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# Pharmacokinetics and Tolerance of Thiamphenicol in Camels and Sheep

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**Abstract:** The pharmacokinetics and tolerance of thiamphenicol (TAP) were investigated in camel and sheep following administration of intravenous dose of 20 mg $kg^{-1}$ body weight. The disposition kinetic of TAP in camel and sheep showed a biexponential profile with elimination half-live of 2.11 h for camel and 1.5 h for sheep and volume of distribution of 1.1 L $kg^{-1}$ for camel and 0.90 L $kg^{-1}$ for sheep indicating extensive tissue penetration which is consistent with TAP lipophilicity. The drug administered at a dose of 40 mg $kg^{-1}$ to camel and sheep produced no changes in haematological and plasma biochemical parameters suggesting that both species can tolerate higher doses of the drug.

**Key words:** Camel, sheep, pharmacokinetics, tolerance, thiamphenicol

## INTRODUCTION

Thiamphenicol (TAP) is a semisynthetic structural analogue of chloramphenicol. The major structural difference between chloramphenicol and TAP is that the para-nitrophenol group has been replaced by the methyl sulfonyl moiety[1]. Thiamphenicol inhibits bacterial protein synthesis at the 50 S ribosomal subunit at the same location as does chloramphenicol. Thiamphenicol has an antimicrobial spectrum of activity similar to chloramphenicol[2]. Thiamphenicol considered to be less toxic than chloramphenicol with no reports linking its use with aplastic anaemia[3], so TAP appears to be a viable substitute for chloramphenicol in veterinary medicine. Few pharmacokinetic studies of TAP have been performed in animals and birds[4]. In dogs ATP was found to be well absorped with half-life of 1.7 h and volume of distribution of 0.66 L $kg^{-1}$[5]. Thiamphenicol is more stable in solution, it is not appreciably protein bound in the body and it does undergo significant biotransformation[6]. The potential usefulness of TAP for treatment of common infection such as mycoplasmae and other chloramphenicol sensitive organisms in animals and poultry requires detailed information on pharmacokinetic properties, bioavailability, distribution and elimination of TAP, especially in the light of the strict regulated use of chloramphenicol due to problem as it may be responsible for severe adverse reactions both in animals and in humans. Such therapeutic restriction of chloramphenicol has led to search for possible substitutes. This study was planned to investigate pharmacokinetic properties and tolerance of TAP in camels and sheep.

## MATERIALS AND METHODS

**Animals and preparations:** For the study of pharmacokinetic of thiamphenicol eight camels (*Camelus dromedarius*) and eight sheep (Ardy breed) were used. The animals had free access to hay and water *ad libitum*. Each animal was weighed before the start of each experiment. Camels and sheep were cannulated under strict aseptic conditions with plastic canulla No. 90 (Portex Ltd, England) for administration of the drug and collection of blood samples.

**Drug administration:** A single dose of TAP injected intravenously (I/v) to each animal at a dose of 20 mg $kg^{-1}$ body weight. The drug was given as thiamphenicol glycinate hydrochloride (25.2 mg $kg^{-1}$; Glitisol, Vilano, Italy).

**Collection of blood samples:** Blood samples of 5 mL pertime-point were collected in heparinizied tubes at 0, 5, 10, 15, 30, 45 min and at 1, 2, 3, 4, 6, 9, 12 and 18 h post-treatment. The blood samples were centrifuged at 2000×g and the plasma was separated and stored at -20°C until analysis.

**Tolerance studies:** Eight camels and six sheep were injected with TAP intravenously (I/v) at a dose of 40 mg $kg^{-1}$ body weight. Blood was collected in EDTA tubes for haematology or heparinized tubes for plasma preparation. Pretreatment Complete Blood Picture (CBP) and serum biochemistry were performed a week prior to starting the project. Then CBP and plasma biochemistry analyses were

repeated at 48, 72 and 98 h post-injection. Clinical signs will also be observed.

