The pharmacokinetics, metabolism and urinary detection time of etamiphylline in camels after intramuscular administration

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The pharmacokinetics of etamiphylline were determined after an intramuscular (i.m.) dose of 3.5 mg/kg body weight in six healthy camels. Furthermore, the metabolites and drug detection time were evaluated. The data obtained median and (range) were as follows: the terminal elimination half-life ($t_{1/2\beta}$, h) was 3.04 (2.03–3.62); apparent total body clearance (Cl/*F*, L/h/kg) was 1.27 (0.74–2.99); the apparent volume of distribution at steady state (V_{ss}/F, L/kg) was 4.94 (3.57–12.54); and renal clearance (Cl_r, L/h/kg) determined in two camels was 0.005 and 0.004, respectively. The detection time of etamiphylline in urine after an i.m. dose of 3.5 mg/kg body weight ranged between 12 and 13 days. Three etamiphylline metabolites were tentatively identified in camels urine: The first one desethyletamiphylline was the main metabolite and resulted from N-deethylation of etamiphylline had a molecular weight of 251, and was detected in urine for about 13–14 days. Theophylline (molecular weight 180) was the second metabolite and resulted from ring N-dealkylation of etamiphylline. It was present in small amounts and was detected for about 5 h after drug administration in urine. The third metabolite, possibly resulted from demethylation of etamiphylline, had a molecular weight of *m/z* 265, and was present in small amounts and was detected in urine for about 5 h after drug administration.

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INTRODUCTION

Etamiphylline 7-(2-Diethylaminoethyl)theophylline (Et) is one of the N-7 theophylline derivatives of the xanthine bronchodilator group. It is used in human medicine as a respiratory stimulant (Addis, 1984; Vazquez *et al.*, 1984; Gleeson *et al.*, 1985). The Et is also used as a respiratory stimulant in the treatment of nitrite toxicity in cattle (Fennell, 1979).

The Et like all the other N-7 theophylline derivatives compounds is not metabolized to theophylline in humans (Zuidema & Merkus, 1978, 1979; Addis, 1984; Vazquez *et al.*, 1984). These derivatives were introduced in an attempt to avoid the gastric irritation from theophylline, and they seem to achieve this (Addis, 1984), however, their bronchodiator effect is at best very weak compared with theophylline (Zuidema & Merkus, 1978; Fleetham *et al.*, 1979; Furakawa *et al.*, 1983). Vazquez & Labayru (1984) found the same clinical results reported earlier by several authors which indicated that theophylline remains the only xanthine useful for the treatment of asthma (Simmons *et al.*, 1975; weinberger & Hendels, 1980; Hendeles *et al.*, 1984).

The dispostion of etamiphylline in camels has not been reported. During the last years we have detected two cases of etamiphylline in postrace urine samples.

The objectives of the study reported here were therefore to characterize the pharmacokinetic parameters of etamiphylline in the camel, and to determine the detection time of etamiphylline and its main metabolite desethyletamiphylline in urine after intramuscular injection (i.m.). Another objective was to estimate its metabolites in camels urine.

MATERIALS AND METHODS

Experimental animals

The study employed six healthy camels (two males and four females) 4–8 years old and ranging in body weight from 400 to 500 kg. The camels were kept in an open pen. None had received any drug for at least 6 months. Good quality lucerne hay was fed once daily, and water was allowed ad libitum.

Experimental design

Lack of pharmacokinetic data for etamiphylline in camels prompted us to use the manufacturer's clinically recommended dose of etamiphylline in horses (3.5 mg/kg body weight).

