

Pharmacokinetics of Chloramphenicol in Sheep after Intramuscular Administration

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This study was carried out to determine the pharmacokinetics of chloramphenicol in eight sheep injected intramuscularly with chloramphenicol sodium succinate (25 mg/kg body weight). The animals were bled at pre-determined time intervals and serum chloramphenicol concentrations monitored using chloramphenicol-ELISA for a period of 30 days post drug administration. Pharmacokinetic evaluation was carried out using a non-compartment analysis. The mean C_{max} values obtained in the eight sheep was 134 ± 34 $\mu\text{g/ml}$ and the time required to reach C_{max} (t_{max}) was 10 ± 0.05 minutes. The mean elimination half-life obtained was 36.37 ± 3.7 h and the mean residence time was 2.83 ± 0.27 h. These findings show that chloramphenicol was absorbed and distributed rapidly from the injection site. At two weeks post drug administration, the drug levels had declined to below the limit of detection of the assay (0.1 ng/ml).

Key words: Chloramphenicol, pharmacokinetics, sheep, ELISA

INTRODUCTION

Chloramphenicol (CAP) is a broad-spectrum antibiotic first isolated from *Streptomyces venezuelae* in 1947 [1-2]. It exerts its antibiotic action via inhibition of protein synthesis in several Gram-negative and Gram-positive bacteria. In veterinary medicine, the use of CAP in food producing animals has been banned because of its serious adverse effects including bone marrow suppression as well as hemolytic and aplastic anaemia [3]. Chloramphenicol toxicity is rare in animals with cats and young animals being the most susceptible [4].

Chloramphenicol induced bone marrow toxicity in humans may not only be caused by therapeutic use but also by ingestion of food products from animals which have been treated with the drug [5-6]. Although the use of CAP in food-producing animals is banned this study was designed to investigate the pharmacokinetics of CAP following current modes of administration. Highly sensitive methods were employed to predict how long after therapeutic use CAP

residues can be expected in edible animal products.

The pharmacokinetics of CAP have been studied in neonatal calves [7], cows [8], goats [9] and sheep [10]. However, these investigations were inconclusive due to the low sensitivity (detection limits of 10-500 ng/ml) of the methods employed. In the present study, a recently developed CAP enzyme-linked immunosorbent assay (ELISA), with a detection limit of 0.1 ng/ml, was used to study the pharmacokinetics of CAP in sheep.

MATERIALS AND METHODS

Experimental animals

Eight red Maasai sheep, all weaned males, weighing between 21 and 25 kg with ages ranging from 9 to 12 months were purchased from Kiserian area of Kenya and used for the experiments. They were housed in a flyproof barnhouse, fed on hay, commercial protein and mineral supplements. The sheep had free access

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to water. A three-week acclimatization period was allowed before the start of the experiments. During that time the sheep were treated for ectoparasites by spraying with 12.5 % w/v amitraz (Triatix, Coopers Animal Ltd, U.K.) and dewormed with albendazole (Norbrook Ltd, Nairobi, Kenya).

Administration of chloramphenicol

A freshly prepared 10 % w/v aqueous solution of CAP sodium succinate (Nabros Pharma, Ahmedabad, India) was administered at a dose of 25 mg/kg body weight by deep intramuscular injection into the right hind limb muscle of each of the experimental animals.

Sample collection and serum preparation

Ten milliliters of whole blood was aseptically collected from all the sheep by jugular venipuncture into plain vacutainer tubes (Becton-Dickinson, Sparks, MD, USA) before and after drug administration. The blood was left to clot at room temperature for 4 h followed by overnight storage at 4 °C. It was then centrifuged at 1200 g for 30 min and the serum separated and stored in 2 ml aliquots at -20 °C until required for analysis.

Blood samples were collected immediately after drug administration and after 5, 10, 15 and 30 min and 1, 2, 4, 6, 8, 12, 24 and 32 h. Thereafter, collection was done twice a day during the first week, daily during second week, on three days during the third week and on two days in the fourth week. The bleeding was strictly done at 8 am and 4 pm so as not to distort the pharmacokinetic parameters during modeling. Pre-treatment blood samples were collected one day before drug administration.

