8.05 ± 0.49 mm in the right eye and 7.75 ± 0.92 mm in the left eye in ambient light. A significant difference was detected between Tbase and T20 min and all the other subsequent time points for both eyes whether with direct light or in ambient light (Fig. 1a,b). No statistical differences were evidenced between the pupillary diameter measured with direct light or in ambient light for both eyes. The pupillary light reflex results were significantly reduced compared with the Tbase values starting from T40 min up to T180 min for both eyes (Fig. 2a). No differences in pupillary light reflex were detected between the two eyes.

In the little owls (Group 2), the maximal pupillary dilation was achieved at T40 min and measured 10.0 ± 0.75 mm in both eyes with direct light and 10.17 ± 0.35 mm in both eyes in ambient light.

A significant difference was detected between Tbase and T10 min and all the other subsequent time points up to T300 min with direct light in both eyes. In ambient light, a significant difference was detected between Tbase and T10 min up to T320 min in both eyes. No statistical differences were evidenced between the pupillary diameters measured with direct light and in ambient light in both eyes (Fig. 3a,b). The pupillary light reflex was significantly reduced in comparison with Tbase from T30 min (right eye) and from T20 min (left eye) up to T110 min in both eyes (Fig. 2b). No differences in the pupillary light reflex were detected between the two eyes.

The pupillary dilation obtained was sufficient for a complete fundus examination in all the birds of each group. No side effects were noticed in the two groups.

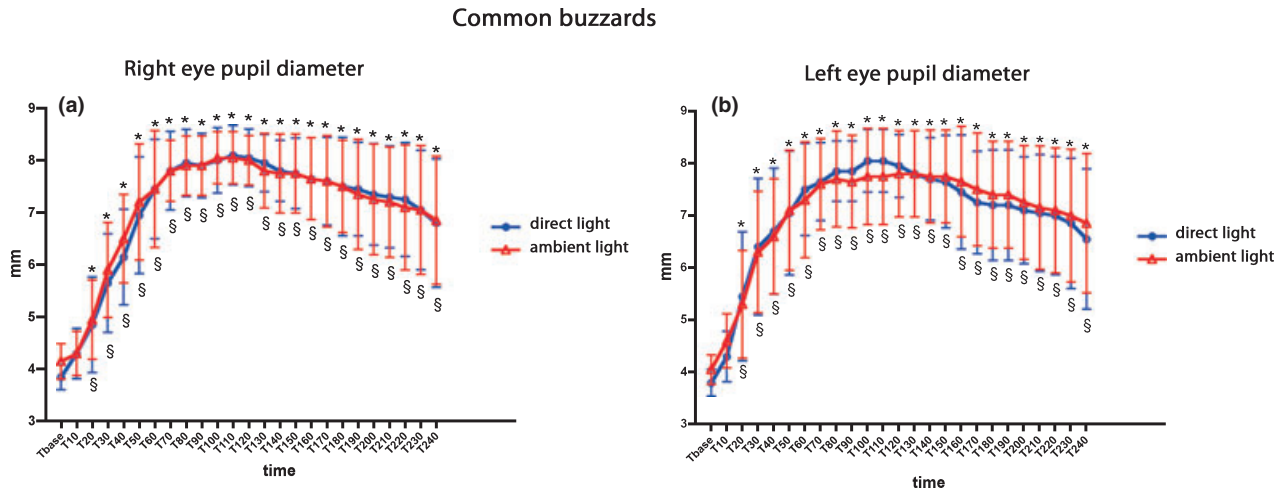

Figure 1. Each line represents average pupil diameters of the right eye (a) and of the left eye (b) in ambient light and with direct light of common buzzards. *Significant difference from Tbase with direct light; §significant difference from Tbase in ambient light.