Etamiphylline camsylate – Et (Millophylline – Dales pharmaceutical Ltd, Skipton N. York, BD33 2RW, UK 140 mg/mL) was administered i.m. in shoulder muscles, as a single dose at 3.5 mg/kg body weight. Blood samples (10 mL) were collected from the jugular vein at 0 (predose) and at 10, 20, 30, 45, and 60 min and at 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 8.0, 14.0 h after drug administration. The plasma was separated by centrifugation and kept at −20°C pending analysis. To determine renal clearance (Cl_r) voided urine samples were collected from two camels up to 8 h as described by (Wasfi *et al.*, 1997, 1999). Voided urine was centrifuged for 10 min at $2000 \times \boldsymbol{g}$. The pH of the urine was measured by a pH-meter (Backman, Palo Alto, CA, USA). Plasma and urine samples were assayed within 10 days.

Assay procedure

The concentration of Et in plasma and urine was measured by gas chromatography/mass spectrometry (GC/MS) as follows: to 0.250 mL sample was added 0.200 mL of chlorpromazine (2.5 μg/mL) as internal standard. Then, 0.050 mL of 2N sodium hydroxide and 2.5 mL of ethyl acetate were added. The mixture was then vortexed for 2 min After centrifugation for 5 min at $15800 \times \boldsymbol{g}$, 2.0 mL of the organic layer was evaporated under nitrogen at 40°C. The residue was dissolved in 0.100 mL of ethyl acetate and 2 μL of the organic solvent was analysed on the GC/MS system. Calibration curves were made from supplemented camels plasma and urine.

GC/MS analysis

Gas chromatography/mass spectrometry analysis was carried out using a Hewlett Packard 5972 mass selective detector (Hewlett Packard, Palo Alto, CA, USA) interfaced to a HP 5890 plus gas chromatograph (Hewlett Packard, Palo Alto, CA, USA) with HP 7673 auto injector and sample tray (Hewlett Packard, Palo Alto, CA, USA). Injections were made in the splitless mode onto a 15-m × 0.25 mm id HP 5MS column. The initial column temperature was 100°C and was programmed to 290°C at 25°C/min. Injection port and interface temperature were 250 and 290°C, respectively. Helium was used as carrier gas. Data were aquired in the selective ion mode (SIM). Monitored ions were at *m/z* 279 and 86 for Et, and ions *m/z* 232, 272, and 318 for chlorpromazine. Results: The linearity of the method was from 0.078 to 10.0 μg/mL for Et supplemented plasma. The percentage recoveries (mean ± SEM) for concentration of 0.625 and 2.5 μg/mL ($n = 10$) were (98.35 ± 0.012) and (99.88 ± 0.119). The intra-assay coefficients of variation for 0.625 and 2.5 μg/mL ($n = 10$) were 6.25 and 2.66%, respectively. The interassay coefficients of variation for 0.625 and 2.5 μg/mL ($n = 10$) were 10.03 and 6.67%, respectively. The limit of detection (LOD) and limit of quantitation (LOQ) were determined by the procedure described by (Armbruster *et al.*, 1994). The LOD, defined as the concentration at which routine GC/MS acceptance critical retention time within 2% of calibrator, ion ratios within 20% of calibrator are met at least 90% of the time was 12.5 ng/mL. The LOQ, defined as the concentration at which all acceptance criteria are met and the quantitative value is within ±20% of the target concentration was 27.5 ng/mL.

Pharmacokinetic analysis

Noncompartmental analysis of the data was performed. Using the statistical moment theory (Gibaldi & Perrier, 1982). The zero moment area [area under the curve (AUC)] and the first moment area [area under the first moment curve (AUMC)] were determined according to the trapezoidal rule up to the last measured concentration and were extrapolated to infinity (Gibaldi & Perrier, 1982). The terminal elimination rate constant (β) was calculated as −2.303 × the slope of the terminal phase of the logarithm of the plasma concentration vs. time curve (using the last 6–4 points) after fitting the line by use of linear regression.

