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Comparative Pharmacokinetic studies of antibiotics (Amoxycillin) and its residues in domestic animals.

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ABSTRACT

The disposition of amoxycillun following intravenous (IV) and oral administration in camel was studied. The kinetic behavior of the drug was best described by two compartment open model. The half-life of distribution was 3.6 ± 0.36 minutes for IV and 15.3 ± 1.9 minutes for oral dosing. The half-life of elimination was 69.3 ± minutes for IV and 80.0 ± 3.4 minutes for oral dosing. The mean peak plasma concentration after oral administration was 2.11 ± 8.3 µg/ml detected at 2 hours after drug administration and the bioavailability was 23.3%.

INTRODUCTION

Antibiotics play an important role in the treatment of various infectious disease in man and animals. An effective concentration of antibiotic must be available at the focus of infection and maintained for adequate time (Gilman *et al*., 1991). The concentration achieved is dependent on the Pharmacokinetic of the drug such as dosage form, routes of administration, half-life and ability to gain access to infection site (tissue target).

Several studies have shown that the Pharmacokinetic behavior, optimal dosage, plasma half-life, renal clearance and urinary excretion of the investigated drugs were different under indigenous conditions when compared with values given in the literature or in the product inserts supplied by manufacturers. For example drug manufacturers give no specific recommendations for the camel. Therefore, the doses used clinically in this species are in general extrapolated from doses recommended for large domestic species. This is not without danger, because toxic effects sometimes occur in camels which are given certain drugs at doses apparently harmless to other species (for review see Al-Dughaym *et al*., 1998; Homeida *et al*., 1981).

An original term “geonetic” has therefore been coined to describe environmental influences on the genetics which are manifested by characteristics biochemical and physiological parameters which ultimately affect

the biodisposition and fate of drugs in population (Muhammed, 1997). Such geonetical influences have been reported for blood and urine pH, blood proteins, drug metabolism and kidney function in buffaloes, cows, sheep, goats and camels (Nawaz *et al*., 1988; Al-Dughaym *et al*., 1998). From these studies it may be concluded that biochemical and physiological parameters are influenced by geonetical conditions which ultimately affect the disposition kinetic, fate and response to drugs. For examples, sulphonamides and antibiotics under indigenous geonetical conditions is deferent from disposition recorded elsewhere (Nawaz and Khan, 1979; Nawaz *et al*., 1989; Homeida, 1999). Therefore, it is imperative that an optimal dosage regimen should be based on the pharmacokinetics data determined in the species and environment in which a drug is to be employed clinically.

Amoxycillin is a semisynthetic penillin with a broad spectrum of antibacterial activity (Gram-positive and Gram-negative organisms) similar to that of ampicillin (Sutherland and Rolinson, 1970), over which it has been shown to have important advantages. In particular, when given orally in a number of species, its bioavailability is approximately twice that of ampicillin and much higher serum concentrations are obtained (Yeoman, 1977). The influence of food on the oral absorption of aminopenicillins has been reviewed by Eshelman and Spyker (1978), and it is been shown that the absorption of ampicillin, but not of amoxycillin, is reduced in the presence of food. It has also

been shown that certain important Gram negative bacilli (including *Escherichia coli*) are destroyed more rapidly by amoxycillin and that experimental infections respond more quickly to amoxycillin than to ampicillin (Hunter *et al*., 1973, Comber *et al*., 1975, Rolinson *et al*., 1977). Amoxycillin is the most frequently prescribed agent for the oral treatment of respiratory tract infections (Woodhead *et al*., 1987). Information regarding to pharmacokinetics of amoxycillin and its residues have not been studied in arabian camels.

Objectives:

This study is planned to investigate pharmacokinetic of amoxycillin in camels.

MATERIALS and METHODS

Animals and Treatments:

Ten healthy adult camels, six males and four femals, of 2-3 years old and 200-230 kg body weight were used for this study. Animals were housed in indivdual pens under natural day length and tempreature and allowed free acces to hay and water. Animals were not fasted prior to administration of the drug. At least one week was allowed to elapse between each experiment.