**Biochemical analysis:** The EDTA samples were analysed within 24 h of collection for haematology variables including White Blood Cell Count (WBC), Red Blood Cell Counts (RBC), Haemoglobin (Hb) and platlet numbers. Differential cell counts were done to estimate the number of neutrophils, lymphocytes, monocytes, eosinophils and basophils. All values were determined using a Roche, Cobas, Minos, Veterinary Automated Haematology Analyser (Roch products, Herts, UK). Plasma urea, alkaline phosphate aspartate aminotransferase were determined using a Roche, Cobas, Minos, Clinical chemistry Analyser (Roch products, Herts, UK).

**Assay of thiamphenicol:** Concentrations of TAP in plasma samples were determined by using an agar plate diffusion method[7], 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 thiamphenicol standards. Standard solutions were prepared in antibiotic-free poled camel or sheep serum by appropriate serial dilutions of powdered TAP standard. Each assay of standard and unknown was carried out in triplicate. The plate were kept at room temperature for 24 h. 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 TAP in control plasma fortified with known concentrations was 6.9% for the range of 0.10-15 $\mu g\ mL^{-1}$. 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 TAP in plasma was 0.10 $\mu g\ mL^{-1}$.

**Determination of serum protein binding of thiamphenicol:** The extent of thiamphenicol binding to serum proteins of camel and sheep was determined *in vitro* by ultrafiltration[8] using camel's or sheep's serum fortified with known concentrations of thiamphenicol rainging between 0.10 and 15 $\mu g\ mL^{-1}$. Five milliliter of each were placed on conditioned semipermeable membrane (Amicon Corp. Lexington, MA; nominal molecular weight cut-off 30 $KD_a$) testing on a porous conical polyethylene support on top of centrifuge tubes. The tubes were centrifuge at room temperature at 1500 g for 50 min. Serum samples and their corresponding ultrafiltrates were assayed to determine the concentration of thiamphenicol. The percentage serum protein binding was calculated as follows: [(serum concentration-ultrafilterate concentration) / serum concentration]×100.

**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[9], where volume of distribution equals dose/intercept at time zero. Kinetic parameters of TAP in camel and sheep were compared using Student's t-test[10]. The probability value $p<0.05$ was considered significant.

## RESULTS

The data of thiamphenicol in camel and sheep (Table 1) was best fitted to a two compartment open model for kinetic analysis. The semi-logarthemic plot illustrating this analysis is shown in Fig. 1. The values of pharmacokinetic parameters are given in Table 2. Higher

Table 1: Peripheral plasma concentration (mean±SD) of thiamphenicol after a single IV bolus of 20 mg $kg^{-1}$ body weight in camel and sheep (n=8 each)

| | Plasma concentration of thiamphenicol ($\mu g\ mL^{-1}$) | |
|---|---|---|
| Time | Camel | Sheep |
| 5 (min) | 72.2±6.2 | 99.0±7.1 |
| 10 | 36.1±4.2 | 37.1±5.1 |
| 15 | 26.2±4.1 | 48.2±4.4 |
| 30 | 18.2±3.1 | 35.1±4.2 |
| 45 | 15.1±2.1 | 22.2±2.2 |
| 1(h) | 13.1±2.1 | 18.1±2.3 |
| 2 | 8.4±1.7 | 11.1±1.8 |
| 3 | 6.3± 1.5 | 7.3±1.5 |
| 4 | 4.1±1.3 | 4.8±1.3 |
| 6 | 1.8±0.51 | 2.5±0.61 |
| 9 | 0.51±0.11 | 0.60±0.04 |
| 12 | 0.12±0.06 | 0.2±0.01 |
| 18 | 0.02±0.01 | 0.08±0. 01 |