Total body clearance (Cl) was calculated by use of the equation D/AUC, where D is the dose of Et. Volume of distribution at steady state (V_{ss}) was estimated by use of the following equation:

$$V_{ss} = (D \times \mathrm{AUMC})/(\mathrm{AUC})^2$$

The terminal elimination half-life ($t_{1/2\beta}$) was calculated as 0.693 divided by β. Renal clearance was estimated by the following equation:

$$\mathrm{Cl_r} = T_u/\mathrm{AUC}_{0-8}$$

where T_u is the total amount of Et excreted in the entire sample collection time (8 h), AUC_{0-8} is the area under the curve from time 0–8 h.

Determination of detection time

One of the objectives of this work was to determine the detection time for Et and its main metabolite desethyletamiphylline in racing camels. This was carried out by collecting urine samples from two treated camels, given Et 3.5 mg/kg body weight as a single i.m. injection. Samples were collected daily at 8 AM for 17. Samples were then subjected to our routine procedure for screening basic drugs in postrace urine samples (Wasfi *et al.*, 1998, 2000a).

Protein binding

The Et plasma protein binding was determined by the use of centrifree filter (Amicon Inc., Beverly, MA, USA). Pooled camel plasma was supplemented with Et at a concentration of 5 μg/mL. The supplemented sample 1.0 mL ($n = 10$) was added to the top part of the microcentrifuge device, which was then centrifuged for 20 min at $1000 \times \boldsymbol{g}$. The concentration of Et on each side of the device was determined by GC/MS method

described above. The percentage protein binding was calculated as follows:

$$\text{Percentage Protein Binding} = (PB_a/PF_a + PB_a) \times 100$$

where PB_a and PF_a are the area of Et bound (upper fraction), and free (lower fraction), respectively.

Determination of Et metabolites

This was performed on urine samples collected 5 h after an i.m. administration of Et, at a dose 3.5 mg/kg. Urine collection method was described before. Samples were analysed by our routine procedure for screening basic, acidic and neutral drugs in postrace urine samples (Wasfi *et al.*, 1998, 2000a). To investigate the extent of glucuronic acid conjugation, urine samples were treated similarly, but without prescreening enzyme hydrolysis. Identification of metabolites was done from their retention time and fragmentation pattern in releation to authenticated materials and standard spectra.

RESULTS

The GC/MS method used for Et analysis proved to be quick and efficient. The disposition curve for etamiphylline was constructed (Fig. 1), and the values for the pharmacokinetic variables were tabulated (Table 1). Renal clearance of Et in two camels was about 0.0044 L/h/kg which roughly accounted for 0.29% of total body clearance. Percentage plasma protein bound Et (mean ± SEM) was estimated to be 64.7 ± 1.7% at a concentration of 5 μg/mL. Desethyletamiphylline [7-(2-Ethylaminoethyl)theophylline] was detected and identified as the main metabolite and it was found in high concentration. Both theophylline (molecular weight 180) and desmethyletamiphylline, suspected molecular weight $m/z = 265$ were also identified as Et metabolites in camels urine after i.m. drug administration. The concentrations of theophylline and desmethyletamipylline compared with that of desethyletamiphylline were very low

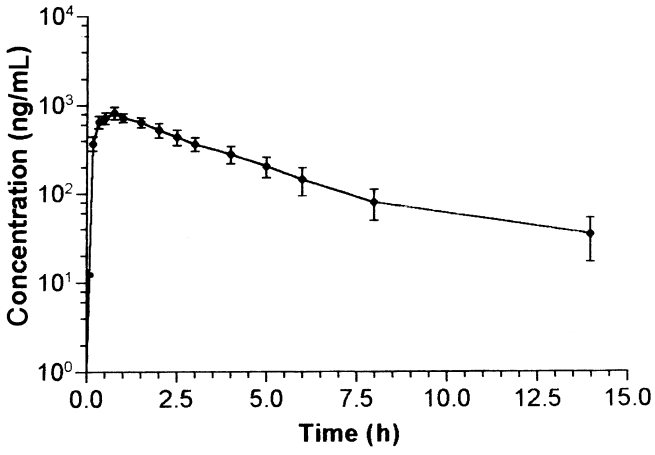

Fig. 1. The Et plasma concentration profile (mean ± SEM) after an i.m. dose of 3.5 mg/kg body weight in six camels.

