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Pharmacokinetics and distribution of thiamphenicol in sheep given repeated intramuscular doses

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Summary — The pharmacokinetics of thiamphenicol and its distribution in various body fluids were studied after repeated intramuscular injection in clinically healthy adult Moroccan crossed Sardi-D'Man sheep. Thiamphenicol was rapidly absorbed from injection sites yielding peak plasma concentrations within 15–30 min. The elimination from the blood was also rapid, with a biological half-life of 1.51 ± 0.51 h. The distribution pattern of thiamphenicol revealed that this antibiotic can penetrate many remote sites of the body. With the exception of the cerebrospinal fluid, concentrations of the drug in other body fluids were higher than the corresponding plasma concentrations.

thiamphenicol / pharmacokinetics / distribution / sheep

Résumé — **Pharmacocinétique et distribution du thiamphénicol chez le mouton après administrations intramusculaires répétées.** *La pharmacocinétique et la distribution du thiamphénicol dans différents fluides biologiques ont été étudiées, après administrations intramusculaires répétées chez le mouton marocain de race croisée Sardi-D'Man. Les résultats obtenus montrent que le thiamphénicol a été rapidement absorbé au niveau du site d'injection avec des concentrations plasmatiques maximales obtenues entre 15 et 30 min. L'élimination du sang a été également rapide puisque la demi-vie d'élimination était de $1,51 \pm 0,51$ h. Le thiamphénicol a largement diffusé dans différents liquides de l'organisme. À l'exception du liquide céphalorachidien, les concentrations de thiamphénicol dans d'autres liquides corporels étaient toujours supérieures aux concentrations plasmatiques correspondantes.*

thiamphénicol / pharmacocinétique / distribution / mouton

* Correspondence and reprints

INTRODUCTION

In veterinary medicine, very stringent controls have been placed upon the use of chloramphenicol, especially in food-producing animals. The main reason is the risk of unexpected aplastic anemia in man, and anemia and cytopenia in animals, which have been associated with chloramphenicol treatment (Best, 1967; Wallerstein *et al*, 1969; Fraunfelder *et al*, 1982; Alavi, 1983; Settepani, 1984). It is hypothesized that this toxicity is due to the reduction of the *p*-nitro group of chloramphenicol to nitrosamine and hydroxylamine products (Murray and Yunis, 1981; Yunis, 1981). Other investigators suggested that marrow damage produced by chloramphenicol may be due to covalent binding of this drug to bone marrow cells (Krishna, 1974) or to its ability to inhibit DNA synthesis (Yunis *et al*, 1974). Therapeutic restrictions of this antibiotic led to investigation of possible substitutes. Thiamphenicol, the methylsulfonyl congener of chloramphenicol, is similar in antibacterial spectrum and clinical efficacy to chloramphenicol (Van Beers *et al*, 1975; Sutter and Finegold, 1976) and appears to be a possible candidate. This drug, lacking the nitro group, has never been reported to cause the devastating aplastic anemia in spite of its extensive use in humans (Yunis, 1988; Manyan *et al*, 1975). So far, studies have been completed regarding the pharmacokinetics of thiamphenicol in cattle and sheep after single intravenous and intramuscular injections (Ashraf, 1989; Abdennebi, 1991, 1994). The main objectives of this report are to study the pharmacokinetics of this drug and to investigate its distribution into various body fluids of sheep after repeated intramuscular injections.

MATERIALS AND METHODS

Animals

Twelve healthy adult Moroccan crossed Sardi-D'Man sheep were selected as homogeneously

as possible with regard to age (9–12 months), sex and weight (30–35 kg). The animals were identified, weighed and kept in a clean environment with a balanced ration and water *ad libitum*.

Drug administration and sampling

The animals were given 4 doses of 20 mg/kg of thiamphenicol (as the glycinate ester, Thiobactin ND, Clin Midy, France) by intramuscular injection at the gluteal muscles, 1 dose every 8 h. Blood samples were taken in heparinized tubes by jugular puncture at 10, 15 and 30 min and at 1, 2, 4, 6 and 8 h following the first drug administration and from 15 min to 8 h after the second and third drug injections. After blood sampling, plasma was collected by centrifugation and immediately frozen pending analysis. Two animals (1 male and 1 female) were slaughtered at 2, 6, 12, 24, 48 and 72 h after the last dose of thiamphenicol. Venous blood and other biological fluids such as bile, pericardial, peritoneal, synovial and cerebrospinal fluids were also sampled from each animal and stored in clean screw cap tubes. All the samples were immediately put in the freezer until analysis.

