Comparative pharmacokinetics of ampicillin trihydrate, gentamicin sulphate and oxytetracycline hydrochloride in Nubian goats and desert sheep

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In this investigation the pharmacokinetics of three commonly used antibiotics, ampicillin trihydrate (10 mg/kg), gentamicin sulphate (3 mg/kg) and oxytetracycline hydrochloride (5 mg/kg), given intravenously, were each studied in five Nubian goats and five desert sheep. The pharmacokinetic parameters were described by a two-compartment open model. The results indicated that there were significant differences between the two species in some kinetic parameters of ampicillin and oxytetracycline but not gentamicin. Ampicillin elimination half life ($t_{1/2\beta}$) in goats (1.20 h) was shorter than that in sheep (2.48 h), and its clearance (*Cl*) significantly higher in goats (2921mL/h·kg) compared to sheep (262 mL/h·kg) ($P < 0.01$). Ampicillin volume of distribution (Vd_{area}) was found to be significantly larger in goats (5673 mL/kg) than in sheep (992 mL/kg) ($P < 0.01$). For oxytetracycline, the $t_{1/2\beta}$ in goats (3.89 h) was significantly shorter than that in sheep (6.30 h) and the *Cl* value in goats (437 mL/h·kg) was significantly higher than in sheep (281 mL/h·kg). The results suggest that when treating sheep and goats, the pharmacokinetic differences between the two species must be considered in order to optimize the therapeutic doses of ampicillin and oxytetracycline.

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INTRODUCTION

Several factors affect the fate of drugs in animals (Gibson & Skett, 1994). These include species differences (Walker, 1980) and variations in environmental conditions (Alvares *et al.*, 1979). In this laboratory we have previously studied species differences between dromedary camels, desert sheep and Nubian goats with respect to drug metabolizing enzymes (Elsheikh *et al.*, 1988; Elsheikh *et al.*, 1991a) and in the pharmacokinetics of antipyrine and sulphadimidine (Elsheikh *et al.*, 1991b). These studies have indicated that goats possess the highest and camels the lowest oxidative and conjugative enzyme activities in liver, kidney and duodenal mucosa. Such differences are expected to cause variations in the pharmacokinetics of drugs and consequently differences in dosage regimens of certain drugs. It has also been shown that goats clear antipyrine and sulphadimidine at a rate significantly faster than the other two species.

In the present work we aimed to extend our previous observations to compare the pharmacokinetic profiles of ampicillin, gentamicin and oxytetracycline in sheep and goats. These are antibiotics with a wide antibacterial spectrum and are commonly used in veterinary practice. Although there is considerable information on the pharmacokinetics of these antibiotics in domestic animals born and reared in the temperate zones (Pilloud, 1973; Haddad *et al.*, 1985; Haddad *et al.*, 1986; Brown & Riviere, 1991), there seems to be comparatively limited data in animals born and reared under tropical conditions (El Baumy & Hassan, 1986; Nawaz & Khan, 1991). Sheep and goats have shown some variations in the disposition kinetics and dosage regimens of certain drugs (Nawaz, 1982; Nawaz & Khan, 1991). Therefore the aim of the present work was to compare the pharmacokinetics of ampicillin trihydrate, gentamicin sulphate and oxytetracycline hydrochloride, given intravenously, in Nubian goats and desert sheep. These two species are usually raised and kept together under similar husbandry conditions. This eliminates any variation because of environmental factors.

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MATERIALS AND METHODS

Animals

Animals used in the present study were clinically healthy young adult males; 15 Nubian goats (10–12 kg body wt; 12–14 months) and 15 desert sheep (16–20 kg body wt; 10–12 months). Animals were obtained commercially and kept in individual pens. They were fed on lucerne, sorghum hay and grains and given drinking water *ad libitum*. They were kept for 15 days before the start of the experiments, for acclimatization, during which period their apparent freedom from parasites and bacterial infections was ensured by clinical examination and faecal examination for parasitic eggs.

