ABSTRACT

The oral tablet formulation of butylscopolamine, which is available without prescription, is commonly used by trainers of racing greyhounds to treat functional urethral obstruction. As medication control of butylscopolamine is therefore required for such use to ensure the integrity of greyhound racing an administration study was performed in six greyhounds to determine the pharmacokinetics of orally administered butylscopolamine. A single dose of one 10 mg butylscopolamine tablet was administered orally to simulate this use in greyhound racing. Blood, urine and faeces were collected at regular intervals from the greyhounds for up to 9 days and butylscopolamine concentrations determined. There was some, but very limited, absorption of butylscopolamine, with rapid elimination from plasma with a mean half-life of 2 hours. Urine concentrations initially declined in a similar manner to the plasma pharmacokinetics but then entered a much longer half-life of approximately 50 hours. Faecal concentrations declined to very low levels between 48 and 120 hours. The use of orally administered butylscopolamine for functional urethral obstruction in greyhounds is unjustified due this very limited drug absorption. Medication control of butylscopolamine’s anti-spasmodic effect on the digestive tract is possible by setting screening limits based on the urinary and faecal drug levels as determined in this study.

Keywords: butylscopolamine, greyhound, pharmacokinetics, plasma, urine

INTRODUCTION

Butylscopolamine is an antimuscarinic quaternary ammonium derivative of scopolamine used for relieving spasm of the smooth muscle of the digestive (Tytgat, 2007) and urinary systems (Papadopoulos et al 2014). It is available for medical and veterinary use in injectable formulations, both alone but also in combination with the non steroidal anti-inflammatory drug metamizole, and in tablet form, and is commonly referred to under its original trade name Buscopan®. Greyhound trainers commonly report that some racing greyhounds ‘tie up’ around racing, this being associated with excitement and characterised by full or partial inability to urinate. This condition has not been well defined (Van Meeuwen, 2009) but is likely to be a functional obstruction such as that defined as detrusor-urethral dyssynergia, with this considered to result from over discharge of acetylcholine to the urethral sphincter leading to contraction of the detrusor muscles (Espineira & Nickel, 1997, Tytgat, 2007).
Butylscopolamine is available as a tablet for oral administration for human medical use without prescription in the United Kingdom, Ireland and Australia, and related to this availability is commonly used by trainers symptomatically to treat full or partial inability to urinate in racing greyhounds.

The aim of this study was characterise the analytical detection, the plasma and the urinary pharmacokinetics of butylscopolamine given orally to greyhound at the dose and formulation commonly used by trainers of racing greyhounds, assess systemic absorption of the drug, the clinical and animal welfare implications, and derive regulatory advice for greyhound racing.

MATERIALS AND METHODS

One 10mg tablet of butylscopolamine bromide (Buscopan® Boehringer Ingelheim) was administered orally on one occasion to six different greyhounds. Three female and three male animals were studied, with a mean dose of 0.31 mg/kg, a mean bodyweight of 32.9 kg and mean age of 4 years. Blood, urine and faecal samples were collected before drug administration. Blood samples were collected after 1, 2, 4, 6, 8, 12, 24, 36, 48, 96, 144 and 192 hours. Urine samples were collected after 2, 4, 6, 8, 12, 24, 36, 48, 72, 96, 120, 144, 168, 192 and 216 hours. Faecal samples were collected at 12, 24, 48, 120, and 192 hours. The blood samples were heparinised and plasma obtained by centrifugation. All samples were stored at -20°C. 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 had access to water at all times. The morning feed was not given on the treatment day prior to drug administration. The study was conducted in accordance to 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. The frozen samples were transported on dry ice to the analytical laboratory.

Concentrations of butylscopolamine were measured in the pre- and post-administration plasma, urine and faecal samples. For extraction, ipratropium was selected as an internal marker due to its similar structure to butylscopolamine. For the urine (1 mL) and faecal samples (300 mg), ammonium bicarbonate (10 mM, pH10) was added and samples were centrifuged prior to solid phase extraction using cartridges (Isolute CBA 500 mg, Biotage EU). These were conditioned, the sample loaded, washed with ammonium bicarbonate and methanol and then eluted with 0.1% formic acid in methanol. For the plasma samples (200 µL), after addition of dichloromethane they were mixed and then centrifuged prior to transferring the organic layer to fresh tubes. Eluents were evaporated to dryness at ambient temperature, and reconstituted with methanol and water. Calibration and quality control samples were used in the range of 10-5,000 pg/mL for urine, 200-50,000 pg/ml for faeces, and 50-20,000 pg/mL for plasma, and the methods were shown to be linear with correlation coefficients greater than 0.98 when weighting factor of 1/x was used. Faecal samples were diluted as required to allow quantification and the final concentration adjusted to express the amount in dry matter.