Thiamphenicol analysis

Thiamphenicol was analyzed by high-pressure liquid chromatography (HPLC) according to the method developed previously (Felice *et al*, 1988). Plasma standards were prepared by spiking pooled blank plasma with appropriate volumes of a freshly prepared working solution of thiamphenicol (Sigma Chemical Co, Saint Louis, USA) to achieve concentrations ranging from 0 to 40 µg/ml. Another set of thiamphenicol standards were prepared in water/methanol (70:30) for the determination of thiamphenicol concentration in the different biological fluids. *N*-Acetyl-*p*-aminophenol (Sigma Chemical Co, Saint Louis, USA) was used as an internal standard using a 500 µg/ml solution prepared in 10% methanol in water. The drug was extracted with ethyl acetate. Briefly, 1 ml of plasma, bile, cerebrospinal, synovial, pericardial or peritoneal fluid was extracted with 10 ml ethyl acetate in 16 x 25 mm screw-cap tubes by mixing on a rotary mixer for 15 min. The sample was centrifuged and the organic phase was transferred to another clean test tube.

The ethyl acetate was evaporated to dryness under a nitrogen stream in a water bath at 35–40°C. The residue was redissolved in 1 ml water/methanol (70:30) and 20 µl of the dissolved residue was injected into the HPLC apparatus. The apparatus used included a Beckman solvent-delivery system, a manual sample injection valve with a 20 µl loop, a variable wavelength detector set at 224 nm, and a calculator integrator. The separation was performed by reverse-phase chromatography using a C-18 column. The mobile phase was 30% methanol in water pumped at 1 ml/min. The thiamphenicol concentration in different body fluids was determined using standard curves by plotting drug concentrations against their corresponding HPLC thiamphenicol/internal standard peak-height ratios. With this method, extraction recoveries of thiamphenicol from plasma were near to 100% with coefficients of variation less than 3% in the range of 1 to 40 µg/ml. The minimum detectable concentration was 0.1 µg/ml.

Pharmacokinetic analysis

Pharmacokinetic analysis was performed for each data set issued from the first drug injection using a pharmacokinetic computer program (Sawchuk, personal communication). In each case, the best fitted model (one vs two-compartment) was

selected according to the Akaike's information criterion (Yamaoka *et al.*, 1978). This analysis allowed the calculation of different kinetic variables such as the constant of absorption (K_a), half-lives of absorption ($t_{1/2}(K_a)$) and elimination ($t_{1/2}(\beta)$), the area under the curve (AUC), the mean residence time (MRT), the maximum plasma concentration (C_{pmax}) and the time at which this concentration was achieved (t_{max}). Since the intravenous route was not used, the plasma clearance (Cl) and the volume of distribution (method of area) were calculated and reported as their true values divided by the bioavailability (F). A value of $V_{d(are)}$ was estimated using 87.5% as the mean value of thiamphenicol bioavailability, determined previously after intramuscular injection (Abdennebi *et al.*, 1994).

RESULTS

The mean plasma concentrations of the antibiotic in plasma after each drug administration are given in table I while selected kinetic parameters, calculated for plasma data obtained after the first drug injection, are presented in table II. In most of the cases, the best-fitted representation for thiamphenicol concentration vs time was obtained using the following equation:

Table I. Mean plasma concentrations of thiamphenicol after repeated intramuscular injection of 20 mg thiamphenicol per kg body weight every 8 h in sheep ($n = 12$).

Time (h)	Thiamphenicol plasma concentration (µg/ml)*		
	After 1st injection	After 2nd injection	After 3rd injection
0.16	8.17 ± 1.34	—	—
0.25	18.88 ± 4.98	22.37 ± 6.13	19.16 ± 5.55
0.50	19.58 ± 4.50	17.18 ± 2.48	19.18 ± 4.50
1	10.86 ± 1.42	9.59 ± 2.14	11.94 ± 2.27
2	6.16 ± 1.58	5.89 ± 1.85	5.90 ± 0.87
4	2.77 ± 1.03	2.04 ± 0.43	2.57 ± 0.95
6	0.99 ± 0.51	0.80 ± 0.44	0.96 ± 0.61
8	0.49 ± 0.34	0.28 ± 0.34	0.33 ± 0.35

* All values are given as the mean ± standard deviation.

Table II. Selected pharmacokinetic parameters calculated for the first intramuscular injection of 20 mg thiamphenicol/kg body weight in sheep ($n = 12$).

Kinetic parameter	Units	Mean \pm SD
Ka	h ⁻¹	11.09 \pm 5.00
t _{1/2} (Ka)	h	0.08 \pm 0.04
A	μ g/ml	30.9 \pm 3.16
α	h ⁻¹	3.38 \pm 1.74
B	μ g/ml	13.0 \pm 4.77
β	h ⁻¹	0.503 \pm 0.159
t _{1/2} (β)	h	1.51 \pm 0.51
Vd _{(area)/F}	l/kg	1.18 \pm 0.39
t _{max}	h	0.37 \pm 0.06
Cp _{max}	μ g/ml	19.98 \pm 3.92
Cl/F	l \cdot kg ⁻¹ \cdot h ⁻¹	0.54 \pm 0.09
MRT	h	2.05 \pm 0.51
AUC	μ g \cdot h/ml	37.68 \pm 5.78

$$C = A \exp(-\alpha t) + B \exp(-\beta t) - (A + B) \exp(-k_{at})$$

Thiamphenicol was rapidly absorbed after intramuscular administration giving rise to peak plasma concentrations of about 20 μ g/ml within the first 30 min. Following this short absorption phase, thiamphenicol plasma concentrations declined progressively with an elimination half-life of 1.51 \pm 0.51 h associated with a MRT of 2.05 \pm 0.51 h. Using 87.5% as the mean value of the intramuscular bioavailability of thiamphenicol (Abdennebi *et al*, 1994), the volume of distribution by area (Vd_(area)) was close to 1 l/kg.