Table 1. Etamiphylline pharmacokinetic variables following intramascular (i.m.) injection to six healthy camels at a dose of 3.5 mg/kg body weight

Pharmacokinetics term	Median	Range
AUC (μg/h/mL)	2.75	1.17–4.73
AUMC (μg/h/mL)	8.56	4.88–24.49
CL/*F* (L/kg/h)	1.27	0.74–2.99
V_{SS}/F (L/kg)	4.94	3.57–12.54
MRT (h)	4.21	2.72–5.19
$t_{1/2\beta}$ (h)	3.04	2.03–3.62
C_{max} (μg/mL)	0.73	0.26–1.18
T_{max} (h)	1.00	0.75–1.50

AUC = area under the curve from time 0 to infinity; AUMC = area under the first moment curve; CL/*F* = apparent total body clearance; V_{ss}/F = volume of distribution at steady state; MRT = main residence time; $t_{1/2\beta}$ = terminal elimination half-life; C_{max} = maximal plasma concentration observed at time T_{max}.

(Fig. 2), but we were able to detect them in urine for about 5 h. Chromatograms obtained from 5 h enzyme hydrolysed sample, with and without methylation, acid-neutral fraction showed no evidence of Et metabolites. Using our routine method for screening postrace urine samples, we were able to detect Et and its main metabolite desethyletamiphylline in camels urine for a period ranging from 12 to 13 days and from 13 to 14 days, respectively.

DISCUSSION

To the author's knowledge the results reported here are the first describing the disposition of Et in camels. The pharmacokinetics of etamiphylline in camels were characterized by a terminal half-life [3.04 (2.03–3.62) h; median and (range)], fast total body clearance [1.25 (0.74–2.99) L/h/kg] and high volume of distribution [4.94 (3.57–12.54) L/kg]. This extensive distribution could be because of the lipid soluble organic base. The degree of protein binding about 65% in camels was not large enough to reflect on the extensive drug distribution.

The renal clearance of etamiphylline estimated in two camels was 4.66 and 4.18 mL/h/kg which accounted for about 0.29% of its total body clearance, the amount of etamiphylline recovered in 8 h was about 2.7% of the administered dose, suggesting that the elimination of this drug is via metabolism rather than by renal clearance.

Three metabolites were identified for Et in camel urine (Fig. 3). The first was desethyletamiphylline, resulting from N-deethylation of Et, with a moleculer ion at m/z 251 (279–28). The second one was theophylline with a molecular ion at m/z 180 resulting from ring-N-dealkylation (279–99). Theophylline, however, was not detected as a metabolite of etamiphylline in humans (Zuidema & Merkus, 1979; Addis, 1984; Vazquez *et al.*, 1984). We could not detect any of the theophylline (xanthine base) metabolites as a result of further theophylline metabolism. This, however, is in contrast with earlier reports that theophylline was metabolized to 1,3

