The pharmacokinetics of orally administered N-butylscopolammonium bromide in the greyhound and its regulatory implications

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ABSTRACT

N-Butylscopolammonium bromide is an antimuscarinic, antispasmodic drug commonly used in racing greyhounds to treat functional urethral obstruction. An oral administration study was performed in six greyhounds to determine the pharmacokinetics of the butylscopolammonium ion and to generate information regarding the detection window for use in medication control. A single dose of one 10-mg N-butylscopolammonium bromide tablet was administered orally to simulate its use in greyhound racing. Blood, urine and faeces were collected at regular intervals from the greyhounds for up to 9 days and N-butylscopolammonium ion concentrations were determined.

The extent of absorption of the N-butylscopolammoniumion ion was very limited and its elimination from plasma was rapid with a harmonic mean half-life of 2 hours. Urine concentrations initially declined in a similar manner to the plasma concentrations but then began a much longer phase characterized by a half-life of approximately 50 hours. Faecal concentrations declined to very low concentrations between 48 and 120 hours.

The very limited drug absorption makes the use of orally administered butylscopolamine for functional urethral obstruction in greyhounds unjustified, although its use in racing greyhounds is likely to continue as the drug is available without prescription. Medication control is now possible by setting a urinary screening limit based on the urinary drug concentrations as determined in this study.

KEYWORDS

Butylscopolammonium bromide; Greyhound; Medication control; Pharmacokinetics; Urine Butylhyoscine bromide; Hyoscine butyl bromide; Hyoscine N-butyl bromide; Butylscopolamine bromide

INTRODUCTION

Trainers commonly report that some racing greyhounds 'tie up' around racing in association with excitement and characterised by full or partial inability to urinate. This condition has not been well characterised (Van Meeuwen, 2009) but is likely to be due to a functional obstruction such as that defined as detrusor-urethral dyssynergia, which is attributed to excessive sympathetic nerve impulses to the urethral sphincter (Espineira & Nickel, 1997).

N-Butylscopolammonium bromide (also known by a variety of synonyms including butylhyoscine bromide, hyoscine butyl bromide, hyoscine N-butyl bromide, butylscopolamine bromide) acts in dogs as it does in other animals and people. It has the potential to relieve spasms of the smooth muscles of the digestive (Tytgat, 2007) and urinary systems (Papadopoulos *et al.* 2014).

N-Butylscopolammonium bromide is available for medical and veterinary use in injectable formulations, both alone and in combination with the analgesic drug metamizole, and in tablet form. N-Butylscopolammonium bromide is commonly referred to by its original trade

name Buscopan®.

N-Butylscopolammonium bromide is available as a tablet for oral administration for human medical use without prescription in the United Kingdom, Ireland and Australia. Trainers commonly use this readily available product to treat full or partial inability to urinate in racing greyhounds. However, the rationale for this use is questionable due to the poor oral absorption of the N-butylscopolammonium ion due to its quaternary ammonium ion structure (Tytgat, 2007) and reports of its limited efficacy in reducing urethral pressure in dogs (Papadopoulos *et al.* 2014).

No administration studies for N-butylscopolammonium bromide in dogs have been found, and there is very limited published pharmacokinetic data (Tytgat, 2007) in the veterinary product information (Veterinary Medicines Directorate, 2011), or from other residue studies (Committee For Veterinary Medicinal Products 1997).

AIM

The aim of this study was characterise the detection, the plasma and the urinary pharmacokinetics of N-butylscopolammonium after oral administration to greyhounds as the bromide salt at the dose and formulation commonly used by trainers of racing greyhounds, assess systemic absorption of the drug, and also consider the regulatory implications for greyhound racing.

MATERIALS AND METHODS

The study was conducted in accordance with the principles of the VICH GCP guidelines (International Co-operation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products, Good Clinical Practice, June 2000, effective July 2001). Ethical approval was obtained from the New South Wales Department of Industry, Skills and Regional Development.

Dogs were fed a commercial dry dog food (Dogpro PLUS Working Dog, Hypro Petcare P/L) with an additional portion of fresh meat, with the daily feed ration as two meals and with ad libitum access to water at all times. The morning feed was not given on the treatment day prior to drug administration.

One 10-mg tablet of N-butylscopolammonium bromide (Buscopan®, Boehringer Ingelheim) was administered orally once to six greyhounds. Three female and three male animals were studied. They had a mean bodyweight of 32.9 kg and mean age of 4 years. The dose was approximately 0.31 mg/kg of body weight.

