Vol 16 No 2, p 143-148

THE DISPOSITION OF HEPTAMINOL IN CAMELS AFTER INTRAVENOUS ADMINISTRATION

I.A. Wasfi, J. Almasri[1], M. Elghazali, Nawal A. Alkatheeri, A.A. Abdel Hadi, F. Altayeb, C.E. Uboh[3], Ishar M. Barezaig, J.A. Rudy[3], L.R. Soma[2] and K. Singh

Camel Racing Laboratory, Forensic Science Laboratory, P.O. Box 253, Abu Dhabi, United Arab Emirates
[1]Veterinary Research Centre, P.O. Box 72437, Al Ain, United Arab Emirates
[2]University of Pennsylvania, School of Veterinary Medicine, New Bolton Centre Campus, Kennett Square, PA 19348, USA,
[3]PA Equine Toxicology & Research Centre, West Chester State University, Department Chemistry, West Chester, PA 19382, USA

ABSTRACT

The present study investigated the disposition of heptaminol after intravenous administration and the effect of diprophylline on the pharmacokinetics of heptaminol in camels. Seven healthy camels (3 males and 4 females) 4-7 years old weighing 300 – 400 kg were used. The study was an open longitudinal design in which each animal served as its own control. In study phase 1, each camel received an intravenous dose of heptaminol (10 mg/kg). Blood samples were collected at 0 (predose) and 5, 10, 15, 30, 45, 60 min and at 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8 and 12 h after drug administration. Phase 2 was conducted one week later in which i.v. doses of heptaminol and diprophylline (10 mg/kg each) were administered and blood samples were collected as in phase 1. Serum concentrations of heptaminol were determined in duplicate by a validated HPLC method. Pharmacokinetic analysis for serum heptaminol for each camel was performed using least squares nonlinear regression analysis. The disposition of heptaminol was best described by a bi-exponential equation. The results obtained (mean ± SD) for heptaminol and heptaminol with diprophylline were, respectively, as follows: Total body clearances (Cl_T) were 645.5 ± 109.9 and 511.1 ± 114.1 ml/kg.h, the elimination half-lives ($t_{1/2b}$) were 2.00 ± 0.21 and 2.09 ± 0.34 h and the steady state volumes of distribution (V_{SS}) were 1432.2 ± 129.5 and 1279.1 ± 310.0 ml/kg, respectively. Diprophylline administration had no significant effect on the pharmacokinetics of heptaminol in camels. Heptaminol and metabolite could be detected in urine samples for 24-48 h after an i.v. dose of 10 mg/kg body weight.

Key words: Camels, clearance, heptaminol, metabolism, pharmacokinetics, racing

Heptaminol (6-amino-2-methyl-2-heptanol) is an amine derivative that is used as a cardiotonic and vasodilator in veterinary medicine. Heptaminol has sympathomimetic effects by inhibiting re-uptake of noradrenaline at adrenergic synapses (Delicado *et al,* 1990). Berthiau *et al* (1989) studied the cardiotonic effect of heptaminol in isolated hearts under different conditions: normoxia, moderate ischaemia and severe ischaemia. In normoxia, heptaminol induced a cyclic increase of systolic left ventricular pressure, associated with an increase in inorganic phosphates but no effect on intracellular pH was observed. When changing from normoxia to moderate ischaemia, the authors observed a decrease of phosphocreatine and systolic left ventricular pressure and a mild intracellular acidification (pH 6.96). Heptaminol induced a restoration of intracellular pH and increased left ventricular pressure. However, in severe ischaemia, the realkalinisation effect and the restoration of left ventricular pressure induced by heptaminol were no longer observed. Because of this cardiotonic and fatigue relieving properties, it could be used in race animals to alter performance.

We have recently detected several heptaminol positive cases in racing camels in the United Arab Emirates (UAE). Veterinary preparations of heptaminol, with diprophylline, are available in UAE. To our knowledge, there are no published pharmacokinetic (PK) studies of heptaminol in camels. The objectives of the present study were, therefore, to characterise the pharmacokinetic parameters of heptaminol in camels, to identify its major metabolites and to determine its detection time in urine after intravenous administration to camels. Furthermore the PK of heptaminol in the camel was evaluated when co-administered with diprophylline to see if the latter would alter the PK of heptaminol.