Experiment 1:

During this experiment amoxycillin as a 5% aqueous solution (Clamoxyl, Smith kline Beecham, Tadworth, UK) at a dose of 10 mg/kg body weight was administered intravenously into the right Jugular Vein. Blood samples were collected serially in sterile heparinizied tubes by venipuncture of the left Jugular Vein at 0, 5, 10, 15, 30 minutes and at 2, 3, 4, 6 and 12 hours after amoxycillin administration. The blood samples were centrifuged at 800 × g for 10 minutes, plasma separated and stored at –30 ^{o}C until analysis.

Experiment 2:

During this experiment, amoxicillin trihydrate as 5% suspension (Clamoxyl Drops, Smith kline Beecham, Tadworth, UK) was administered via orogastric tube at a dose of 20 mg/kg body weight. Fifty ml of water was administered after the drug to wash out the orogastric tube before withdrawing it. Blood samples were collected and handled as described before.

Assay of amoxicillin:

Concentrations of TAP in plasma samples were determined by using an agar plate diffusion method (Bennett *et al*. 1966), using *Bacillus subtillis* (ATCC 6633) as test organism, growing on Muller-Hinton agar (Mast Group Ltd Mersyside, UK). Wells of 8 mm in diameter containing 25 ml seeded agar were prepared and filled with either the test sample or amoxicillin standards. Standard solutions were prepared in antibiotic-free poled camel serum by appropriate serial dilutions of powdered amoxicillin standard. Each assay of standrd and unknown was carried out in triplicate. The plate were kept at room temperature for 24 hours. Mean zone diameters were measured and plasma concentrations were determined using the curve constructed from the results of standard samples. The intra-assay coefficient of variation for amoxicillin in control plasma fortified with known concentrations was 6.9% for the range of 0.10-15 μg/ml. A linear relationship existed between the zone of inhibition and TAP concentrations in plasma with a correlation coefficient of 0.99. The detecting limit of amoxicillin in plasma was 0.10 μg/ml.

Pharmacokinetic calculations:

A computerized curve-stripping program (R Strip, Micro Math Research, Saint louis, MO, USA) was used to analysed the concentration-time curves for each animal. The relevant pharmacokinetics parameters were

calculated using conventional equations associated with compartmental analysis (Gibaldi and Pirrier, 1982), where volume of distribution equals dose / intercept at time zero. Kinetic parameters of amoxicillin in camel were compared using Student's *t-test* (Kirkwood, 1988). The probability value $P < 0.05$ was considered significant.

RESULTS

The disposition curves describing the decline in plasma concentrations of amoxycillin after IV and oral administration (Fig. 1). The kinetic behaviour of the drug was best described by an open two-compartment open model. The initial part of the curve describing the disappearance of amoxycillin from plasma was steep reflecting the processes involved in distribution of the drug from central to peripheral compartment. The value of half-life of distribution was only 3.6 ± o.36 minutes for IV and 15.3 ± 1.9 minutes for oral dosing. The later portion of the curve reflected the elemination of the injected amoxycillin from the central compartment. The elemination half-life was 69.3 ± 2.6 minutes for IV and 80.0 ± 3.4 minutes for oral dosing (Table I and II).

After orogastric administration of amoxycillin the antibiotic was rapidly but incompletely absorped with $t_{1/2}$ of absorption of 15.3 ± 1.9 minutes. The mean peak plasma concentration was 2.11 ± 0.83 μg/ml was detected at 2 hours after drug administration. The mean estimated systemic availability (F) was 23.3 ± 1.2 % (Table II).