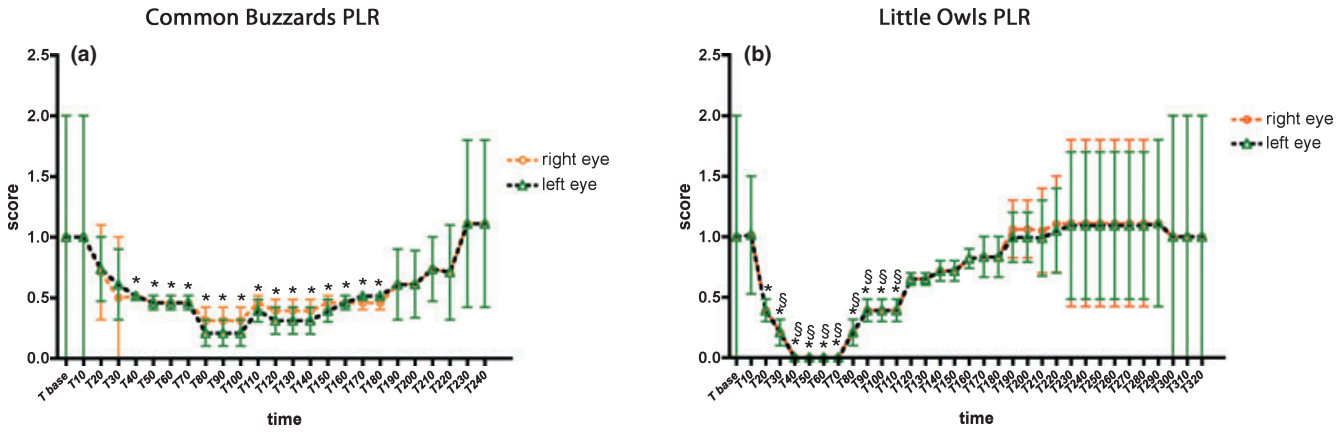

Figure 2. Light pupillary reflexes average score for Group 1 (a) and for Group 2 (b). *Significant difference from Tbase.

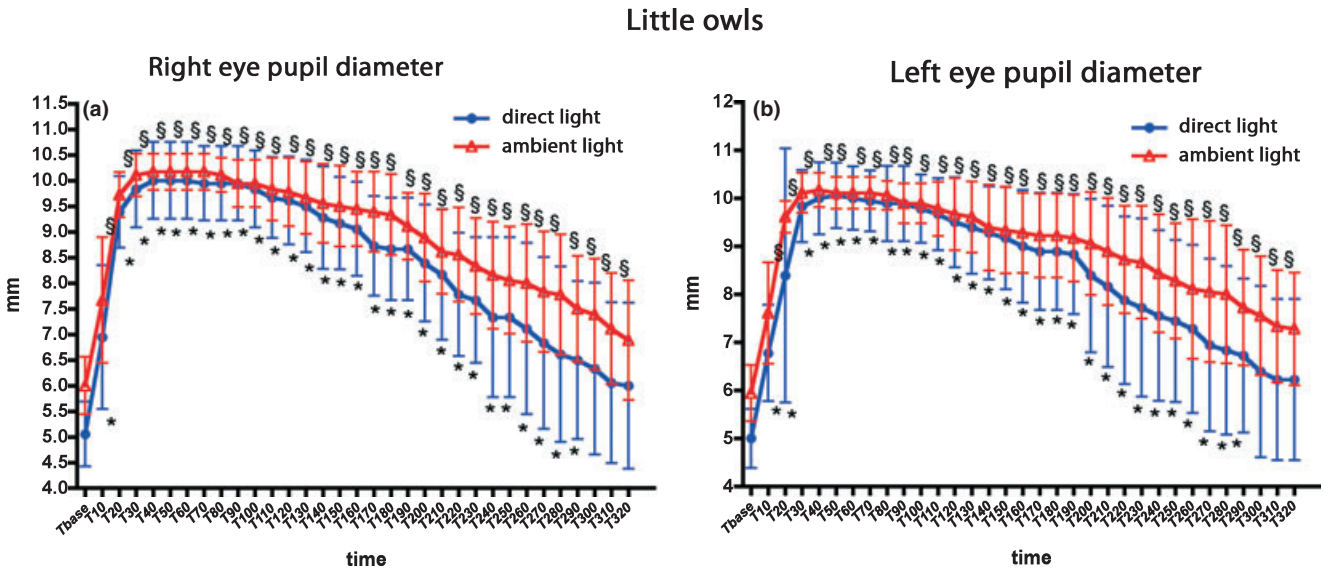

Figure 3. Each line represents average pupil diameters of the right eye (a) and of the left eye (b) in ambient light and with direct light of little owls. *Significant difference from Tbase with direct light; §significant difference from Tbase in ambient light.

DISCUSSION

A single topical administration of rocuronium bromide to both eyes of birds of Group 1 and Group 2 provided a consistent bilateral mydriasis without noticeable side effects. This is the most important result of this study as to the authors' knowledge the safety of the concurrent bilateral topical application of any neuromuscular blocking agent to the eyes of birds of prey has not been investigated to date.

In all the birds of both groups, the pupillary light reflexes disappeared and the achieved mydriasis was consistent (≥8 mm) in common buzzards (Figs 4, 5, 6 and 7) and maximal (≥10 mm) with an almost complete disappearance of the iris in little owls (Figs 8, 9 and 10).

Even though the statistical differences between Groups 1 and 2 are not really comparable because of the different doses of rocuronium employed in each group of this study, the obtained results suggest some differences in the speed of onset and the duration of the drug effect among the two species of birds of prey. With regard to the speed of onset, the effect of rocuronium varied by groups. In fact, the higher degree of pupillary dilation was obtained at T110 min in Group 1 and at T40 min in Group 2. This difference is probably due to the different iris pigmentation of the birds of Groups 1 and 2. Common buzzards and little owls have brownish and yellow irises, respectively. As reported by others,[10,11] iris pigmentation could influence the availability of a curariform agent within the eye due to a probable pigment binding effect.

Figure 4. Right normal eye of an adult common buzzard. Note the brownish pigmentation of iris and the diameter of the pupil at around 4 mm, under direct light stimulation.

Figure 5. Left normal eye of the same adult common buzzard of Fig. 4.