Table 2: Pharmacokinetic parameters (mean±SD) for two-compartment open model of thiamphenicol after a single intravenous (i.v) bolus of 20 mg $kg^{-1}$ body weight in camel and sheep (n=8 each)

| | Values | |
|---|---|---|
| Disposition parameters | Camel | Sheep |
| A ($\mu g\ mL^{-1}$) | 87.3±5.2 | 112.0±6.1 |
| $\alpha$ ($h^{-1}$) | 4.20±0.42 | 4.3±0.41 |
| $t_{1/2}\ \alpha$ (h) | 0.165±0.006 | 0.151±0.004 |
| B ($\mu g\ mL^{-1}$) | 22.1±1.2 | 30.1±2.1 |
| $\beta$ ($h^{-1}$) | 0.328±0.001 | 0.46±0.001 |
| $t_{1/2}\ \beta$ (h) | 2.11±0.31 | 1.5±0.32 |
| $Vd_{(area)}$ (L $kg^{-1}$) | 1.10±0.15 | 0.90±0.34 |
| $Vd_{ss}$ (L $kg^{-1}$) | 0.97±0.21 | 0.68±0.31 |
| $Cl_B$ (L/h/kg) | 0.318±0.03 | 0.36±0.004 |

A = Extrapolated zero time plasma drug concentration of the $\alpha$ phase, obtained by the method of residuals;
$\alpha$ and $\beta$ = distribution and elimination constant;
$t_{1/2}\ \alpha$ and $t_{1/2}\ \beta$ = distribution and elimination half-lives;
$V_{d\ (area)}$ = volume of drug distribution;
$Vd_{ss}$ = volume of drug distribution at steady state;
$Cl_B$ = total body clearance

Table 3: Haematological and plasma concentration of biochemical parameters in camel and sheep treated with thiamphenicol (mean±SD) intravenous bolus of 20 mg $kg^{-1}$ body weight (n=8 each)

| | Camel | | Sheep | |
|---|---|---|---|---|
| Parameter | Saline treated | TAP treated | Saline treated | TAP treated |
| Haematocrit | 0.31±0.03 | 0.29±0.02 | 0.33±0.03 | 0.32±0.02 |
| Total WBC ($10^6$ $mm^{-3}$) | 11.4±0.40 | 11.8±0.41 | 11.8±0.06 | 11.2±0.05 |
| Lymphocytes ($10^6$ $mm^{-3}$) | 6.10±0.30 | 6.1±0.30 | 6.1±0.20 | 5.7±0.40 |
| Neutrophils ($10^6$ $mm^{-3}$) | 5.30±0.20 | 4.9±0.31 | 5.1±0.20 | 5.1±0.30 |
| Eosinophils ($10^6$ $mm^{-3}$) | 0.41±0.02 | 0.42±0.02 | 0.42±0.03 | 0.46±0.02 |
| Monocytes ($10^6$ $mm^{-3}$) | 0.40±0.01 | 0.46±0.023 | 0.40±0.02 | 0.44±0.03 |
| RBC ($10^{12}$ $mm^{-3}$) | 8.3±0.20 | 8.1±0.60 | 8.3±0.50 | 8.6±0.40 |
| Hb (g $dL^{-1}$) | 12.6±0.20 | 12.3±0.40 | 12.6±0.60 | 12.1±0.50 |
| Total protein (g $dL^{-1}$) | 5.4±0.30 | 5.1±0.30 | 5.6±0.20 | 5.80±0.30 |
| Urea nitrogen (mg $dL^{-1}$) | 12.0±1.3 | 11.9±0.30 | 13.1±0.60 | 12.8±0.50 |
| Glucose (mg $dL^{-1}$) | 121.2±16.1 | 117.0±9.30 | 130.3±12.0 | 128.3±12.0 |
| Creatine (mg $dL^{-1}$) | 0.83±0.03 | 0.81±0.03 | 0.71±0.03 | 0.76±0.03 |
| Creatine kinase (IU $L^{-1}$) | 65.3±3.20 | 62.2±4.10 | 70.1±4.10 | 68.1±3.10 |
| Aspartate aminotransferase (IU $L^{-1}$) | 54.1±5.10 | 55.2± 3.60 | 62.1±3.10 | 66.3±4.10 |
| Lactic dehydrogenase (IU $L^{-1}$) | 142.0±12.8 | 145.0±14.0 | 150.3±13.0 | 155.1±14.0 |