Drug treatments

Sheep and goats were each allocated randomly into three equal groups (Groups 1, 2 and 3). Ampicillin trihydrate (Penbritin, Beecham Animal Health, Bristol, UK), gentamicin sulphate (Pharmaceutical and Chemical Works Co. Ltd, Budapest, Hungary) and oxytetracycline hydrochloride (Farvet, Holland) were injected intravenously (i.v.) in sheep and goats in Groups 1, 2 and 3 at a dosage of 10, 3 and 5 mg/kg body wt, respectively. Blood (5 mL) was collected from the jugular vein using heparinized syringes before treatment and at 5, 10, 20, 40 min and at 1, 2, 3, 4, 5, 6, 8, 10, 12, 18, and 24 h post-injection. Plasma was separated after centrifugation of blood at 900 ***g*** for 10 min at 5°C and stored at -20°C prior to analysis.

Analytical procedure

The antibiotic concentrations in the plasma samples were determined by microbiological assay with an agar-diffusion method according to Bennett *et al.* (1966) and Arret *et al.* (1971). The test organism was *Bacillus subtilis* (Difco Laboratories, Detroit, USA) for ampicillin and gentamicin and *Bacillus cereus* var. *mycoides* (Difco Laboratories, Detroit, USA) for oxytetracycline. Standard solutions were prepared in sheep or goat plasma by appropriate serial dilution of the powdered drug standard (Sigma, MO, USA). Each assay of standard and unknown was carried out in triplicate. The inhibition zone sizes for the plasma unknowns were converted to concentrations using standard curves developed for each drug. Limits of quantification were 0.08 μg/mL for ampicillin, 0.15 μg/mL for gentamicin and 0.1 μg/mL for oxytetracycline. The coefficient of variation for each assay was less than 5%.

Pharmacokinetic analysis

Pharmacokinetic analysis of the data was performed with the aid of a computer program for non-linear regression analysis (Med. USA). Ampicillin, gentamicin and oxytetracycline data were analysed using a two-compartment open model. The plasma elimination curves were described by the biexponential equation:

$$C_t = Ae^{-\alpha t} + Be^{-\beta t}$$

where C_t is the plasma concentration at time t. The values of the pharmacokinetic constants [zero-time plasma intercept for the distribution (A) and elimination (B) phases and the rate constants from the distribution (α) and elimination (β) phases] calculated by this analysis were used to compute the distribution ($t_{1/2\alpha}$) and elimination ($t_{1/2\beta}$) half-lives, the volume of the central compartment (V_c), the volume of distribution (V_{darea}), the body clearance (Cl_B), the extrapolated concentration of the drug at zero time (C_{op}) and the area under concentration vs. time curve (AUC) according to the equations previously described by Baggot (1977) and Gibaldi and Perrier (1982). The equations used were:

(1) $t_{1/2} = 0.693/\text{rate constant}$
(2) $V_c = \text{Dose}/C_{op}$
(3) $Cl\text{B} = \text{Dose}/AUC$
(4) $V\text{darea} = \text{Dose}/AUC\text{x}\beta$
(5) $AUC = (A/\alpha) + (B/\beta$

Statistical analysis

The harmonic mean for half-lives and means ± SEM of the other parameters were calculated. The Wilcoxon's Rank Sum Test (Gad & Weil, 1986) was used to evaluate the significance of difference between half-lives. Student's t-test and analysis of variance were used to determine the significance of difference between means of other parameters. A level of $P \leqslant 0.05$ was considered significant.

RESULTS

Ampicillin

The mean plasma concentrations of ampicillin plotted logarithmically against time, after the i.v. administration of the drug in sheep and goats are illustrated in Fig. 1, and the pharmacokinetic parameters are presented in Table 1. Significant differences were observed between sheep and goats in the pharmacokinetic variables of ampicillin. The V_d was significantly greater ($P<0.01$) in goats than in sheep. The apparent elimination rate constant was significantly higher in goats ($P<0.01$) compared to sheep, which was consistent with the shorter elimination half life time (harmonic mean). Figure 1 suggests significantly lower values of the AUC ($P<0.001$) in goats indicating higher distribution of the drug

Gentamicin

The mean plasma concentrations of gentamicin plotted logarithmically against time, after i.v. administration in goats and sheep are shown in Fig. 2. No significant differences were observed between sheep and goats in any of the pharmacokinetic parameters studied (Table 2).