SEND REPRINT REQUEST TO IBRAHIM A. WASFI email: iawasfi@yahoo.com

Materials and Methods

Experimental Animals

Seven healthy Arabian camels (3 males, 4 females), 4-7 years old weighing 300 to 400 kg were used. The camels were kept in open pens. None of the experimental animals had been administered drugs for at least 6 months. Good quality hay and lucerne (alfa-alfa) were fed once daily and water was allowed *ad libitum*.

Experimental design

The study was a 2 phase open longitudinal design in which each animal served as its own control. Heptaminol (Sigma, Missouri, USA; dissolved in normal saline at a concentration of 100 mg/ ml) was administered to camels as a bolus i.v. injection into the jugular vein at a dose of 10 mg/kg body weight.

Blood samples (10 ml) were collected from the opposite jugular vein at 0 min (predose) and at 5, 10, 15, 30, 45 and 60 min and at 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 6.0 and 8.0, 12, and 24 h after administration of the drug. The blood samples were allowed to clot, and the serum was separated by centrifugation (2000 x g for 10 min) and stored at -20°C. The serum samples were assayed within 10 days of collection.

The second phase of the experiment was conducted one week later in which the same camels were used. The heptaminol-diprophylline injectable (Frecardyl, containing 50 mg/ml heptaminol, 50 mg/ml diprophylline, and 25 mg/ml diethylphenyl citrate; Buenos Aires, Argentina) was administered to the camels as a bolus i.v. injection into the jugular vein at a dose of 10 mg/kg body weight recommended by the manufacturer.

Blood samples were collected and processed as in phase 1.

Assay procedures

The concentration of heptaminol in the serum was measured as reported by Cociglio *et al* (1984) using n-propylamine as internal standard. Following extraction, both amines were derivatised in acetonitrile with o-phthaldialdehyde and 2-mercaptoethanol. The adducts were quantitated by reversed-phase high-performance liquid chromatography using calibration curves constructed from the analysis of camel serum supplemented with heptaminol standard (Sigma, Missouri, USA). Under our experimental conditions, the linearity of the method was from 0.025 to 10 µg/ ml of heptaminol in supplemented camel serum (r^2 >0.995). The intra assay coefficients of variation at 0.3125 and 2.5 mg/ ml (n = 20) were 1.89% and 2.27%, respectively. The inter assay coefficients of variation at 0.25 µg/ml (n= 6), 1.0 µg/ml (n = 6) and 5.0 µg/ml (n = 6) were 1.45%, 5.66% and 2.33%, respectively. The recovery (mean + SEM) of heptaminol of 0.3125 and 2.5 µg/ml in supplemented camel serum (n = 10) were 83.2% and 82.7%, respectively. The limit of quantification (LOQ) based on a signal to noise ratio ≥ 5 was 19 ng/ml of heptaminol in supplemented camel serum.

Pharmacokinetic analysis

Pharmacokinetic analysis of serum heptaminol concentrations for each animal was performed using least – squares non-linear regression analysis program (WinNonLin Standard edition, version 1.5, USA). One, two and three–compartment models were tested for the best fit to the i.v. administration data. Weighting was achieved according to the variance modes: $var(t) = {}^{1}/Y_{observed}{}^{2}$ where var (t) is the variance of the residual error of drug concentration at time t and $Y_{observed}$ is the observed drug concentration at time t. The best fit was based on Akaike's Information Criterion (Yamaoka *et al,* 1978), Schwarz's (1978) Criterion, analysis of residual plots and correlation matrix.

Determination of detection time

The detection time for heptaminol in racing camels was determined by collecting daily urine samples from the treated camels at about 08.00 hours daily for 5 days. These urine samples were then subjected to routine procedure for screening basic drugs in post race urine samples (Wasfi *et al,* 1998).

Extraction and analysis of metabolites

GC/MS

Heptaminol metabolites were extracted by solid phase extraction and analysed by gas chromatography/mass spectrometry as reported previously (Wasfi *et al,* 2003).