The urine and plasma methods produced a lower limit of quantification (LLOQ) of 10 and 50 pg/mL, respectively, and they were shown to be accurate and reproducible with acceptable inter-batch variability of precision and accuracy. The precision of the urine method was demonstrated to be within ±19.4% at LLOQ and within ±12.3% at higher QC concentrations (30, 2,500 and 4,500 pg/mL), whilst the precision of the plasma method was within ±8.2% at all QC concentrations (50, 100, 10,000 and 16,000 pg/mL). The accuracy of the urine method was within ±9.8% at all QC concentrations, whilst the accuracy of the plasma method was within ±13.0% at all QC.
concentrations. Urine QC samples diluted 1-in-100 for urine samples also produced acceptable results with precision within ±5.6% and accuracy within ±8.2%.

The LLOQ of faecal method was 0.2 ng/g. The precision of the method in a single batch was within ±6.9% at QC concentrations of 0.2, 20 and 40 ng/g, whilst the accuracy was within ±11.9% at these QC concentrations.

Sample analysis was performed by Aqcuity I-Class Ultra performance liquid chromatography (Waters UK) and Xevo TQ-S triple quadrupole mass spectrometry, 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 butylscopolamine using the precursor ion of m/z 360.3 and the production ions of m/z 138.1 (for quantification), m/z 103.1, m/z 121.4 and m/z 194.2 (for qualification) at cone voltage of 30 V and collision energy 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 ipratropium.

Chromatographic separation was achieved on an Acquity HSS T3 (100 mm x 2.1 mm, 1.8 µm) column using 0.1 % formic acid in methanol (A) and 0.1 % formic acid in water (B) as mobile phases. Gradient was operated at 50 °C and at a flow rate of 0.4 mL/min. It was started at 10 % organic for 0.5 minutes followed by 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 curve to infinity (AUC_{0-inf}) was calculated using the log-linear trapezoidal rule with the AUC for the last time point to infinity determined from the final plasma concentration divided by the elimination rate constant for the terminal phase.

RESULTS

Following oral administration to six greyhounds of butylscopolamine bromide plasma pharmacokinetic parameters for are summarised in Table 1, mean plasma and urine concentrations of butylscopolamine are illustrated in Figure 1, and concentrations of butylscopolamine in faeces given in Table 2.

DISCUSSION

Greyhound trainers commonly use butylscopolamine tablets obtained without prescription when racing greyhounds ‘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 reported detection time studies for butylscopolamine in dogs have been found, and there is very limited pharmacokinetic data published, (Tytgat, 2007), in the veterinary product information, (Veterinary Medicines Directorate, 2011), or from other residue studies, (Committee For Veterinary Medicinal Products 1997).

Butylscopolamine entering the duodenum, 10 mg in 500 mL of fluid, will lead to an approximate concentration of 20 micrograms per mL. If just 5% of butylscopolamine is absorbed then the initial concentration that the plexus will observe in the gut is approximately 1000 ng/mL. This value is
similar to the M₃ receptor potency determined for guinea pig (Tytgat, 2007) and indicates an antispasmodic effect on the gut. However, 1000 ng/mL is massively higher than the peak plasma concentrations observed from this greyhound oral administration study which are in the low ng/mL range (Figure 1). The AUC₀₋∞ values are very low and lead to a very high oral clearance for butylscopolamine. 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% (Gibaldi and Perrier, 1982). This study confirms the very low oral bioavailability observed for butylscopolamine in previous animal studies and no systemic anti-muscarinic effect. This, coupled with limited efficacy, would indicate its use for functional urethral obstruction such as that defined as detrusor-urethral dysynergia, is unjustified.

Butylscopolamine urine concentrations peak at 4 hours before initially declining in a similar manner to the plasma pharmacokinetics (Figure 1). The ratio of urine to plasma butylscopolamine concentrations between 4 hours (peak urine concentration) and 12 hours is very large with a value of approximately 1000. A very large ratio of this magnitude indicates that butylscopolamine undergoes rapid renal clearance that approaches renal blood flow in the dog. This seems likely as butylscopolamine is mainly excreted in the urine after parenteral administration (Committee For Veterinary Medicinal Products, 1997). Moreover, tiotropium (another quaternary ammonium drug) undergoes renal clearance that exceeds creatinine clearance indicating active secretion into the urine (Durham, 2004). After 2 days, the urine concentrations enter 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 leading to a large terminal volume of distribution. Similar properties of other quaternary ammonium compounds suggests binding in tissues does occur (Leusch et al 2001).

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 levels of a drug that are no longer therapeutically effective (Morris 2015). This study also indicates that orally administered butylscopolamine could be a rational therapy for relieving spasm of the smooth muscle of the digestive tract. As such medication control is required for orally administered butylscopolamine 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 butylscopolamine fall to ng/mL levels between 48 and 120 hours after oral administration (Table 2), and gut receptor potency (Tytgat, 2007) is a thousand times higher. This would indicate a urine screening level in the range of 100-10 pg/mL (Figure 1) should be the starting point for regulatory considerations to control the use of orally administered butylscopolamine in racing greyhounds. Such screening limits are set independently by regulatory authorities, guided by scientific information and analytical considerations (Morris 2015).