Drug Analysis

Chloramphenicol Enzyme-Linked Immunosorbent Assay (ELISA): Sera prepared from sheep following CAP administration were tested using the CAP-ELISA assay method as previously described [11]. The assay involved coating 96 well microtitre plates with anti-CAP antibody raised in camel and frozen at 620 °C.

The coated plates were then thawed and washed five times using a solution containing phosphate buffered saline and Tween 20 (PBST). Aliquots of 100 μ l of CAP conjugate (diluted in PBST) and the test sample were added to each of the 96 wells. The plates were stored at 4 °C overnight. After 12 h, any unbound materials were washed off the microtitre plates. Tetramethylbenzidine-hydrogen peroxide (TMB/H₂O₂) was added to serve as the chromogen-substrate system. The reaction was stopped using orthophosphoric acid and the optical densities (OD) read using an ELISA reader connected to a desktop computer.

The limit of detection (0.1 ng/ml) of the assay was taken as the concentration corresponding to the mean OD of 30 negative control sheep sera minus two standard deviations.

Pharmacokinetic evaluation

Non-compartment analysis was used for the pharmacokinetic analysis of CAP concentrations versus time data. This is the model that best described the experimental data. Pharmacokinetic parameters were calculated using the WinNonlin® version 1.1 software package (Scientific Consulting Inc., Cary, NC, USA). The pharmacokinetic parameters were determined using the formulae described by Gilbert and Perrier [12].

The elimination half-life was calculated using the equation

$$t_{1/2}\beta=0.693/\beta$$

Where β is the elimination rate constant.

The area under the CAP plasma concentration versus time curve extrapolated to infinite time, AUC_{0-∞} (ng h/ml), and the area under the moments curve, AUMC_{0-∞} (ng h²/ml), were estimated by the log-trapezoidal rule from the serum drug concentration versus time plots. The mean residence time (MRT) which is the time the drug spends in the body from administration to its final elimination was calculated as follows:

$$MRT=AUMC_{0-\infty}/AUC_{0-\infty}$$

Statistical analysis

Descriptive analysis of the duplicates of individual drug concentration and pharmacokinetic parameters were calculated using drug ELISA databases based on Microsoft Access and Microsoft Excel, respectively.

RESULTS

The mean serum CAP concentration versus time plot obtained following intramuscular administration of the drug in sheep is shown in figure 1. Seven days after drug administration, a mean serum CAP concentration of 0.26 ± 0.03

ng/ml was still detectable. However, by day 9 post-administration, serum drug concentrations were very close to the detection limit of 0.1 ng/ml in all the animals.

Table 1 gives the pharmacokinetic parameters obtained. A mean ($n=8$) peak serum CAP concentration (C_{max}) of 134 ± 34 $\mu\text{g/ml}$ (range 28.7-312.8 $\mu\text{g/ml}$) was obtained at time (t_{max}) 0.18 ± 0.05 h (range 0.08-0.50 h). The estimated mean apparent elimination half-life ($t_{1/2\beta}$) was 36.4 ± 3.66 h (range 17-49.9 h). The mean values for the $AUC_{0-\infty}$ and the $AUMC_{0-\infty}$ were estimated as 124.5 $\mu\text{gh/ml}$ and 357.3 $\mu\text{gh}^2/\text{ml}$, respectively, giving an $MRT_{0-\infty}$ of 2.83 ± 0.27 h.

