Wildlife Science

Efficacy of topical rocuronium bromide as a mydriatic agent in domestic pigeons (*Columba livia*)

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**ABSTRACT.** This study was conducted to investigate the efficacy of rocuronium bromide as mydriatic agent in domestic pigeons (*Columba livia*). This study was done in two phases. In the first phase, rocuronium bromide (0.20 mg/20 µl) was topically instilled to the right eye (OD) of eight domestic pigeons. Pupil diameter was measured before instillation (T0), and at 5 (T05) and 10 (T10) min after instillation, and every 10 min thereafter until 160 (T160) min. Pupillary light reflex (PLR) was assessed using a scoring system at the same time points. In the second phase, the same dosage was instilled twice in the span of 10 min into both eyes (OU) of four pigeons (eight eyes). Measurements were done accordingly. The iris color in the first phase were: gravel, pearl and bull eye. All irises in the second phase were bull eye. Mydriasis were observed in 6/8 (75%) pigeons in the first phase. Maximal mydriasis was observed at T30 (mean pupil diameter=4.62 ± 0.13 mm). Pupil diameter in the treated eye was significantly different from contralateral eye and from T0 since T05 (*P*=0.017 and *P*=0.006, respectively)−T120 (*P*=0.043 and *P*=0.044, respectively). PLR was disappeared from T10 (*P*=0.034) to T90 (*P*=0.041). In the second phase, mydriasis was only observed in 2/8 eyes. This study suggested that rocuronium bromide was able to produce mydriasis in pigeons other than bull eye iris.

**KEY WORDS:** *Columba livia*, iris color, mydriasis, pigeon, rocuronium bromide

Mydriatic agents are important diagnostic tools in ophthalmic examinations, especially for evaluating the posterior segment of the eye [13]. In exotic animals, including birds, the small globe increases the difficulty of critical examination of the lens and posterior segment such as vitreous body, retina, and pecten; therefore, pharmacologic mydriasis may facilitate the entire eye examination [8]. In avian patients, it could be troublesome to induce mydriasis since the irises of the birds have a different muscular system than those of the mammals, which possess both striated muscle fibers and nonstriated fibers [3, 8, 19]. Therefore, parasympatholytic and sympathomimetic agents that are commonly used to induce mydriasis in mammals’ irises do not work in avian patients as the latter have predominantly striated fibers in nature [11].

In pigeons, several compounds have been investigated to induce mydriasis including *d*-tubocurarine [26], curare, and gallamine [6]. The use of *d*-tubocurarine was considered to be impractical as it needs to be administered via intracameral injection which could predispose the eyes to several complications such as intraocular infections and cataract formation [26]. Conversely, topical applications of curare and gallamine are easy to apply but they give fairly inconsistent results even though benzalkonium chloride is added to help reduce the surface tension of the cornea [6].

Recently, rocuronium bromide has been reported as an efficient mydriatic agent in several species of birds, including Hispaniolan Amazon parrots (*Amazona ventralis*) [1, 21], common buzzard (*Buteo buteo*), little owl (*Athene noctua*), tawny owls (*Strix aluco*) [2, 3], and European kestrels (*Falco tinnunculus*) [4]. Topical application of rocuronium bromide was reported to be relatively safe, with the majority of bird species experiencing no major side effects, except one report of corneal ulceration [1] and a transient lower eyelid paresis in Hispaniolan Amazon parrots [21]. Despite such abundance of reports in other species, rocuronium bromide has not been investigated in pigeons. This study aimed to investigate the efficacy and safety of rocuronium bromide as a mydriatic agent in pigeons.
MATERIALS AND METHODS

Twelve healthy adult domestic pigeons (Columba livia) of undetermined sex were used in this study. This study was approved by the Seoul National University Institutional Animal Care and Use Committee (SNU-181224-5). The eyes and periorcular region were examined for gross abnormalities in a well-lit room. Intraocular pressure (IOP) measurements (TonoVet®; Icare; Tiolat, Helsinki, Finland; rebound tonometer) were obtained and the adnexa and anterior segment were examined with slit-lamp biomicroscopy (Keeler® PSL One Portable Slit Lamp; Keeler Ltd., Windsor, UK).