Oxytetracycline

The mean plasma concentrations of oxytetracycline plotted

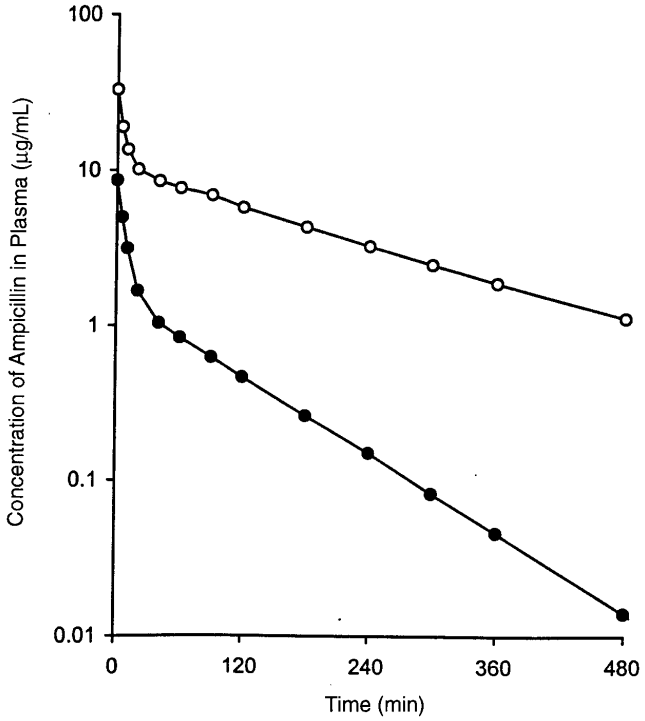

Fig. 1. Semilogarithmic plot of mean plasma concentrations of ampicillin trihydrate vs. time after intravenous administration (10 mg/kg body weight) in five goats (●) and five sheep (○). The SEM (not drawn) represented 10% or less of the mean values.

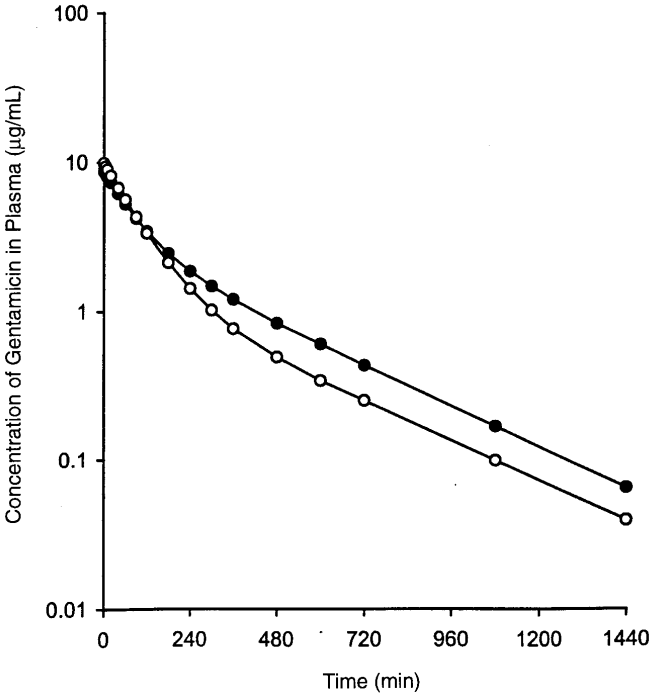

Fig. 2. Semilogarithmic plot of mean plasma concentrations of gentamicin sulphate vs. time after intravenous administration (3 mg/kg body weight) in five goats (●) and five sheep (○). The SEM (not drawn) represented 10% or less of the mean values.

Table 1. The pharmacokinetic parameters of ampicillin trihydrate in plasma of goats and sheep after a single intravenous injection at a dosage of 10 mg/kg body weight

Pharmacokinetic parameter	Goats (n = 5)	Sheep (n = 5)
C_{op} (μg/mL)	8.61 ± 0.86	32.85 ± 3.61*
α (h^{-1})	8.36 ± 0.92	11.01 ± 1.67
β (h^{-1})	0.58 ± 0.10	0.28 ± 0.04*
A (μg/mL)	7.13 ± 0.72	22.63 ± 2.22*
B (μg/mL)	1.48 ± 0.20	10.22 ± 1.48*
$t_{1/2\alpha}$ (h)	0.083 (harmonic mean)	0.063 (harmonic mean)
$t_{1/2\beta}$ (h)	1.195 (harmonic mean)	2.476 (harmonic mean)
V_{darea} (L/kg)	5.67 ± 0.83	0.99 ± 0.12*
Cl_B (mL/h·kg)	2921.4 ± 300.5	262.2 ± 19.7*
AUC (μg/mL·h)	3.59 ± 0.42	38.98 ± 2.84*

Values in the Table (other than the half-lives values) are means ± SEM.
*Significantly different from goats ($P < 0.01$).