LC/MS

A heptaminol calibration curve (10 – 250 µg/ ml) was prepared in preinjection camel urine 100 µl of samples and calibrators were diluted into 9.9 ml of 0.1% formic acid. Aliquots of these samples were analysed directly by LCMS(MS). LC/MS analyses of heptaminol was performed on Thermo Electron Deca XP plus, using Surveyor autosampler and liquid chromatograph (Thermo Quest, 355, San Jose, CA, USA). Injections were made onto ACE 5 C8 columns (5 micron, 50x3 mm, Chadds Ford, PA, USA) using C8 guard columns. A solvent gradient

was programmed (Table 1) using a mixture of (A) 2.33 mm formic acid, pH 3.0 and (B) 0.1% formic acid in acetonitrile. The mass spectrometer was operated in positive ion electrospray mode. The spray voltage was set at 5 kV and the heated capillary was operated at 250°C. Sheath and auxiliary gases (nitrogen) were set at 24 and 2.95 arbitrary units, respectively. Helium was used as collision gas. Data were acquired in MS mode, scanning from 140-170 Da, and in MSMS mode, scanning from 50-170 Da. The precursors used were m/z146.1 (heptaminol) and 162.1 (hydroxy heptaminol) with an isolation width of 1.3 Da, Q value of 0.3 and activation time of 30 ms in wideband activation mode. The collision energy was 35%.

Statistical analysis of data

The values of the pharmacokinetic parameters were expressed as means ± SD. Statistical comparison between PK parameters of the 2 phases of the experiment was carried out applying student paired t test. The level of significance was $p < 0.05$.

Table 1. The non-linear gradient solvent program of solvent A (2.33 mm formic acid, pH 3.0) and solvent B (0.1% formic acid in acetonitrile) used for LC/MS analysis.

	Time	%A	%B	ul/min
1	0.0	90	10	20
2	0.2	90	10	20
3	0.3	90	10	200
4	3.0	0	100	200
5	3.3	0	100	200
6	3.31	90	10	400
7	4.0	90	10	400
8	4.01	90	10	20
9	4.2	90	10	20

Results

Plasma pharmacokinetic data

The disposition of heptaminol in camels was best described by a bi-exponential equation (2 compartment open model) (Fig 1). The values of the pharmacokinetic parameters are listed in Table 2. Heptaminol was characterised by a fast clearance, a large volume of distribution and a short elimination half-life. Diprophylline had no effect on heptaminol pharmacokinetic parameters when co-administered with heptaminol (Fig 1, Table 2).

Detection time of heptaminol

Heptaminol was detected for 1-2 days in camel urine samples.

Urinary metabolism data

A tentative identification of heptaminol metabolites was based on the presence of chromatographic peaks present in the urine samples after administration of heptaminol, in comparison with urine samples obtained before administration of heptaminol. The methylated acid-neutral fraction showed no evidence for the presence of metabolites.

The basic fraction showed one metabolite in addition to heptaminol. Both analytes appeared to be excreted in unconjugated form because the GC and LC peaks of heptaminol and its metabolite in the non- hydrolysed urine samples were not remarkably different when compared to those of glucuronidase-treated samples. This observation is further supported by the fact that no evidence of conjugation was detected when the urine samples were diluted and directly analysed by LC/MS. The metabolite was tentatively identified as hydroxy heptaminol. By LC/

Table 2. Pharmacokinetic parameters of heptaminol following intravenous administration to 7 healthy camels (3 males and 4 females) at a dose of 10 mg/kg body weight and heptaminol with diprophylline at an intravenous dose of 10 mg/kg each. Data are expressed as mean ±SD.

Variable	Heptaminol	Heptaminol with diprophylline	Significance
$T_{½\alpha}$ (hour)	0.19±0.09	0.16±0.08	N.S
$T_{½\beta}$ (hour)	2.00±0.21	2.09±0.34	N.S
AUC (ug $hour^{-1}$ per mL)	15.8±2.51	20.3±4.23	N.S
Cl_T (mL $hour^{-1}$ per kg)	645.5±109.9	511.1±114.1	N.S
Vss (mL kg^{-1})	1432.2±129.5	1279.1±310.0	N.S
Vc (mL kg^{-1})	540.5±185.7	484.3±162.5	N.S
AUMC (µg $hour^{-2}$ per mL)	36.07±9.89	52.4±16.3	N.S
MRT (hour)	2.25±0.27	2.54±0.53	N.S

$t_{1/2\alpha}$= half–life of distribution phase; $t_{½\beta}$= half–life of elimination phase; AUC= area under the curve to infinity; Vss= volume of distribution at steady state; Cl_T= total body clearance; Vc= volume of central compartment; AUMC= area under first moment curve; MRT= mean residence time.