This study was done in two phases. In the first phase of the study, rocuronium bromide (0.20 mg/20 µl) was instilled once into the right eye (OD) of eight birds. The pupil diameter prior to the administration of rocuronium bromide was measured at the time base (T0). The birds were manually restrained and held in lateral recumbency during the instillation of rocuronium bromide with the cornea positioned upward and were maintained in that position for at least 15 sec to facilitate drug absorption. After the instillation of rocuronium bromide, the pupil diameter was measured at 5 (T05) and 10 (T10) min time points and every 10 min thereafter, until the pupillary light reflex (PLR) briskly returned at 160 (T160) min. The measurement was performed by placing a ruler in contact with the dorsal surface of the eye at a distance of 2 mm from the upper eyelid and its photograph was obtained using a cellular iPhone® SE camera without the use of flash under the same room light condition. The measurement was also performed at the contralateral eye (OS) at every time point.

Direct PLR was evaluated using a finnoff (WA 41100®; Welch Allyn, Skaneateles Falls, NY, USA) in a darkened room at every time point after the measurement of pupil diameter. The direct PLR was evaluated using a scoring system (0: normal, 1: decreased, 2: almost disappeared, 3: absent).

To further examine the ability of rocuronium bromide to induce mydriasis in pigeon with bull eye colored iris, the second phase of the study was conducted. In the second phase of the study, rocuronium bromide (0.20 mg/20 µl) was instilled into both eyes OD and OS (left eye) of four birds (eight eyes). The PLR and diameter of the pupil were measured the same way as phase one of the study. In this phase of the study, when rocuronium bromide were failed to induce the mydriasis after 10 min, the second dosage were instilled and the measurement were done accordingly.

Throughout the course of the experiment, the animals were monitored for any sign of side effects, including ocular irritation, lacrimation, blepharospasm, conjunctival hyperemia, chemosis, and paralysis of the eyelid, wing, hind limb, or neck.

Data were presented as the mean ± standard deviation (SD). Statistical analysis was performed using IBM SPSS Statistics 23.0 (IBM Corp., Armonk, NY, USA). A paired student’s t-test was used to compare pupillary diameter at all time points to T0 and the contralateral eye. The Wilcoxon signed-rank test was used to evaluate the PLR at every time point compared with T0. Differences were considered statistically significant at P≤0.05.

RESULTS

General ophthalmic examination of the experimental pigeons was normal. IOP ranged from 10 to 15 mmHg. Rocuronium bromide successfully induced mydriasis in six out of eight pigeons used in the first phase of this study. In order to investigate the degree of mydriatic effect induced by rocuronium bromide in pigeons, only the data from the six pigeons in which the drug successfully induced mydriasis were included in the statistical analysis. The effect was immediately observed 5 min after the application of rocuronium bromide in pigeons in which mydriasis was induced. Optimal mydriasis was observed at T30 with a mean diameter of 4.62 ± 0.13 mm (Fig 1). Pupillary diameter OD was significantly different from that at T0, starting from T05 (P=0.006) up to T120 (P=0.044; Fig 1; Table 1). Pupillary diameter OD also showed significant differences compared with the OS starting from T05 (P=0.017) up to T120 (P=0.043; Fig 1; Table 1). The PLR was disappeared from T10 (P=0.034) to T90 (P=0.041; Table 2).

In the first phase of the study, three different colors of iris were observed among the eight birds: gravel (1/8), pearl (3/8), and bull eye (4/8). The two eyes in which rocuronium bromide were failed to induce mydriasis were both had bull eye color. In the second phase of the study, all of the birds had bull eye colored irises (8 eyes out of four birds). In this phase of the study, the first instillation of rocuronium bromide was failed to induced mydriasis in all of the eyes. The second instillation of rocuronium bromide were managed to induce mydriasis in two of the eight eyes. Because of the lack of efficacy, statistical analysis was not performed for the second phase of the study.

During the topical instillation of the drug, all pigeons in this study experienced irritation of variable degrees with three pigeons reacting more strongly as compared with the others. All pigeons remained alert and responsive during the experiment. Five pigeons in the first phase of the study had transient lower eyelid paresis, which resulted in transient lower eyelid elevation during which the ability of blinking was retained. This was not observed during the second phase of the study. Besides these, no other local or systemic adverse effects were observed.