Table 2. The pharmacokinetic parameters of gentamicin sulphate in plasma of goats and sheep after a single intravenous injection at a dose rate of 3 mg/kg body weight

Pharmacokinetic parameter	Goats (n = 5)	Sheep (n = 5)
C_{op} (μg/mL)	8.74 ± 1.17	9.87 ± 1.76
α (h^{-1})	0.75 ± 0.15	0.67 ± 0.11
β (h^{-1})	0.16 ± 0.02	0.15 ± 0.02
A (μg/mL)	5.81 ± 0.81	8.35 ± 1.48
B (μg/mL)	2.92 ± 0.43	1.52 ± 0.28
$t_{1/2\alpha}$ (h)	0.924 (harmonic mean)	1.041 (harmonic mean)
$t_{1/2\beta}$ (h)	4.20 (harmonic mean)	4.54 (harmonic mean)
V_{darea} (L/kg)	0.27 ± 0.02	0.22 ± 0.01
Cl_B (mL/h·kg)	115.33 ± 12.67	134.99 ± 5.43
AUC (μg/mL·h)	27.09 ± 2.48	22.36 ± 0.87

Values in the Table (other than the half-lives values) are means ± SEM.

logarithmically against time, after i.v. administration to sheep and goats are shown in Fig. 3 and the pharmacokinetic parameters obtained are given in Table 3.

The antibiotic was more rapidly eliminated in goats compared to sheep, as evidenced by the shorter time of elimination (harmonic mean) and the greater Cl values ($P<0.05$). Differences in the AUC also suggest faster elimination of oxytetracycline in goats than in sheep ($P<0.05$).

DISCUSSION

The results obtained for pharmacokinetics of gentamicin indicated no significant differences between goats and sheep. This might be related to similar distribution and elimination characteristics of the drug in the different species. Gentamicin is minimally bound to plasma proteins and eliminated mainly via the kidney (Brown & Riviere, 1991).

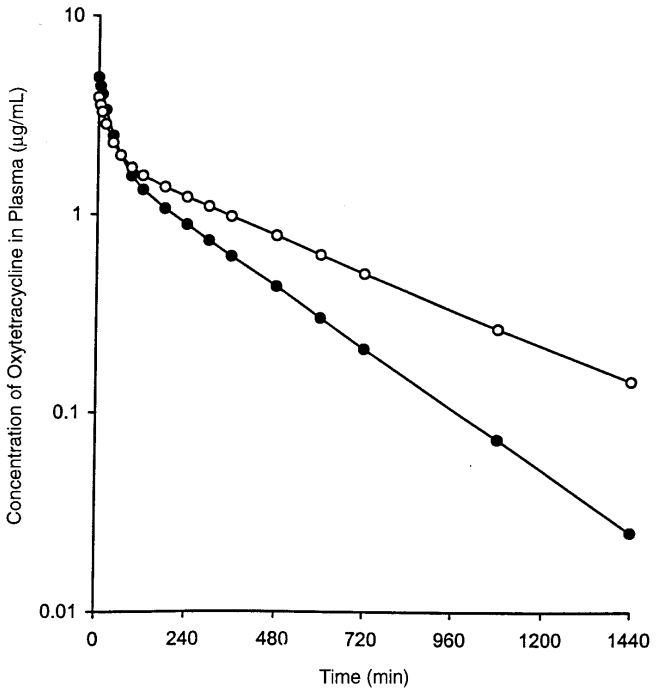

Fig. 3. Semilogarithmic plot of mean plasma concentrations of oxytetracycline hydrochloride vs. time after intravenous administration (5 mg/kg body weight) in five goats (●) and five sheep (○). The SEM (not drawn) represented 10% or less of the mean values.