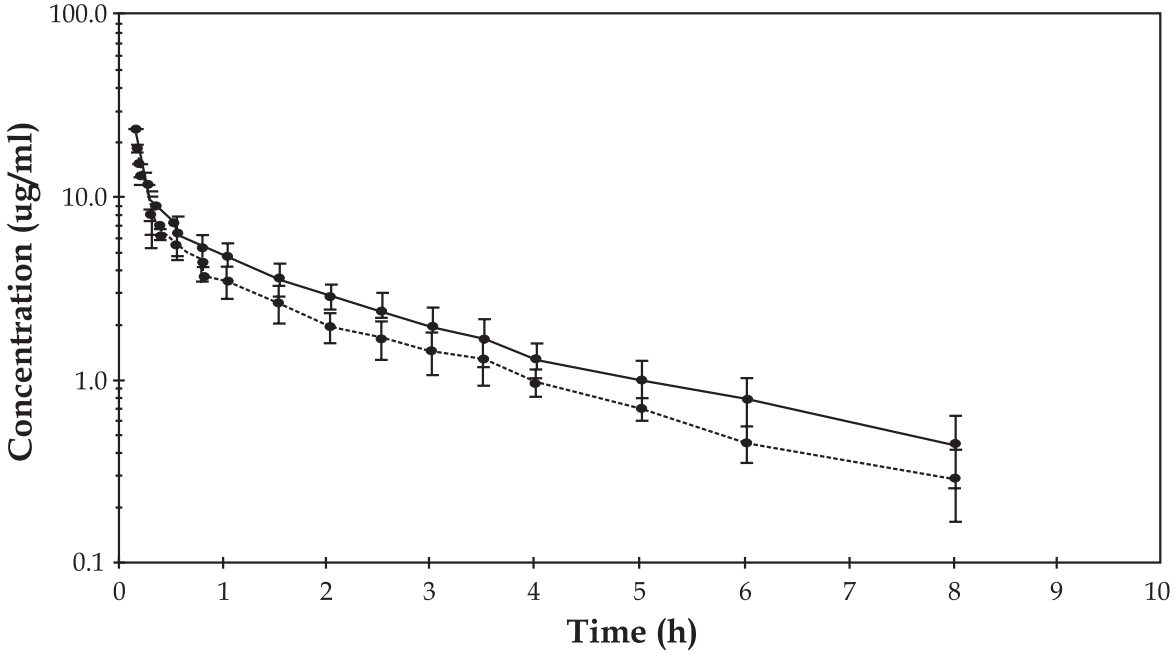

Fig 1. Heptaminol serum concentration (mean ± SEM) of 7 camels after an intravenous dose of 10 mg/kg body weight alone (----●----) and with diprophylline at an intravenous dose of 10 mg/kg body weight each (—●—).