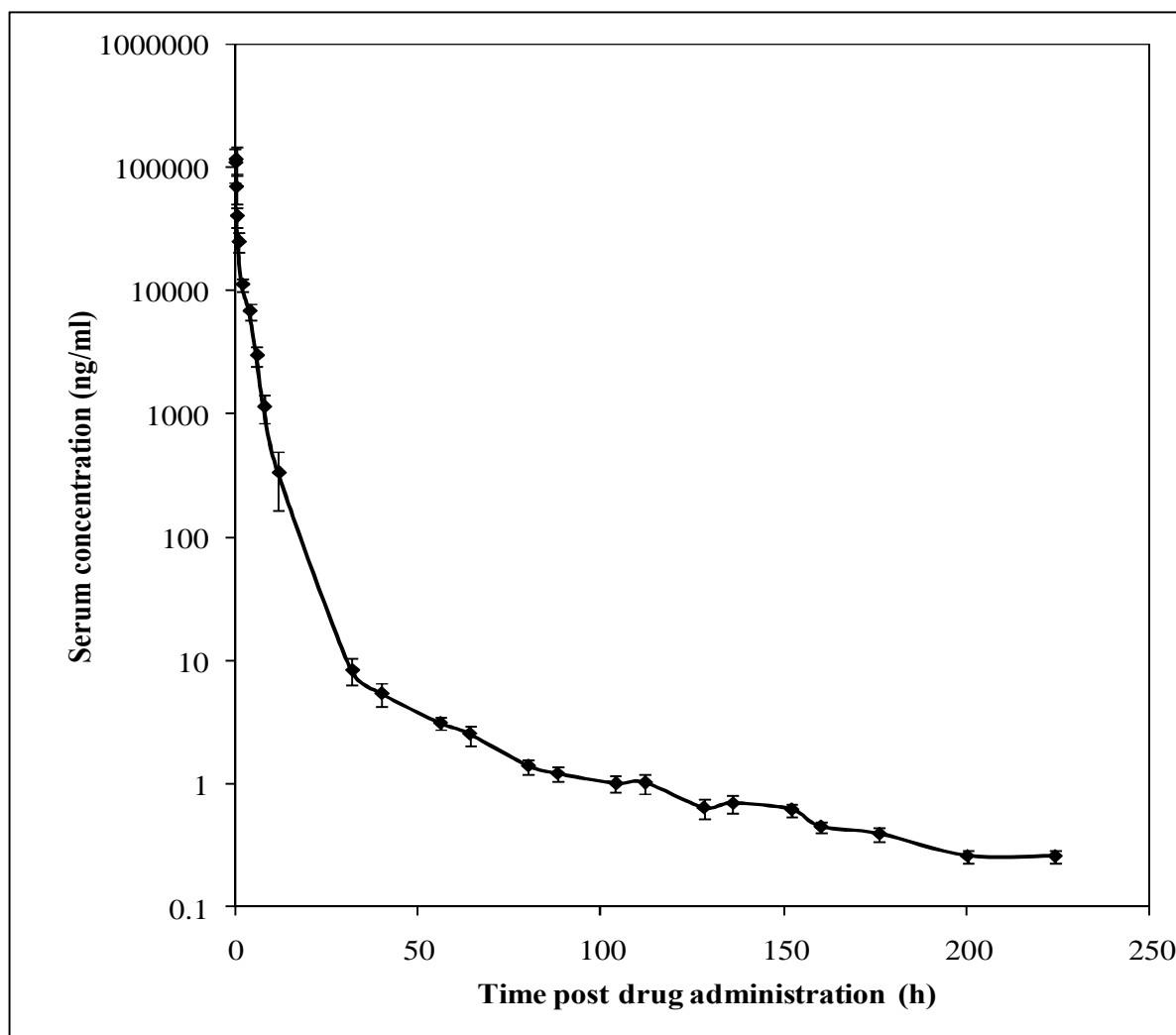


Fig. 1: Mean semi-log serum concentrations of chloramphenicol versus time following intramuscular administration of a single dose of 25 mg/kg body weight to healthy sheep ($n=8$).

Table 1: Individual pharmacokinetic parameters for sheep injected intramuscularly with 25 mg/kg body weight of CAP sodium succinate.