DISCUSSION

Rocuronium bromide has been widely reported to be a safe and efficient topical mydriatic agent to induce mydriasis in several species of birds, including Hispaniolan Amazon parrots [1, 21], common buzzard, little owl, tawny owl [2, 3], and European kestrels [4]. In previous reports, rocuronium bromide managed to produce consistent mydriasis in 100% of the birds [1–4, 21]. In the first phase of the present study, however, rocuronium bromide was only effective in 6/8 (75%) of the pigeons. With striated
muscle fibers known to be predominant in the birds' irises, neuromuscular blocking agents are expected to produce mydriasis in birds [11, 24]. However, there seem to be variations in the arrangement and development of the various muscular components among species of birds [1, 19, 20, 24], which is probably why the efficacy of rocuronium bromide in pigeons in this study was different from that reported previously [1–4, 21]. Another possible explanation for the lack of efficiency of rocuronium bromide in inducing mydriasis in this study might be related to the different iris coloration among the pigeons used. Three iris types are known to exist in pigeons: the wild type or "gravel", the white or "pearl", and the "bull eye", which is almost black [25]; all three variations existed in pigeons in the first

**Table 1.** Comparison of mean pupil diameter of domestic pigeons OD after application of rocuronium bromide

| Time (min) | Mean pupil diameter, mm | P value | Compared to T0 | Compared to OS |
|------------|-------------------------|---------|----------------|---------------|
|            | OD                      | OS      |                |               |
| 0          | 3.04 ± 0.18             | 3.16 ± 0.30 | 0.296         |               |
| 5          | 3.91 ± 0.37             | 2.95 ± 0.18 | 0.006*        | 0.017*        |
| 10         | 4.25 ± 0.38             | 2.95 ± 0.29 | 0.001*        | 0.003*        |
| 20         | 4.58 ± 0.12             | 2.95 ± 0.18 | 0.000*        | 0.000*        |
| 30         | 4.62 ± 0.13             | 2.95 ± 0.29 | 0.000*        | 0.000*        |
| 40         | 4.50 ± 0.27             | 2.91 ± 0.12 | 0.000*        | 0.000*        |
| 50         | 4.50 ± 0.38             | 3.08 ± 0.20 | 0.001*        | 0.001*        |
| 60         | 4.45 ± 0.40             | 3.12 ± 0.20 | 0.001*        | 0.001*        |
| 70         | 4.37 ± 0.44             | 2.95 ± 0.29 | 0.001*        | 0.002*        |
| 80         | 4.33 ± 0.43             | 3.00 ± 0.27 | 0.002*        | 0.005*        |
| 90         | 4.20 ± 0.55             | 2.91 ± 0.20 | 0.005*        | 0.007*        |
| 100        | 4.12 ± 0.86             | 2.87 ± 0.13 | 0.032*        | 0.019*        |
| 110        | 4.08 ± 0.75             | 2.91 ± 0.12 | 0.022*        | 0.016*        |
| 120        | 3.91 ± 0.75             | 2.91 ± 0.20 | 0.044*        | 0.043*        |
| 130        | 3.87 ± 0.80             | 3.04 ± 0.18 | 0.061         | 0.070         |
| 140        | 3.70 ± 0.74             | 2.95 ± 0.10 | 0.112         | 0.080         |
| 150        | 3.79 ± 0.90             | 3.00 ± 0.15 | 0.118         | 0.112         |
| 160        | 3.58 ± 0.73             | 2.91 ± 0.12 | 0.143         | 0.116         |

**Table 2.** Comparison of mean PLR scores of domestic pigeons in the right eye (OD) after application of rocuronium bromide from T0 min

| Time (min) | PLR | P value |
|------------|-----|---------|
| 0          | 0.00 ± 0.00 |         |
| 5          | 2.20 ± 1.30 | 0.059   |
| 10         | 2.80 ± 0.44 | 0.034*  |
| 20         | 3.00 ± 0.00 | 0.025*  |
| 30         | 3.00 ± 0.00 | 0.025*  |
| 40         | 3.00 ± 0.00 | 0.025*  |
| 50         | 3.00 ± 0.00 | 0.025*  |
| 60         | 3.20 ± 1.09 | 0.039*  |
| 70         | 2.40 ± 0.54 | 0.038*  |
| 80         | 2.00 ± 1.00 | 0.041*  |
| 90         | 2.00 ± 1.00 | 0.041*  |
| 100        | 1.60 ± 1.34 | 0.063   |
| 110        | 1.60 ± 1.34 | 0.063   |
| 120        | 1.20 ± 1.30 | 0.109   |
| 130        | 0.60 ± 0.89 | 0.180   |
| 140        | 0.60 ± 0.89 | 0.180   |
| 150        | 0.40 ± 0.89 | 0.317   |
| 160        | 0.20 ± 0.44 | 0.317   |

a) Mean ± SD. PLR: Pupillary light reflex (0: normal, 1: decreased, 2: almost disappeared, 3: absent). b) *P<0.05, statistically significant.
phase of the study. The two pigeons that did not respond to rocuronium bromide had a greyish brown-colored iris or what is known as “bull eye”. In the second phase of the study, all of the eyes also had bull eye irises. These different responses from different iris colors to rocuronium bromide are similar to the observation from its parent compound, vecuronium bromide, when used as a mydriatic agent in double-crested cormorant (*Phalacrocorax auritus*) in which vecuronium bromide was effective in inducing mydriasis in an adult cormorant with a blue-colored iris but was only marginally effective and highly inconsistent when used in juvenile cormorants with a brown-colored iris [12].