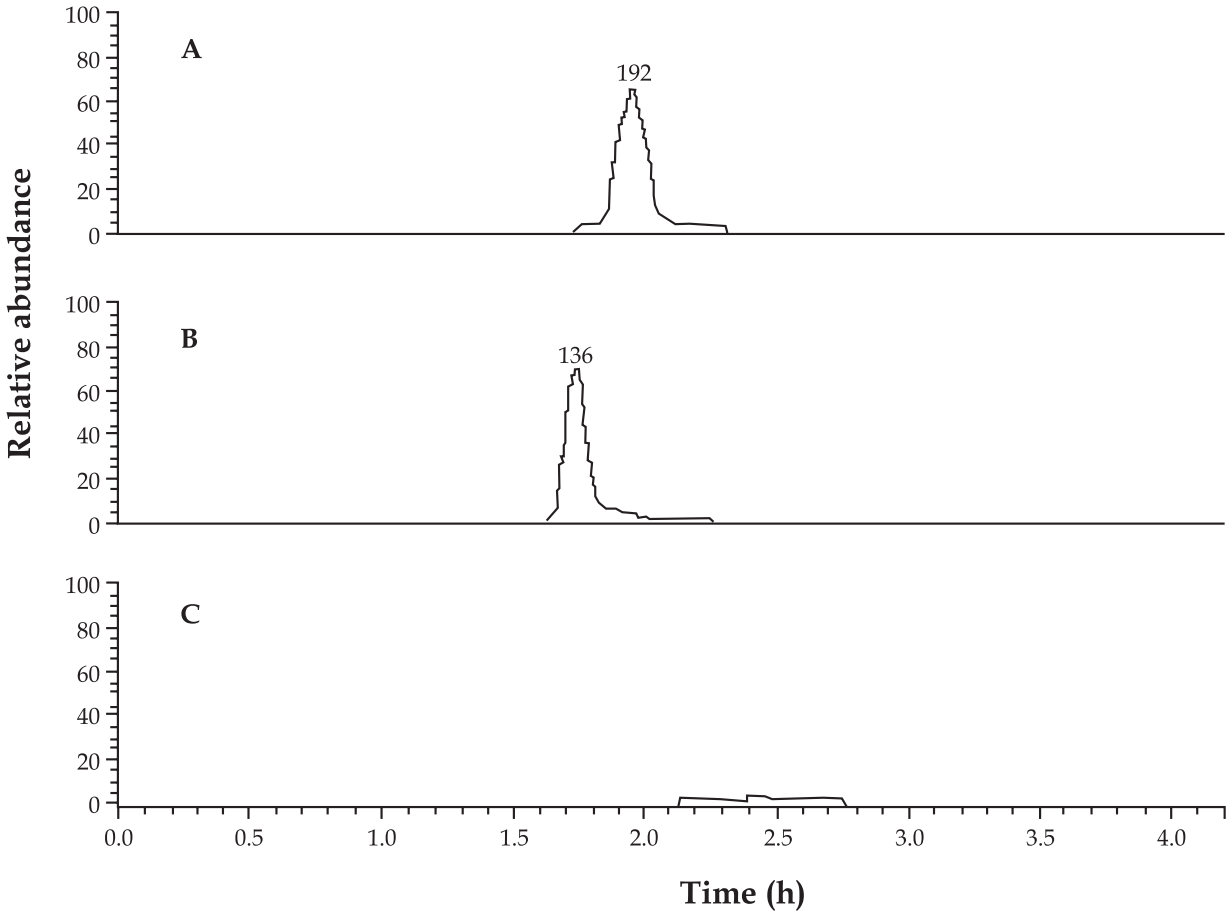

Fig 2. Total ion chromatogram of a urine sample 3 hours after intravenous administration of heptaminol; A, heptaminol; B, hydroxy-heptaminol and C, solvent blank.