Blood, urine and faecal samples were collected before drug administration. Blood samples were collected 1, 2, 4, 6, 8, 12, 24, 36, 48, 96, 144 and 192 hours after dosing. Urine samples were collected 2, 4, 6, 8, 12, 24, 36, 48, 72, 96, 120, 144, 168, 192 and 216 hours after dosing. Faecal samples were collected at 12, 24, 48, 120 and 192 hours after dosing. The blood samples were heparinised and plasma obtained by centrifugation. The frozen samples were transported on dry ice to the analytical laboratory. All samples were stored at -20ºC.

Concentrations of N-butylscopolammonium were determined in the pre- and post-administration plasma and urine samples. For extraction, ipratropium bromide was selected as an internal marker due to the similarity of its structure to N-butylscopolammonium. For extraction of the urine and faecal samples, ammonium bicarbonate (10 mM, pH 10) was added and samples were centrifuged before weak cation exchange solid phase extraction (Isolute® CBA 500 mg, Biotage EU). The cartridges were conditioned, the sample loaded, washed with ammonium bicarbonate and methanol and then eluted with 0.1% formic acid in methanol. For extraction of the plasma samples (200 µL), after addition of dichloromethane they were mixed and then centrifuged before transferring the organic layer to fresh tubes. Eluents were evaporated to dryness at ambient temperature, and dissolved in methanol and water. Calibration and quality control samples were used in the range of 10–5,000 pg/ mL for urine, 200–50,000pg/mL for faeces and 50–20,000pg/ mL for plasma, and the methods were shown to be linear with cor- relation coefficients >0.98 when weighting factor of 1/x was used. Faecal samples were diluted as required into the range of the calibration line and the final concentration were normalised to the dry weight.

Sample analysis was performed by ultra-performance liquid chromatography (Acquity I-Class, Waters, UK) and triple quadrupole mass spectrometry (Xevo TQ-S, Waters, UK) in positive electrospray mode at a capillary voltage of 0.9 K, a source temperature of 150°C and a desolvation gas temperature at 500°C. Selective reaction monitoring (SRM) was performed for the N-butylscopolammonium ion using the precursor ion of m/z 360.3 and the product ions of m/z 138.1 (for quantification), m/z 103.1, m/z 121.4 and m/z 194.2 (for qualification) at a cone voltage of 30 V and collision energies of 22, 46, 26 and 20 eV, respectively. The SRM transition of 332.3 > 166.3 (collision energy 22 eV, cone 3 V) was used for the ipratropium ion.

Chromatographic separation was achieved on a reversed phase column (Acquity HSS T3, 100 mm x 2.1 mm, 1.8 µm) using 0.1 % formic acid in methanol (A) and 0.1 % formic acid in water (B) as mobile phases. Gradient was operated at 50ºC and at a flow rate of 0.4 mL/min. The gradient started at 10 % A for 0.5 minutes followed by an increase to 99.9 % A at 3 minutes. This was held for 1 minute before resuming the initial

conditions and re-equilibrating.

Pharmacokinetic parameters were estimated using non-compartmental analysis with Phoenix WinNonlin 7.0 (Pharsight Corporation, Cary, NC). The area under the plasma concentration versus time curve to infinity (AUC0-∞) was calculated using the log-linear trapezoidal method with the AUC from the last time point to infinity estimated from the final plasma concentration divided by the elimination rate constant for the terminal phase.

RESULTS

Plasma pharmacokinetic parameters after oral administration of N-butylscopolammonium bromide to six greyhounds are summarised in Table 1; mean plasma and urine concentrations of N-butylscopolammonium are shown in Figure 1, and concentrations of N-butylscopolammonium in faeces are reported in Table 2.