Interestingly, not all pigeons with greyish brown-colored iris or “bull eye” in this study failed to respond to single instillation of rocuronium. It is important to note that even though only eight pigeons were included in the first phase of the study, during the preliminary study, a single instillation of rocuronium bromide was effectively induced mydriasis in all pigeons with such iris color. Another possible reason for this failure could be that the volume of the drugs instilled could not be maximally absorbed. Although it was ensured that all dosages were retained at the eye fissure during the instillation it was observed that several pigeons made the swallowing gesture several times, which might have resulted in the drug being rapidly drained through the nasolacrimal duct before it was absorbed in the eyes. However, observing the result from the second phase of the study, in which rocuronium were given twice, we were inclined to believe that the different iris coloration might be the reason for these different results.

In the cormorant study, it was suspected that the different pigmentation might have resulted in pigment-binding effect of the vecuronium in the brown-colored iris, affecting the availability of the drug as it bound to the melanin and therefore, failed to induce effective mydriasis [12]. The tendency of the drug compound to bind to the melanin has been shown in several experiments and is known to influence ocular pharmacokinetics of the drugs that it is usually associated with prolonged drug retention in the pigmented tissue leading to prolonged drug responses [23, 24]. In pigeons, different iris coloration is associated with the presence or absence of the pigments, guanine and pteridine, in the anterior surface of the iris [17, 18]. In pigeons, the color gravel (yellowish red; Fig. 2A, 2B) is associated with the presence of both guanine and pteridine pigments while the color pearl (white with varying tinges of red to pink variations due to abundant blood vessels) (Fig. 2C) is associated with the guanine pigment [17, 18]. The absence of both the pigments in the anterior surface of the iris results in the “bull eye”, (Fig. 2D) in which the posterior hexagonal brown or black pigment shows through and thus produces the black color effect [5].

Considering that the same iris color in different species of birds could be caused by different mechanisms [5, 24], whether it is the existence of certain pigment or the lack of it [5], it is difficult to conclude whether the failure of rocuronium to induce mydriasis in a pigeon with “bull eye” iris is caused due to the same reason as vecuronium’s lack of effect in juvenile cormorant, without the knowledge of the nature of cormorant iris color pigmentation. In fact, the majority of black or very dark brown iris in birds owe their dark color to the presence of anterior iris pigment and are different from the “bull eye” irises in pigeons [5]. That being said, it will be interesting to investigate the effect of rocuronium on the same species of birds with a different iris

![Image](https://example.com/image.jpg)

**Fig. 2.** The iris colors in pigeons. (A) Gravel colored iris (partial) during miosis, (B) Partially gravel colored iris during mydriasis, (C) Pearl colored iris during mydriasis, (D) Bull eye iris during miosis.
color to understand whether rocuronium has the tendency to bind to certain iris pigment and whether or not it is affecting the pharmacokinetics of the rocuronium. In the present study, the pigeons were obtained randomly from a pigeon farm; therefore, the variation in color of the irises in these pigeons could not be controlled. Furthermore, because of the small number of pigeons used in this study, there is also a possibility for rocuronium to fail to induce mydriasis in pigeons with gravel and pearl eyes even though, in this experiment, mydriasis was induced successfully in irises of those colors.

The dosage of rocuronium used in this study was established based on a previously published study on other species [1–4, 21]. Smaller dosage was recommended with regard to safety precautions over the possible side effects since neuromuscular blocking agents are known to have caused systemic adverse effects such as eyelid, neck, and hind-limb muscle paralysis when used as mydriatic agents in birds [4, 15]. Previous studies on rocuronium bromide on other species did confirm the safety profile of rocuronium bromide as a mydriatic [1–4, 21]; the only side effects reported were superficial corneal ulceration, either caused by restrain or by corneal irritation from the acidity of the drugs [1] and a transient lower eyelid paresis [21]. Rocuronium bromide is known to have a low pH [1] and therefore, might cause irritation when applied topically to the eyes. During the instillation of the drugs in this study, all pigeons seemed to have experienced a variable degree of irritation, with three pigeons reacting more strongly as compared with the others.