It is concluded that the use of orally administered butylscopolamine for functional urethral obstruction in greyhounds is unjustified, and that medication control of its anti-spasmodic effect on the digestive tract will be possible by setting a urinary screening limit based on the urinary and faecal drug levels as determined in this study.

ACKNOWLEDGEMENTS
Steven Karamatic of Greyhound Racing Victoria provided advice on hyoscine butylscopolamine use in greyhound racing Australia. Boehringer Ingelheim Ltd confirmed Buscopan® tablet formulation equivalence between Europe and Australia.

CONFLICT OF INTEREST STATEMENT

The Greyhound Board of Great Britain 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. Tim 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, Simon Gower is Veterinary Director at the Greyhound Board of Great Britain and receives payment this activity, Marjaana Viljanto and Simon Hudson are employees of LGC, Stuart Paine is an employee of the University of Nottingham and has received fees for advice from the Greyhound Board of Great Britain and Melissa Pritorino and Sally Colgan are employees of Eurofins SCEC.

AUTHOR CONTRIBUTION STATEMENT

Tim Morris designed, coordinated, reported and interpreted this study, Stuart Paine performed pharmacokinetic analysis and interpretation, Marjaana Viljanto developed methodology and performed chemical analysis with Simon Hudson as the principal investigator, Simon Gower provided clinical and regulatory interpretation, Melissa Pritorino developed methodology and performed the in-vivo study with Sally Colgan as principal investigator. All authors contributed to and reviewed the manuscript and are accountable for its contents.

REFERENCES


Figure 1. Mean, with standard deviation, plasma and urine concentrations in pg/mL of butylscopolamine following a single oral 10mg dose to 6 greyhounds.
Table 1: Plasma pharmacokinetic parameters for butylscopolamine following a single oral 10 mg dose to 6 greyhounds using a non-compartmental PK analysis. Cl/F represents the oral clearance of butylscopolamine and HL the half-life. * = Geometric mean

<table>
<thead>
<tr>
<th>Animal</th>
<th>Weight (Kg)</th>
<th>$\text{AUC}_{0-\infty}$ (pg.h/mL)</th>
<th>Cl/F (mL/min/kg)</th>
<th>HL (hrs)</th>
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<tbody>
<tr>
<td>Dog 1</td>
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</tr>
<tr>
<td>Dog 2</td>
<td>32.3</td>
<td>4650</td>
<td>1039</td>
<td>1.9</td>
</tr>
<tr>
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<td>38.8</td>
<td>716</td>
<td>8608</td>
<td>1.6</td>
</tr>
<tr>
<td>Dog 4</td>
<td>33.5</td>
<td>1110</td>
<td>4506</td>
<td>3.2</td>
</tr>
<tr>
<td>Dog 5</td>
<td>31.5</td>
<td>6815</td>
<td>783</td>
<td>2.0</td>
</tr>
<tr>
<td>Dog 6</td>
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<td>2013</td>
<td>2566</td>
<td>1.7</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td><strong>32.9</strong></td>
<td><strong>2907</strong></td>
<td><strong>3255</strong></td>
<td><strong>2.0</strong>*</td>
</tr>
<tr>
<td><strong>Median</strong></td>
<td><strong>32.9</strong></td>
<td><strong>2075</strong></td>
<td><strong>2566</strong></td>
<td><strong>1.9</strong></td>
</tr>
</tbody>
</table>

Table 2. Concentrations of butylscopolamine in faeces, in ng/g dry matter, after following a single oral 10 mg dose to 6 greyhounds. SAT = Saturation of detection despite 1/1000 dilution, ND = Not Detected, 0.02 ng/mL is the Lower Limit of Quantification. Dogs are numbered in order of the time of drug administration.

<table>
<thead>
<tr>
<th>Hours after administration</th>
<th>Dog 1</th>
<th>Dog 2</th>
<th>Dog 3</th>
<th>Dog 4</th>
<th>Dog 5=</th>
<th>Dog 5=</th>
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<td>0</td>
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<td>0.59</td>
<td>ND</td>
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<td>ND</td>
</tr>
<tr>
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<td>9.15</td>
<td>SAT</td>
<td>47.95</td>
<td>11255</td>
<td>SAT</td>
<td>SAT</td>
</tr>
<tr>
<td>48</td>
<td>14.93</td>
<td>177.49</td>
<td>112.15</td>
<td>45.59</td>
<td>6.2</td>
<td>542.62</td>
</tr>
<tr>
<td>120</td>
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<td>0.22</td>
<td>0.38</td>
<td>1.84</td>
</tr>
<tr>
<td>196</td>
<td>0.62</td>
<td>0.2</td>
<td>ND</td>
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<td>&lt;0.02</td>
<td>0.6</td>
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