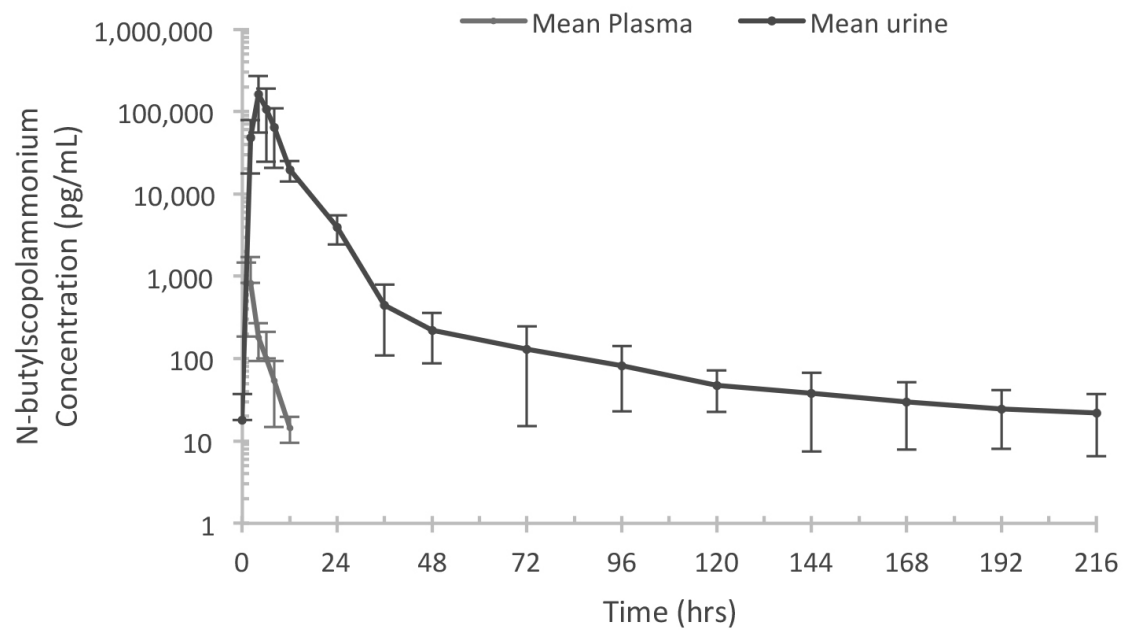
Table 1 : Plasma pharmacokinetic parameters for N-butylscopolammonium following a single oral 10 mg dose as the bromide salt to 6 greyhounds by non-compartmental PK analysis. Cl/F represents the oral clearance of N-butylscopolammonium and HL the half-life. * = Geometric mean

Animal	Weight (Kg)	$AUC_{0-\infty}$ (pg.h/mL)	C1/F (mL/min/kg)	HL (hrs)
Dog 1	34.0	2136	2029	2.5
Dog 2	32.3	4650	1039	1.9
Dog 3	38.8	716	8608	1.6
Dog 4	33.5	1110	4506	3.2
Dog 5	31.5	6815	783	2.0
Dog 6	27.2	2013	2566	1.7
Mean	32.9	2907	3255	2.0*
Median	32.9	2075	2566	1.9

Table 2 : Concentrations of N-butylscopolammonium in faeces, in ng/g dry matter, after a single oral 10 mg dose as the bromide salt to 6 greyhounds. SAT = Saturation of detector despite 1/1000 dilution, ND = Not Detected, 0.02 ng/mL is the Lower Limit of Quantification. Dogs are numbered in order of the date of drug administration.

Hours after administration	Dog 1	Dog 2	Dog 3	Dog 4	Dog 5=	Dog 5=
0	ND	1.04	0.59	ND	2.04	ND
12	9.15	SAT	47.95	11255	SAT	SAT
48	14.93	177.49	112.15	45.59	6.2	542.62
120	0.32	2.01	<0.02	0.22	0.38	1.84
196	0.62	0.2	ND	1.28	<0.02	0.6

Figure 1: Mean (± standard deviation) plasma and urine concentrations in pg/mL of N-butylscopolammonium following a single oral 10 mg dose of the bromide salt to 6 greyhounds.



DISCUSSION

Greyhound trainers commonly administer over the counter N-butylscopolammonium bromide tablets to racing greyhounds when they 'tie up' around racing. The rationale for this use is questionable given the poor oral absorption of quaternary ammonium compounds (Leusch *et al.* 2001; Tytgat, 2007) and reports of its limited efficacy in reducing urethral obstruction or pressure in dogs (Murakami *et al.* 200; Papadopoulos *et al.* 2014).

No reports of detection time studies for N-butylscopolammonium in dogs have been found, and very limited pharmacokinetic data has been published, (Tytgat, 2007), in the veterinary product information, (Veterinary Medicines Directorate, 2011), or from other residue studies, (Committee For Veterinary Medicinal Products 1997).