In the first phase of the study, five pigeons experienced transient lower eyelid paresis, in which the lower eyelid was partially elevated. During this transient lower eyelid paresis, the ability to blink was not affected, which was similar to the observation in Hispaniolan Amazon parrots [21]. None of the birds in the second phase of the study experienced this complication. Lower eyelid paresis was also reported in a study of another neuromuscular blocking agent, alcuronium in Hispaniolan Amazon parrots in which full neck and hind-limb muscle paralysis was also observed [15]. During the course of the present study, transient lower eyelid paresis without any other side effects, except irritation, were observed.

Hodos et al. used vecuronium in their study to produce mydriasis in pigeons through a series of drops every 60 sec during the course of 6–10 min and managed to induce mydriasis of 5.75–6.0 mm in diameter [7]. In the present study, the maximum mydriasis in pigeons induced by a single topical application of rocuronium bromide resulted in a median of 4.62 ± 0.13 mm in diameter with one pigeon achieving a maximum of 5.25 mm of mydriasis. Compared to vecuronium, rocuronium is known to only have one-eighth of the vecuronium potency [10], which might explain the superior mydriasis achieved in the former study. Conversely, although it is impossible to exactly know the dosage of vecuronium in the previous study [7], when we considered one drop to be equal to 0.05 ml, each pigeon in the previous research received approximately 3–5 mg of vecuronium compared with only 0.20 mg rocuronium in the present study, which is probably the reason for the wider diameter of the pupil mydriasis in the former study.

Although rocuronium lacks potency compared to its parent compound, rocuronium is generally known to have a more rapid onset of action [10]. In theory, having a lower potency means that rocuronium is safer to be applied in a larger dosage than vecuronium, and since both drugs have similar molecular weights, the application of a higher dosage of rocuronium will introduce more molecules into the neuromuscular junctions, increasing the availability of rocuronium to bind to the ACh receptors, resulting in a more rapid onset of neuromuscular blockade [9]. The problem with that design is the small size of pigeon eyes. The rocuronium used in this study had a concentration of 10 mg/ml, which was the same concentration used in another study [2, 21] and 0.20 mg was contained in 20 µl. The maximum amount of fluid reported to be able to be held in the human palpebral fissure was only 25–30 µl [16, 22]. Taking that into consideration, even the dosage used in the current study was unlikely to be retained and absorbed completely by the pigeon [22]. That being said, it was interesting to note that the average time needed for rocuronium to produce 4.00–4.75 mm mydriasis in the present study was 10 min after the drug application compared with the previous study in which mydriasis was achieved at 10 min after the last drop, which meant 20 min after the 1st drug application. A study comparing a single and two consecutive instillations of rocuronium bromide in tawny owls showed no significant differences in mydriasis onset, time for maximal effect, or maximal pupillary diameter [2]. However, given that in the present study, a single instillation of rocuronium bromide failed to induce 100% mydriasis in all pigeons, further studies to investigate repeated instillation of rocuronium bromide in pigeons might be beneficial.

In the first phase of this study, rocuronium bromide only successfully induced mydriasis in 6/8 of the pigeon (75%) and only the data from those six pigeons were used in the statistical analysis in order to investigate the degree of mydriasis produced by rocuronium bromide in pigeons. By doing so, it can be concluded that although rocuronium did not always successfully induce mydriasis in pigeons, when it successfully induced mydriasis, the degree of the dilated pupil was significantly different from the normal pupil.

The duration of mydriasis induced by rocuronium bromide in this study was relatively short compared to previous studies on other birds, which was only less than 3 hr compared to 6 hr in Hispaniolan Amazon parrots [21]. The short mydriatic duration would be favorable as it could minimize unnecessary light exposure from a prolonged mydriasis. Even though it was reported that there was no evidence of animals undergoing mydriasis (using tropicamide or atropine) to suffer retinal damage from the ambient light level [14], it has been demonstrated in laboratory animals [27].

In conclusion, rocuronium bromide produced moderate mydriasis in pigeons in this study without significant side effects. In the first phase of study population with all iris colors, a single instillation of rocuronium bromide was effective in 6/8 (75%) of pigeons. In the second phase of the study population with only bull eye iris color, a single instillation of rocuronium bromide was not effective in inducing the mydriasis with only 2/8 eyes being induced after twice instillations. While the mydriasis produced by rocuronium bromide was only moderately effective in the first phase of the study, the degree of mydriasis achieved allowed for fundus examinations with direct ophthalmoscopy.
CONFLICT OF INTEREST. The authors declare no conflicts of interest and there was no financial support of manufacturer associated with the products used in this study

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