In biological fluids, the mean concentrations of the antibiotic decreased over time to become undetectable after 24 h (fig 1). With the exception of the cerebrospinal fluid (CSF), concentrations of thiamphenicol were always higher than the corresponding plasma concentrations. The mean values from all body fluids ranged from 9.75–31.25

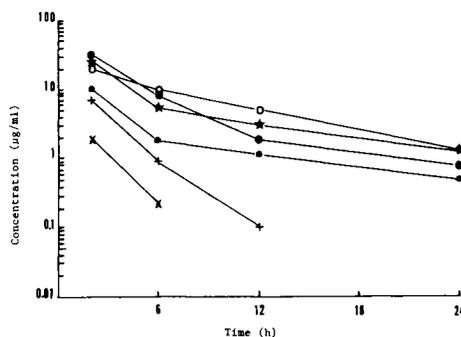


Fig 1. Semilogarithmic graph of mean thiamphenicol concentrations in plasma (+), bile (O), synovial (●), pericardial (★), peritoneal (•) and cerebrospinal (X) fluids of sheep following 4 doses of thiamphenicol (20 mg/kg every 8 h).

μ g/ml after 2 h to 0.5–1.25 μ g/ml at 24 h. However, in the CSF, the drug was detected only in the 2nd and 6th hour samples with CSF/plasma concentration ratios of 0.42 and 0.24, respectively. The concentrations of thiamphenicol were recorded, in decreasing order, in biliary, pericardial, synovial and peritoneal fluids followed by plasma and cerebrospinal fluid.

DISCUSSION

Pharmacokinetic analysis indicated that maximum drug concentrations occurred shortly after intramuscular injection. Such a phenomenon suggests the very rapid absorption of this antibiotic from the injection site. In previous studies, it was observed that the bioavailability of thiamphenicol glycinate after single intravenous and intramuscular administrations was 85% in veal calves (Ashraf, 1989) and 87.5% in sheep (Abdennebi *et al*, 1994). The rapid absorption of thiamphenicol associated with its high bioavailability indicates that the intramuscular route of administration may be preferred to the intravenous route. The plasma

half-life of about 1.5 h is similar to that observed when the drug was given intravenously either to sheep (Abdennebi *et al*, 1994) or to veal calves (Ashraf, 1989; Abdennebi, 1991).

Concerning the diffusion of thiamphenicol, the estimated $V_{d(\text{area})}$ (1 l/kg) is similar to that of chloramphenicol (Davis *et al*, 1972), and is greater than distribution volumes of other antibacterial agents currently used in veterinary medicine such as penicillin and aminoglycosides (Baggot, 1980). This suggests that thiamphenicol is more concentrated in extravascular tissues and, therefore, may provide sufficient concentrations at the site of infection.

The drug appears to be relatively concentrated in biliary, pericardial and synovial fluids. In contrast, thiamphenicol was weakly detected in the CSF. This observation agrees with that of Ashraf (1989), who studied thiamphenicol distribution in calves under steady-state conditions and Adams *et al* (1987) with florfenicol in the same species. Plomp *et al* (1981) reported concentrations of thiamphenicol in human CSF which were 5–10% of the corresponding serum concentrations, while Pfenninger *et al* (1977) found that concentrations of thiamphenicol in the CSF were 20% of those of the serum in healthy human subjects but may increase to 50% in patients with meningitis. The distribution pattern of thiamphenicol in other biological fluids has not been studied in animals, but this study suggests that the antibiotic can well penetrate synovial, pericardial and peritoneal fluids, at least. Minimum inhibitory concentrations (MIC) of thiamphenicol have been reported as 4 µg/ml or less for most strains of Gram-positive cocci, and 12.5 µg/ml or less for most strains of Gram-positive and Gram-negative bacilli (Laplassotte and Brunaud 1961; Van Beers *et al*, 1975; Plomp, 1979). These MIC levels were reached in most of the analyzed biological fluids. Consequently, it appears evident that the drug would be of significant

value against many infections located in various part of the body. Finally, it must be emphasized that the present data on thiamphenicol were collected in clinically healthy animals. The distribution pattern of the drug may be altered in the face of disease. Many investigators have demonstrated that significant pharmacokinetic changes may occur in diseased animals (Baggot, 1980; Ames *et al*, 1983).

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