Table 3. The pharmacokinetic parameters of oxytetracycline hydrochloride in plasma of goats and sheep after a single intravenous injection at a dose rate of 5 mg/kg body weight

Pharmacokinetic parameter	Goats (n = 5)	Sheep (n = 5)
C_{op} (µg/mL)	4.90 ± 0.58	3.88 ± 0.41
α (h^{-1})	1.88 ± 0.33	1.95 ± 0.23
β (h^{-1})	0.18 ± 0.02	0.11 ± 0.01*
A (µg/mL)	3.11 ± 0.41	1.99 ± 0.21*
B (µg/mL)	1.79 ± 0.22	1.89 ± 0.21
$t_{1/2\alpha}$ (h)	0.37 (harmonic mean)	0.36 (harmonic mean)
$t_{1/2\beta}$ (h)	3.89 (harmonic mean)	6.30 (harmonic mean)
V_{darea} (L/kg)	2.53 ± 0.29	2.67 ± 0.39
Cl_B (mL/h·kg)	436.99 ± 47.94	281.31 + 25.01*
AUC (µg/mL·h)	12.08 ± 1.50	18.37 ± 1.68*

Values in the Table (other than the half-lives values) are means ± SEM.
*Significantly different from goats ($P < 0.05$).

Different results were obtained for oxytetracycline and ampicillin. Goats exhibited enhanced ability to clear the two drugs from the body compared with sheep. The relatively higher values of V_d for ampicillin in goats might indicate excellent penetration of the drug into body tissues and fluids.

Nawaz and Khan (1991) reported considerable differences between local (Pakistani) sheep and goats in the disposition kinetics of ampicillin. Ampicillin is eliminated in urine, largely as unchanged ampicillin, and only a small proportion appears in the form of biologically inactive penicilloic acid (Rolinson & Sutherland, 1973). Variations between different animal species in elimination parameters might be related to differences in renal clearance and urinary excretion of ampicillin, as this drug is minimally bound to plasma protein. Ampicillin elimination, $t_{1/2\beta}$, in goats in the present study (55.2 min) is close to that in dwarf goats (60 min) (Anika *et al.*, 1986). However, it is different from that reported in the Nubian goat by Ibrahim (1988) (102 min). These differences might be attributable, at least in part, to differences in inter-animal and inter-laboratory variations or to other unknown causes. Moreover, Ibrahim (1988) used a spectrophotometric procedure for analysis, while in the present work a microbiological assay was used. In sheep, the elimination $t_{1/2\beta}$ of ampicillin in the present study (149 min) was significantly longer than that reported in lacaune sheep (19.2 min) (Tufenkji *et al.*, 1991). Again the differences in the animal breed, in the analytical methods or other unknown factors may be responsible for these differences. Previous reports indicated that there is wide species differences in ampicillin elimination kinetics. For example, buffalo calves (Jayakumar *et al.*, 1986), sheep (Brown *et al.*, 1986; Tufenkji *et al.*, 1991), horses (Sarasola & McKellar, 1994) and pigs (Galtier & Charpenteu, 1979) all have different kinetic variables, probably reflecting, at least partially, differences in their renal clearance of the drug.

The differences in the disposition kinetics of oxytetracycline between sheep and goats in the present study could be attributed to the variations in protein binding and renal excretion of the drug. Oxytetracycline is excreted by glomerular filtration with little metabolism and tubular secretion. The correlation between renal clearance and protein binding has been established for various tetracycline derivatives which are excreted almost exclusively by glomerular filtration (Kunin, 1967). Kirkwood and Widdowson (1990) reported a greater than ten fold difference between different species in plasma half-life of oxytetracycline, during the elimination phase, from 79 min in rabbits to 942 min in the horse.

Marked species differences were also reported in the literature for the $t_{1/2\beta}$ of oxytetracycline which was 630 min in horses (Pilloud, 1973), 420 min in camels (El-Gendi *et al.*, 1983), 546 min in cattle (Yoder & Packer, 1954), 217 min in buffalo (Varma & Paul, 1983) and 221 min in Merino sheep (Anika *et al.*, 1986). The latter is shorter than our present result (378 min).

Determination of the glomerular filtration rate, body water regulation, and biliary excretion of oxytetracycline and ampicillin might have been of value in assessing the difference in clearance of these drugs in sheep and goats. The sheep and goats used in the present work are essentially desert animals and their body water regulation may differ from that of animals born and reared in temperate zones. This may be particularly relevant in the case of ampicillin which is mainly excreted in urine. The present findings suggest that when treating sheep and goats, species differences must be taken into account in order to optimize the therapeutic dose of the antibiotic used, and to minimize the possibility of overdosing (which may lead to toxicity), or underdosing (which may lead to failure of therapy).

This study stresses the need to undertake pharmacokinetic studies in the target animal, in order to determine the optimum therapeutic dose and to minimize dependence on extrapolation of dosage regimens from one animal to another.

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