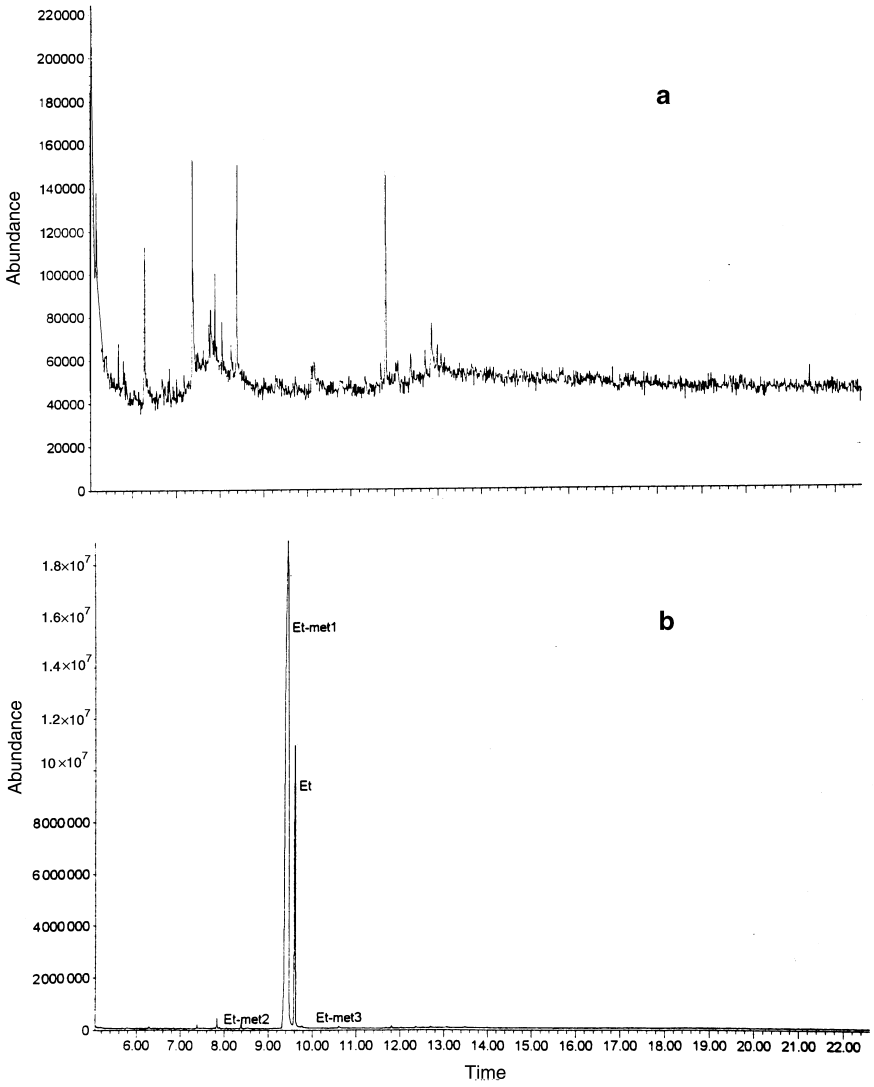

Fig. 2. Total ion chromatogram of urine extract of camel C (a) preadministration (b) 4.5 h after Et administration. Et, parent compound; Et-met1, desethyletamiphylline; Et-met2, theophylline; Et-met3, demetyl etamiphylline.

dimethyl uric acid and 3 methyl xanthine in horses (Harkins *et al.*, 1998) and to caffeine in camels (Wasfi *et al.*, 1999, 2000b). This may be attributable to several reasons: (1) the volume of urine sample used was 5–10 mL; (2) the concentration of theophylline as Et metabolite was very small; (3) the elimination of theophylline via caffeine metabolism was very low 0.01% of the administered theophylline dose (Wasfi *et al.*, 1999). The third metabolite resulted form demethylation of Et and has a suspected molecular ion of *m/z* 265 (279–14).

Our results indicate that N-dealkylation, a phase 1 metabolic pathway, is the major route for etamiphylline elimination in camels. There were no differences in the concentrations of Et and its main metabolite following enzyme hydrolysis in comparison with the unhydrolised samples indicating that conjugation of Et and its main metabolite did not occur. Perviously N-dealkylation of theophylline (xanthine bronchdilator) has been reported and that cytochrome P450 (CYP) IAE catalysed 80–90% of theophylline N-dealkylation *in vitro* (Robson *et al.*, 1988; Rasmussen *et al.*, 1995). In horses and camels, theophylline is persumably metabolized by CYP IAE and CYP 2EI (Harkins *et al.*, 1998; Wasfi *et al.*, 1999). The same enzymes are, persumably, responsible for the major route of Et N-dealkylation in camel.