It is important to note that whilst calibration and quality control samples were used in the ranges described the method was not formally validated (Racing Medication and Testing Consortium 2018). The cost of such formal validation was considered both unnecessary and prohibitive in the context of the study's objectives and the regulator's priorities and the methods used were already in use for formal confirmations. The regulators, as in most animal sports jurisdictions, also use Screening Limits rather than quantification in their medication control of therapeutic substances.

The AUC0-∞ values reflect low absorption and high oral clearance for the N-butylscopolammonium ion. The oral clearance is approximately 75 times greater than hepatic blood flow in the dog (approximately 40 mL/min/kg) suggesting a bioavailability of less than or equal to 1%. This study confirms the very low oral bioavailability observed for N-butylscopolammonium in previous animal studies and the absence of a systemic anti-muscarinic effect. This, coupled with limited efficacy, would indicate its use for functional urethral obstruction such as that defined as detrusor-urethral dyssynergia, is not justified. This, in itself, is a welfare issue that requires further communication to trainers.

N-Butylscopolammonium urine concentrations peaked at 4 hours before initially declining in a similar manner to the plasma concentrations (Table 1). After 2 days, the urine concentrations entered a phase characterized by a much longer half-life of approximately 50 hours (Figure 1). It is not clear what is driving this long terminal phase in the urine but may be due to either some recirculation via the enterohepatic system or a small amount of the drug residing in a specific tissue. Similar properties of other quaternary ammonium compounds suggest binding in tissues does occur (Leusch *et al.* 2001). This extended terminal phase may have regulatory implications.

Medication control rules for animals in competitions rely either on an effect on body systems, a direct or indirect effect on performance, or relate the substance to its presence on a defined list. Medication control can be managed by the use of screening limits, based on an understanding of the concentrations of a drug that are no longer therapeutically effective (Morris 2015). This study also indicates that orally administered N-butylscopolammonium bromide could be a rational therapy for relieving spasm of the smooth muscles of the digestive tract due to local concentrations exceeding approximately 1000 ng/mL, a concentration that is similar to the M3 receptor potency determined for guinea pig (Tytgat, 2007) and indicates an antispasmodic effect on the gut. As such medication control is required for orally administered N-butylscopolammonium in racing greyhounds, based on measures of blood or urinary concentrations. The total gastrointestinal tract transit time in dogs ranges from 22 to 57 hours (Boillat *et al.* 2010). Faecal concentrations of N-butylscopolammonium fell to ng/mL concentrations between 48 and 120 hours after oral administration (Table 2) but gut receptors require concentrations thousands of times higher to be affected (Tytgat, 2007).

These findings suggest that a urinary screening limit in the range of 100-10 pg/mL (Figure 1) could be used to control the use of orally administered N-butylscopolammonium bromide in racing greyhounds. Given the rapid elimination of N-butylscopolammonium ion from plasma (Figure 1), and in the context that in the targeted method used here the lower limit of quantification was 50 pg/mL, any detection of the drug in plasma is likely to indicate exposure within the last 24 hours.

As compared to oral exposure, the parenteral administration of N-butylscopolammonium bromide, given the much greater bioavailability by this route, would be expected to lead to very much higher urinary concentration in both the initial and the terminal phases of urinary excretion. The concentration found in urine, given the nearly 100-fold difference in bioavailability, is therefore useful to understand if N-butylscopolammonium bromide has been given by the oral or parenteral route. Given the terminal urinary half-life of approximately 50 hours, higher concentrations are likely to be detected by routine screening methods for at least 7 days after dose administration.

CONCLUSION

It is concluded that the use of orally administered N-butylscopolammonium bromide for functional urethral obstruction in greyhounds is not justified and its use may be a welfare issue. Medication control of its anti-spasmodic effect on the digestive tract will be possible by setting a urinary screening limit based on the urinary concentrations as determined in this study.

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CONFLICT OF INTEREST DECLARATION

The Greyhound Board of Great Britain commissioned and funded this study, the administration study was performed at Eurofins SCEC, the chemical analysis was performed at LGC and the pharmacokinetic analysis was performed at the University of Nottingham. T Morris is Independent Scientific Adviser to the Greyhound Board of Great Britain and receives fees for this activity and holds an unpaid appointment the University of Nottingham, S Gower is Veterinary Director at the Greyhound Board of Great Britain and receives payment for this activity, M Viljanto and S Hudson are employees of LGC, S Paine is an employee of the University of Nottingham and has received fees for advice from the Greyhound Board of Great Britain and M Pittorino and S. Colgan are employees of Eurofins SCEC.

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