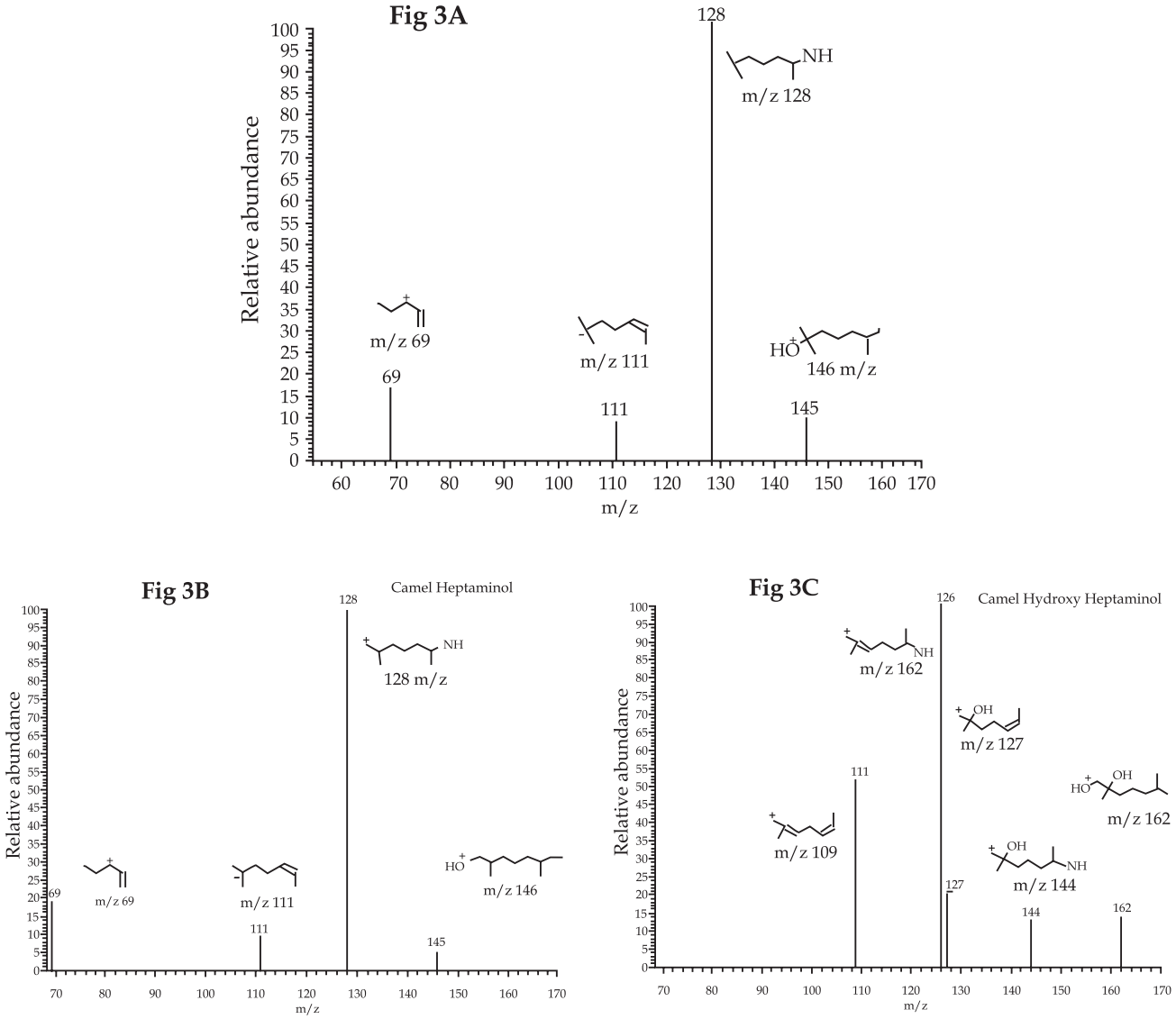

Fig 3. Proposed fragmentation pattern of heptaminol reference standard, A; heptaminol from camel urine, B and hydroxy heptaminol, C.

MS analysis, this metabolite would have a molecular weight of 161 and with a pseudomolecular ion of m/z 162 [M+1] it had a retention time of 1.74 min compared to 1.92 min for heptaminol (Fig 2). The proposed fragmentation pattern of heptaminol and hydroxy heptaminol from camel urine and heptaminol reference standard are shown in Figs 3B, 3C and 3A, respectively.

Discussion

Under our experimental conditions, heptaminol was well tolerated by camels as there no adverse were observed. The pharmacokinetics of heptaminol in camels was characterised by a short terminal elimination half-life, a rapid clearance and a large volume distribution. The pharmacokinetic parameters of heptaminol in camels were comparable to those reported in man and dogs (Kees *et al,* 1987; Cociglio *et al,* 1984).

In humans, nearly all the dose of heptaminol given was recovered unchanged in urine within 24 h, indicating renal elimination by glomerular filtration and tubular secretion without metabolism (Kees *et al,* 1987). However, as in camels, the rat metabolised heptaminol to a hydroxylated metabolite, 6-amino-2-methyl-1,2-heptanediol, which was excreted unconjugated in urine with evidence of saturation of metabolism at high doses (Chanoine *et al,* 1981).

We have observed that co-administration of diprophylline with heptaminol did not affect the pharmacokinetics of the latter. Diprophylline, however, is not metabolised and is eliminated unchanged in urine (Hasegawa *et al,* 1991; Nadai *et*

al, 1992). Co-administration of probenecid decreased the total body clearance of diprophylline and the renal clearance of diprophylline decreased as the probenecid concentration increased, a result indicating that probenecid inhibits the tubular secretion of diprophylline (Nadai *et al,* 1992). It, therefore, appears that the renal elimination of heptaminol in camels is insignificant as its pharmaokinetics were not affected by co-administration of diprophylline.

Irvine (1994) evaluated the cardiovascular effects of heptaminol in race horses. He used total doses of 5, 10 and 20 g which is roughly equivalent to 10, 20 and 40 mg/kg dose. Only the 20 g dose resulted in detectable changes of restlessness, tachycardia and marked P and T wave changes and these effects did not last more than 20 minutes. It is unlikely that the dose we used in camels (10 mg/kg) would have any cardiovascular effects that would affect performance. Indeed, we did not observe any sympathomimetic effect of heptaminol during the experiment.