One of the objectives of this study was to determine the detection time of Et and its main metabolite in camels. We were able to detect Et and desethyletamiphylline in enzyme hydrolysed urine samples for a period ranging from 12 to 14 days after Et i.m. administration. This long detection time might be because of the enzyme hydrolysis we performed on urine samples as a prescreening step to free conjugated drugs and metabolites. Also the alkaline nature of camel urine (Wasfi *et al.*, 1998) where Et and its metabolite were expected to be in the unionized form, and thus, reabsorbed in the renal tubules, and would extend their detection time in camels. This may also be because of the fact that daily urine volume of camels is very small about 1.0 L and the glomerular filtration rate is lower than that of other animals 0.55–0.65 mL/kg/min (Wilson, 1984), allowing time for conjugate hydrolysis and reabsorbtion. The screening procedure used is GC/MS method with a sensitivity of >10 ng/mL for most drugs. All of these factors explain this extended detection time for

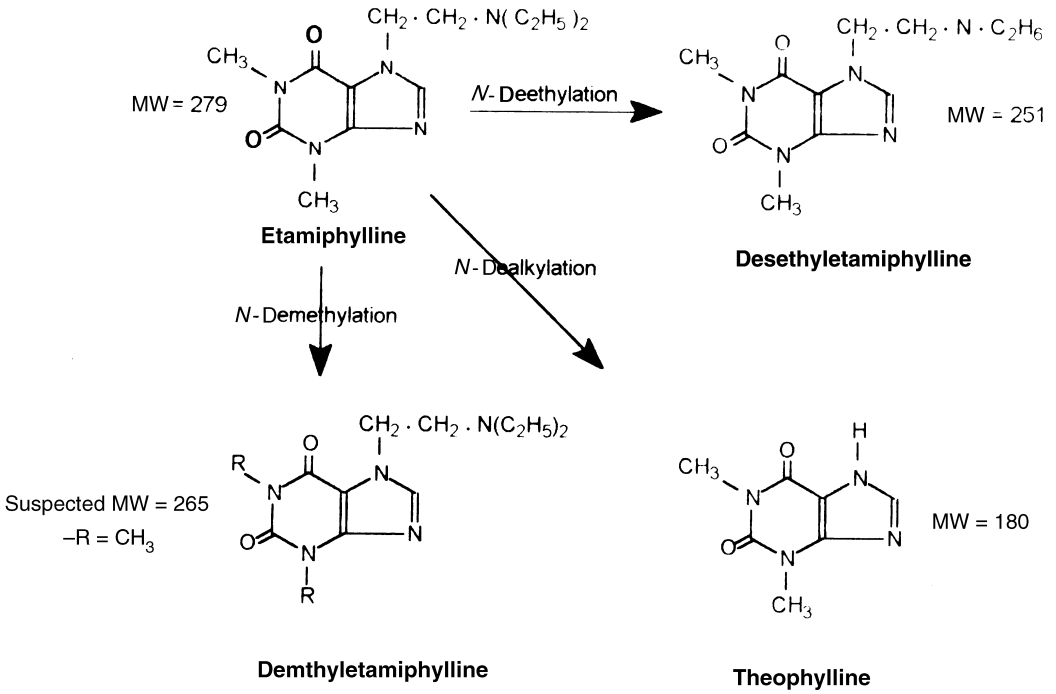

Fig. 3. Etamiphylline metabolic pathway in camels after an i.m. dose of 3.5 mg/kg body weight.

Et and its main metabolite in camel urine. Therefore, taking in considration that the camel racing commission in UAE uses a zero drug concentration rule (that is, it does not permit presence of any drug and/or metabolite at the time of racing), for precautionary measures we recommend withholding Et administration before racing for a minimum period of 15 days.

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