Figure 6. Iris dilatation of the right eye of Fig. 4. A single topical dose of 0.40 mg of rocuronium bromide determines a good mydriasis.

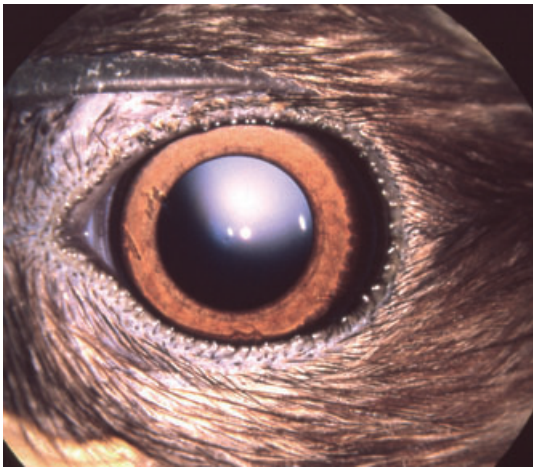

Figure 7. Iris dilatation of the left eye of Fig. 5. A single topical dose of 0.40 mg of rocuronium bromide determines a good mydriasis.

Figure 8. Both eyes of an adult little owl. Note the yellow iris and the diameters of the pupil at around 5 mm, under direct light stimulation.

Figure 9. Bilateral concurrent mydriasis in the little owl of Fig. 8. A single topical dose of 0.20 mg of rocuronium bromide in each eye determines a consistent mydriasis.

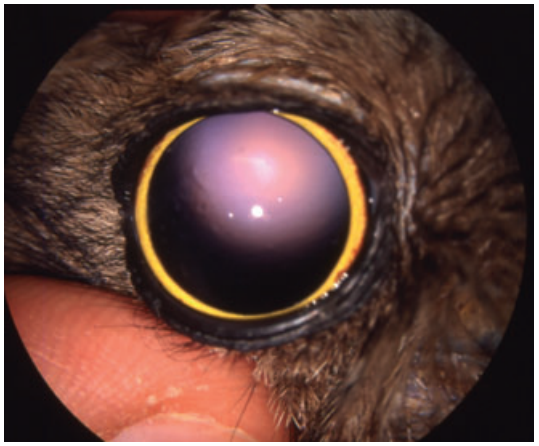

Figure 10. Maximal mydriasis in a little owl post-administration of a single dose of 0.20 mg of rocuronium bromide. Note the almost complete disappearance of the iris.

The duration of effect of rocuronium was not directly comparable because measurements were stopped at two different time points in the two groups and the employed doses of the drug were different. However, the results suggest that mydriasis lasted longer in Group 1 where the pupil diameter at T240 min decreased more slowly compared with Group 2. This fact seems to confirm the pigment-binding effect[10,11] that could cause an initial accumulation of the drug with a delay of mydriasis onset and then a slow release within the eye with a prolonged duration of action, but it could also be related to the different species of birds evaluated.

Rocuronium bromide appears to be effective and safe at the doses used in this study, because the achieved mydriasis was sufficient for an entire indirect ophthalmic examination and no general or local side effects developed in the birds of both groups.

As previously reported,[11] the authors of this study confirm the importance of third eyelid retention during the topical administration of the drug to avoid its rapid elimination from the ocular surface and consequently its lack of action. In the authors' opinion, this can reduce the necessity of repeated doses which may in turn reduce the risk of severe side effects associated with multiple dosing. In the veterinary literature, the adverse effects related to the topical use of NMBAs, include eyelid, neck and hind limb muscle paralysis, and death[8–10].

In the past, other topically applied NMBAs have been tested in diurnal birds of prey and only vecuronium bromide was considered efficacious and safe[8]. The achieved mydriasis was good but only after three repeated administrations. Also the drug was applied to one eye only.

More recently, rocuronium bromide was evaluated as a topical mydriatic drug in nocturnal birds of prey but also in this study, the application was restricted to a single eye.[11] The authors compared the mydriatic effect of a single dose vs. two doses (15 min-interval) of 0.35 mg of rocuronium bromide administered topically to one eye and they concluded that a single topical administration to the eyes of the examined birds was sufficient to cause good pupillary dilation, therefore concluding that a second topical administration was unnecessary. In the two protocols studied, the drug did not cause any adverse effects, thus the authors suggested that the administration of rocuronium simultaneously in both eyes could be safe.

As previously reported,[11] the results of this study confirm that rocuronium bromide is a safe and efficacious topical mydriatic drug in birds of prey and that a single dose of the drug applied to each eye is sufficient to induce consistent pupillary dilation. With the doses employed in this study, it was also possible to induce concurrent, bilateral mydriasis in common buzzards, and little owls without any adverse effects.

To the authors' knowledge this is the first report discussing concurrent bilateral mydriasis in birds of prey using a topically applied NMBA. It represents a significant improvement in avian ophthalmology, describing the possibility of examining both eyes at the same time, thus allowing a reduction of the bird's handling-time and its stress-related consequences.

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