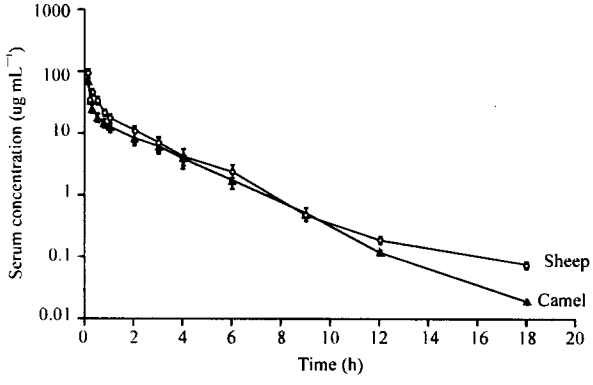


Fig. 1: Mean semi-log serum concentrations of thiamphenicol versus time following intravenous (i.v) administration of a single dose of 20 mg $kg^{-1}$ body weights to healthy camel and sheep. (n=8 each)

values ($p<0.05$) of half-life and volume of distribution, but lower values ($p<0.05$) of zero-time intercept for distribution and elimination phase and for total body clearance were shown for camel compared to sheep.

The protein binding percentage of TAP in camel serum was 11.1±0.60 and sheep serum was 10.2±0.50.

The indices of WBC, RBC, haematocrit and haemoglobin and the plasma concentration of metabolites and enzyme following the injection of TAP at a dose 40 mg $kg^{-1}$ body weight in camel and sheep remained within the expected normal ranges. Each sampling occasion compared with saline treated controls (Table 3).

## DISCUSSION

The disposition kinetics of thiamphenicol in camels and sheep showed a biexponential profile. Similar profile has been reported for human, goats, cattle, dogs and pigs[4-6,11-15]. The initial half-life of distribution ($t_{1/2}$ α) is similar to that of other species. However, longer half-life of elimination ($t_{1/2}$ β) was reported for camel (2.11 h) compared to sheep ( 1.5 h). Values in literature reported for other species included cattle (1.75 h) by Abdennebi *et al.*[13], sheep (1.02 h) by Lavy *et al.*[4], dogs (1.74 h) by Castells *et al.*[5] and pigs (1.39 h) by Haritova *et al.*[15], suggestive of faster elimination of antibiotic in most species compared to camel. Furthermore, TAP shows low protein binding in camel and sheep, about 10%, similar to other species. The drug is not metabolized and mainly excreted in its active form by renal glomerular filteration[16,17]. Under normal conditions the glomerular filtration rate and renal plasma flow expressed in relation to body weight are two to four times higher in sheep[18] than in camel[19]. These features may explain the relative longer half-life of TAP elimination and lower body clearance of TAP in camels compared to sheep. The volume of distribution of TAP in camel (0.9-1 L $kg^{-1}$) exceeded the volume of central compartment (0.410 L $kg^{-1}$) and total body water of the camel[20] suggesting extensive tissue penetration. This is expected because of the lipophilic properties of TAP.

Previous studies showed that the concentration of TAP in tissue was similar to that found in plasma[21]. The minimum effective concentration inhibiting bacterial growth (MIC) against most of thiamphenicol-sensitive micro-organisms have been determined to be approximately 0.5 μg $mL^{-1}$[22]. The data presented here suggest that when TAP is administered i.v. at 20 mg $kg^{-1}$ body weight, mean plasma concentrations higher than MIC values are obtained at 9 h post-injection.

There were no significant changes observed in haematological or plasma biochemical parameters suggesting that TAP given i.v. at a dose of 40 mg $kg^{-1}$

body weight is tolerable by camel and sheep. It is likely that the wide therapeutic index of TAP[23] would allow a considerable increase in drug dosage to achieve high tissue concentration. However, some side effects may not have been observed in this study as a result of administration of one dose of TAP.

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