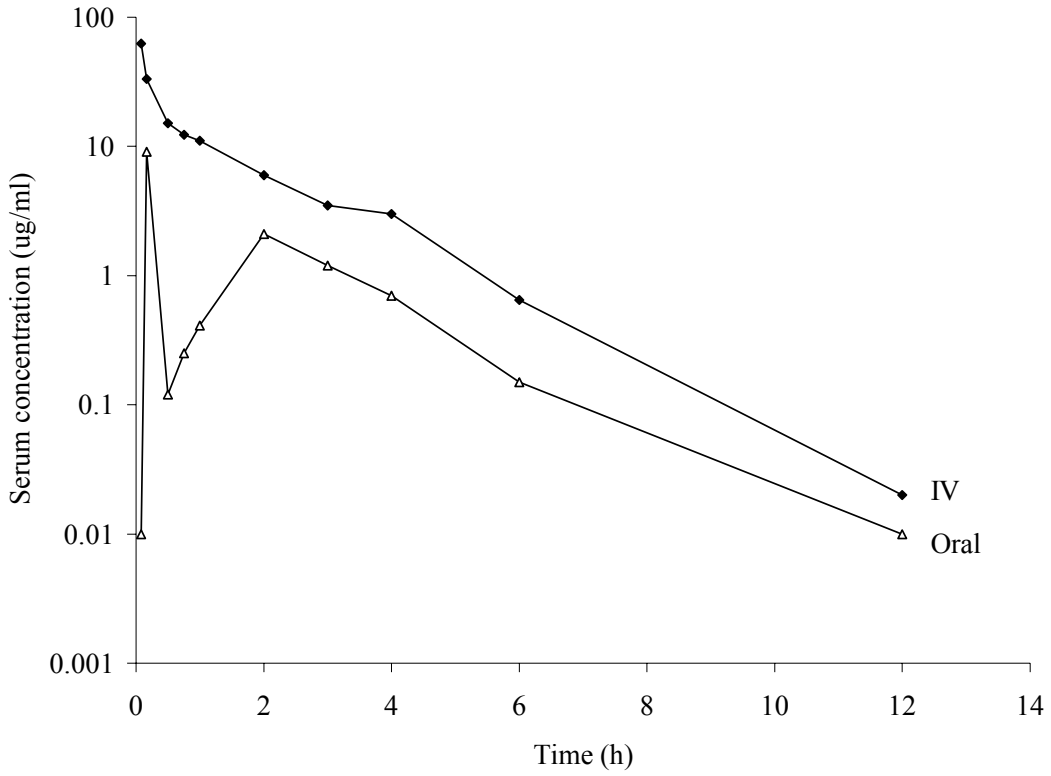

Figure 1: Mean semi-log serum concentrations of amoxycillin versus time following intravenous (i.v) administration of a single dose of 10 mg/kg body weights or 20 mg/kg body weight orally to healthy camel. (n. = 10 each).

Table-I: Peripheral plasma concentration (mean ± SD) of amoxycillin in camels after a single intravenous bolus of 10 mg/kg body weight or 20 mg/kg body weight orally. (n=10 each).

Time	**Plasma concentration of amoxycillin (μg/ml)**	
	IV	**Oral**
5 **(min)**	62.3 ± 3.1	0.01
10	33.2 ± 1.2	0.06
30	15.11 ± 0.6	0.12 ± 0.01
45	12.3 ± 0.5	0.25 ± 0.01
1**(h)**	11.10 ± 0.4	0.41 ± 0.01
2	6.00 ± 0.03	2.1 ± 0.06
3	3.50 ± 0.02	1.2 ± 0.1
4	3.00 ± 0.02	0.70 ± 0.01
6	0.65 ± 0.01	0.15 ± 0.01
12	0.02	0.01

Table- II: Pharmacokinetic parameters of amoxycillin given at a single intravenous dose of 10 mg/kg body weight to healthy camels. (n. = 10).