One of the objectives of the present study was to determine how long heptaminol remained detectable in camel urine. We were able to detect heptaminol in enzymically treated urine samples collected from 24-48 h after administration of heptaminol. In the 72 h samples neither heptaminol nor hydroxy heptaminol could be detected in camel urine. The long detection time of heptaminol despite to its short half – life (2.0 h) and rapid clearance (645 mL/h/kg) may be due to the large Vss (1432 mL/kg) and the alkaline nature of camel urine which may result in hydrolysis of the unstable glucuronide conjugates and recycling of the parent compound *in vivo*. This is rendered more likely since camels have small urine volume (about 1.0 L/day; Wasfi *et al,* 1998) and a low glomerular filteration rate (0.55 – 0.65 ml/kg/ min; Wilson, 1984), allowing ample time for *in vivo* hydrolysis and re-absorption. We routinely perform enzymic or alkaline hydrolysis on post race samples and we screen by very sensitive GC/MS methods (Wasfi *et al,* 1997). Therefore, as a precautionary measure, we recommend withholding administration of heptaminol for a minimum period of 3 days before racing.

Acknowledgement

Support received from Lt Col Dr.Ahmad Hassan Alawadhi, Director of the Forensic Science Laboratory and technical assistance given by B. Agha, S. Wajid, S. Mousa, Asmaa M. Kamil, O. Mohammed, N. Ali of Photography Department is gratefully acknowledged.

References

Berthiau F, Garnier D, Argibay JA, Seguin F, Pourrias B, Grivet JP and le Pape A (1989). Decrease in internal H^+ and positive inotropic effect of heptaminol hydrochloride: a 31P n.m.r. spectroscopy study in rat isolated heart. British Journal of Pharmacology 98:1233-1240.

Chanoine F, Benedetti MS, Ancher JF and Dostert P (1981). Absorption, distribution, elimination and metabolism of 14C-heptaminol hydrochloride in rat. Arzneimittelforschung 31:430-435.

Cociglio M, Sauvaire D and Alric R (1984). Liquid chromatographic assay of heptaminol in serum and its oral pharmacokinetics in the dog. Journal of Chromatography 307:351-359.

Delicado EG, Fideu MD, Miras-Portugal MT, Pourrias B and Aunis D (1990). Effect of tuamine, heptaminol and two analogues on uptake and release of catecholamines in cultured chromaffin cells. Biochemical Pharmacology 40:821-825.

Hasegawa T, Nadai M, Apichartpichean R, Muraoka I, Nabeshima T and Takagi K (1991). Pharmacokinetic characteristics of N7-substituted theophylline derivatives and their interaction with quinolone in rats. Journal of Pharmaceutical Science 80:962-965.

Irvine CHG (1994). Effect of heptaminol on cardiovascular function in racehorses. Proceddings of the 11th International Conference of Racing Analysts and Veterinarians, Queensland, Australia. pp 249-251.

Kees F, Dominiak P and Grobecker H (1987). Bioavailability of heptaminol in healthy subjects. Arzneimittelforschung 37:1182-1185.

Nadai M, Apichartpichean R, Hasegawa T and Nabeshima T (1992). Pharmacokinetics and the effect of probenecid on the renal excretion mechanism of diprophylline. Journal of Pharmaceutical Science 81:1024-1027.

Schwarz G (1978). Estimating the dimension of a model. Analytical Statistics 6:461-464.

Wasfi IA, Hadi AAA, Zorob O Osman ME, and Boni NS (1997). Pharmacokinetics of phenylbutazone in camels. American Journal of Veterinary Research 58:636-640.

Wasfi IA, Boni NS, Abdel Hadi AA, El Ghazali M, Zorob O, Alkatheeri NA and Barazaiq IM (1998). Pharmacokinetics, metabolism and urinary detection time of flunixin after intravenous administration in camels. Journal of Veterinary Pharmacology and Therapeutics 21:203-208.

Wasfi IA, Abdel Hadi AA, Elghazali M, Nawal AA, Hussain MM and Hamid AM (2003). Comparative pharmacokinetics of diphenhydramine in camels and horses after intravenous administration. Veterinary Research Communications 27:463-473.

Wilson RT (1984). The Camel (The Print House Pte., Singapore). pp 74.

Yamaoka K, Nakagawa T and Uno T (1978). Application of Akaike's Information Criterion (AIC) in the evaluation of linear pharmacokinetic equations. Journal of Pharmacokinetics Biopharmaceutics 6:165–175.