Parameter	Animal number								Mean±SE
	1	2	3	4	5	6	7	8	
R ²	1	0.89	1	0.98	0.99	0.88	0.82	0.96	0.94±0.02
t _{max}	0.16	0.08	0.08	0.16	0.5	0.08	0.16	0.25	0.18±0.05
C _{max}	86	29	45	133	40	313	221	207	134±34
t _{last}	248	200	272	272	344	272	296	272	272.00±13.42
C _{last}	0.05	0.23	0.15	0.16	0.02	0.22	0.02	0.17	0.13±0.03
AUC _{0-last} (nghml ⁻¹)	79.2	58.3	76.7	108.8	119.0	186.6	168	199	124.5±19
β	0.03	0.02	0.02	0.02	0.04	0.02	0.02	0.01	0.02±0.0
t _{1/2β} (h)	23.18	43.22	39.27	34.47	17.1	44.13	39.64	49.94	36.37±3.66
AUC _{0-∞} (observed) (μghml ⁻¹)	79	58	77	109	119	187	168	199	124.5±19
AUC _{0-∞} (predicted) (μghml ⁻¹)	79	58	76	109	119	187	168	199	124.5±19
AUMC _{0-last} (μgh ² ml ⁻¹)	143	176	182	275	485	390	651	536	356±67
AUMC _{0-∞} (observed) (μgh ² ml ⁻¹)	143	180	185	278	486	395	652	539	357±67
AUMC _{0-∞} (predicted) (μgh ² ml ⁻¹)	143	180	185	278	486	392	653	539	341±□7
MRT _{0-last} (h)	1.8	3.03	2.37	2.53	4.08	2.09	3.88	2.69	2.81±0.27
MRT _{0-∞} (observed) (h)	1.81	3.09	2.41	2.56	4.08	2.12	3.88	2.71	2.83±0.27
MRT _{0-∞} (predicted) (h)	1.81	3.1	2.41	2.56	4.08	2.1	3.88	2.71	2.83±0.27

DISCUSSION

The CAP ELISA used to study the pharmacokinetics of CAP in sheep serum as part of the assay validation has been described previously [11]. Following a 25 mg/kg intramuscular dose of CAP, the drug was absorbed rapidly from the injection site and by one week post drug administration the mean serum concentration was 0.26±0.03 ng/ml. Significant (p<0.05) individual differences were observed in the serum drug concentrations and pharmacokinetic parameters.

In the present study, the experimental sheep had peak drug concentrations of 134±34 μg/ml (27-313 μg/ml) attained at a t_{max} of 10 min (5 to 10 min) post drug administration indicating rapid drug absorption from the injection site. The

elimination half-life of CAP was 36 h indicating relatively rapid elimination of the drug from the system while the MRT was 2.83±0.27h. The short MRT values obtained could be attributed to the rapid attainment of high serum CAP concentrations, which are then maintained for a very short period.

A pharmacokinetic study of CAP in goats injected intramuscularly with a dose of 25 mg/kg bodyweight has been reported [10]. In this study the t_{max} was 5 min, the elimination half-life 3.63 h and the MRT 6 h. The elimination half-life and the MRT reported were shorter than those obtained in the present study. These differences could be attributed to the different analytical method used. An HPLC method, which involves long extraction procedures, thus reducing the percentage recovery and accuracy of results, was used in the previous study.

A pharmacokinetic study in sheep injected intramuscularly with a 25mg/kg bodyweight dose of CAP has been carried out [13] giving a t_{\max} of 2 ± 0.55 h, a C_{\max} of 15.57 ± 3.95 $\mu\text{g/ml}$, an elimination half life of 5.75 ± 1.2 h and an AUC of 146.67 ± 41.8 $\mu\text{g.h/ml}$. The authors reported a longer t_{\max} , a lower C_{\max} and a higher AUC compared to the parameters obtained in the present study. These differences in pharmacokinetic parameters could be due to the different dose rates and analytical method used. Unlike in the present study that employed CAP ELISA, an HPLC method was used.

CONCLUSION

The CAP ELISA developed in the present study can successfully be used to study pharmacokinetics in animals but a deconjugation step must be included in order to separate chloramphenicol from its major metabolites. A single 25 mg/kg body weight intramuscular dose of CAP to red Maasai sheep resulted in serum

levels of 0.0004 $\mu\text{g/ml}$ for up to seven days post drug administration and the maintenance of a therapeutic level of 6 $\mu\text{g/ml}$ (3.8-10.6 $\mu\text{g/ml}$) for up to 4 h post drug administration.

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