Kinetic parameters	Mean ± SD
A (μg/ml)	56.17 ± 9.1
B (μg/ml)	16.29 ± 4.1
α (min^{-1})	0.190 ± 0.006
β (min^{-1})	0.010 ±0.0.002
$t_{1/2}$ (α) (min)	3.647 ± 0.36
$t_{1/2}$ (β) (min)	69.3 ± 2.6
V_d (area) (ml/kg)	543.4 ± 13.2
Cl_B (ml/min/kg)	5.434 ± 0.312
AUC (μg/h/ml)	30.1 ± 3.1

A = zero-time intercept of distribution phase; B = zero-time intercept of elimination phase; α = distribution constant; β = elimination constant; $t_{1/2}$ (α) = half-life of distribution phase; $t_{1/2}$ (β) = half-life of elimination phase; V_d (area) = volume of drug distribution; Cl_B = total body clearance of the drug; AUC = area under the concentration-time curve.

Table- III: Pharmacokinetic parameters of amoxycillin given at a single oral dose of 20 mg/kg body weight, to healthy camels. (n. = 10).

Kinetic parameters	Mean ± SD
C_{max} (μg/ml)	3.11 ± 0.83
T_{max} (h)	2.1 ± 0.39
$t_{1/2}$ (α) (μg/ml)	15.3 ± 1.9
$t_{1/2}$ (β) (μg/ml)	80.0 ± 3.4
AUC (μg/ml/min)	14.1 ± 2.1
F% (0→12h)	23.3 ± 1.2

C_{max} = peak concentration; T_{max} = time maximum concentration; $t_{1/2}$ (α) = half-life of distribution phase; $t_{1/2}$ (β) = half-life of elimination phase; AUC = area under the concentration-time curve; F (0→12h) = bioavailability during administration [AUC oral/AUC intravenous × dose IV / dose oral].

DISCUSSION

The disposition of amoxicillin in camel after both intravenous and oral administration was best described by a two-compartment model with a short half-life. Similar results have been reported in man (Spyker *et al*., 1977), horse (Wilson *et al*., 1988; Ensink *et al*., 1992), sheep and goat (raigmill *et al.*, 1992), rat (Torres-Molina *et al*., 1992) and dog (Kung and Wanner,1994).

The short distribution half-life of amoxicillin indicates that the drug moves rapidly from central to peripheral compartment. The volume of distribution of the drug exceeds the volume of the central compartment (410 ml/kg) suggesting extensive tissue penetration (Wilson, 1984). The rapid elimination phase suggests that, like other penicillin's, amoxicillin is eliminated rapidly by renal tubular secretion (Gilman *et al*., 1991). This is consistent with the fact that the nephron in the camel is twice as long as in cows or goat (Abdalla and Abdalla, 1979).

The high body clearance (5.4 ml/min/kg) and short elimination half-life amoxycillin indicate that IV administration of these soluble forms to camels may have some limitations for treating bacterial infections in camel practice. In complete absorption and rapid elimination after oral administration were responsible for the small area under plasma concentration time curve so that bioavailability was only 23%. The low plasma concentrations of amoxycillin

seen may be because of chemical reduction by rumen microflora (Knoppert *et al*., 1988), poor solubility in aqueous rumen contents (Shoaf *et al*., 1987) or extensive first pass effect (Ratz *et al*., 1995). As camelid and ruminant forestomach share similarities in fermentive capacities and digestive qualities, similar causes for poor absorption of amoxycillin in camels might be expected. Furthermore, amoxycillin has been reported to be absorbed across the intestinal mucosa by both passive diffusion and active transport. The active transport is most likely mediated through the oligopeptide carrier localized at the apical enterocyte membrane which is a symport carrier transporting a substrate with a proton across the apical enterocyte membrane (Westphal *et al*., 1990; 1991; Lennernas *et al*., 2002). The existence in camels of a carrier-mediated transport that can become saturated at high dosage, could explain the low oral bioavailability of amoxycillin reported here and elsewhere (Agerso and Friis, 1998).

Throughout a course of treatment is highly desirable to maintain therapeutic concentrations of antibiotic in the body. A minimum inhibitory concentration (MIC) of amoxycillin 0.5 μg/ml has been reported Hyatt *et al*. (1995). In the present study, a mean plasma amoxycillin concentration of 0.5μg/ml or more was maintained for at least 4-6 hours after a single IV or oral dosing. It seems likely that such a dose value may need to be given more than once every 24 hours.

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