



Original Contribution

PHARMACOKINETICS OF DICLOXACILLIN SODIUM AFTER INTRAVENOUS AND INTRAMUSCULAR ADMINISTRATION TO SHEEP

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ABSTRACT

The disposition of dicloxacillin-sodium, given intravenous (i.v.) or intramuscular (i.m.) at 25 mg/kg body weight was studied in a total of six locally-bred sheep, aged between 2-3 years and weighing between 40 and 45 kg. Data were analyzed by compartmental and non-compartmental models. The intravenous and intramuscular serum concentration curves were best described by one-compartment pharmacokinetic open model. Mean elimination half-lives ($t_{1/2\beta}$) after i.v. and i.m. administration were 0.66 h and 1.90 h, respectively. The absorption of intramuscularly-administered sodium-dicloxacillin in sheep was fast: the maximum serum concentration ($C_{\max}$) ($9.81 \pm 0.86 \mu\text{g/ml}$) was reached relatively quicker ($t_{\max} = 0.39$ h). The bioavailability was 71.72 %.

Key words: Pharmacokinetics, dicloxacillin, sheep.

INTRODUCTION

Dicloxacillin is a narrow-spectrum isoxazolyl penicillin antibiotic used for the therapy of severe gram-positive aerobic bacterial infections (2; 12; 19; 21; 23). Although dicloxacillin has been partly used in veterinary medicine, few kinetic studies have been performed (9; 18; 20). Therefore essential pharmacokinetic data, necessary for development of rational dosage regimens, are still lacking.

The purpose of the present study was to describe the disposition kinetics of dicloxacillin after a single bolus i.v. and i.m. injection in sheep

MATERIALS AND METHODS

Animals

Six, 2-3 year old locally-bred sheep weighing between 40 and 45 kg were used. All the animals were in good health and had not received any drugs for at least 6 weeks. They

were kept indoors under uniform conditions and were fed twice daily with green fodder of the season (months of May and June) and the water was provided *ad libitum*. In all experiments, prior to drug administration, a control blood sample was collected. The animals were weighed the day before administration of sodium dicloxacillin.

Drug application

Before the experiment jugular flexible heparinized cannula (Venflon-2, Viggo AB, Sweden) was inserted in right *v. jugularis* following local anesthesia. Dicloxacillin sodium salt (generously supplied by Balkanpharma-Razgrad Co., Razgrad, Bulgaria) was dissolved *ex tempore* in sterile water for injection at a concentration that produced a 10 percent solution. Prior to drug administration 2 ml of blood was taken. Then the drug was injected as a bolus dose of 25 mg/kg body weight into the left *v. jugularis*. 2 ml blood samples were taken subsequently at 10, 20, 30 min and at 1, 2, 3, 4, 5, 6 and 8 h. After 3-week "washout" period the experiment was repeated using the same dose of dicloxacillin sodium but this time by i.m. injection into the neck muscles.

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Antibiotic assay

Blood samples were allowed to clot and then centrifuged at 1500g for 15 min. Serum was removed and frozen at -20° C. Assays were done within 1 day of sample collection.

Serum concentrations of dicloxacillin were determined microbiologically, using agar-well diffusion method (Bennett et al., 1966) and *Bacillus mycoides* HB₂ as test organism. Pure substance (kindly supplied by *Balkanpharma, Razgrad, Bulgaria*) diluted in blank sheep serum was used as reference standard. Plates were incubated at 37 °C for 18-20 h. Standard solutions were prepared at the same time as sample collection and stored at -20°C pending assay (within 24 h). Inhibition zones were read with digital callipers. Assay validation for dicloxacillin indicated a limit of quantification (LOQ) of 0.03 µg/ml, linearity (r^2 of 0.988), intra-assay coefficient of variation (CV) of 2.45 (n=12), and inter-assay CV of 3.64 (n=36). The recovery rate was higher than 93 % for the compound.

Pharmacokinetic analysis

Dicloxacillin serum concentration for each sheep was analyzed by means of versus time compartmental (1) and non-compartmental models (10) and by use a non-linear curve

fitting program. The serum curves obtained after i.v. administration were fitted at 5 sheep to the one-compartment open model by computer pharmacokinetic program TopFit® (version 2.0.) using Akaike's information criterion (AIC) and Schwartz' s test (Sc) (11; 22; 24). One sheep was fitted using a two-compartment open model. The area under the concentration-time curve ($AUC_{0 \rightarrow \infty}$) was calculated by trapezoidal rule from time 0 to the last sample time, and was extrapolated to infinity as the ratio between the last measured serum concentration and the terminal slope of the serum concentration versus time curve. The other pharmacokinetic parameters were obtained using classical equations (1; 10).

The mean residence time ($MRT_{i.m.}$) was determined by the equation: $MRT_{i.m.} = AUMC/AUC$ and the mean absorption time (MAT) - according to following equation $MAT = MRT_{i.m.} - MRT_{i.v.}$ (10). Bioavailability (F) was obtained as the ratio between the $AUC_{0 \rightarrow \infty}$ obtained after i.m. administration and $AUC_{0 \rightarrow \infty}$ after i.v. injection:

$$F = (AUC_{0 \rightarrow \infty i.m.} / AUC_{0 \rightarrow \infty i.v.}) \times 100$$

All values were expressed as the means for the six animals $\pm$ SEM.

Table 1. : Serum dicloxacillin levels (µg/ml) in sheep after single i.v. or i.m. administration of dicloxacillin sodium at a dose of 25 mg/kg of body weight (Mean $\pm$ SEM)

Time (h)	Dicloxacillin levels (µg/ml)	
	Intravenous administration	Intramuscular administration
0.17	45.18 $\pm$ 5.80	6.22 $\pm$ 0.89
0.33	30.95 $\pm$ 3.26	8.88 $\pm$ 1.26
0.50	22.36 $\pm$ 2.39	9.33 $\pm$ 1.02
1	15.44 $\pm$ 2.14	7.59 $\pm$ 0.84
2	5.22 $\pm$ 0.80	5.59 $\pm$ 0.51
3	1.52 $\pm$ 0.24	4.24 $\pm$ 0.36
4	0.57 $\pm$ 0.05	2.79 $\pm$ 0.29
5	0.35 $\pm$ 0.01	1.80 $\pm$ 0.25
6	< 0.13	1.13 $\pm$ 0.20
8	< 0.13	0.63 $\pm$ 0.12

RESULTS

After i.v. bolus dicloxacillin injection the mean serum levels of antibiotic were 45.18 $\pm$ 5.80 µg/ml at 0.17 h and decreased to 0.35 $\pm$ 0.01 µg/ml at 5 h. The results of the serum levels are found on **Table 1**. The data presented on **Table 1** show the means and standard errors for all sheep after i.v. and i.m. routes of administration. The concentration-

time profile of dicloxacillin in 6 sheep given 25 mg/kg intravenously is presented in **Figure 1**. The pharmacokinetic parameters calculated from these data are presented on **Table 2**. The data obtained after i.v. administration in 5 of the sheep best fitted to a one-compartment open model, and data in one sheep (№ 6) was fitted using a two-compartment open model. The mean elimination half-life ($t_{1/2\beta}$) of dicloxacillin after i.v. administration was

0.66±0.03 h. The apparent volume of distribution ($V_{d_{area}}$) was 623.44±90.54 ml/kg of body weight and the total body clearance (Cl_B) was 10.97±1.62 ml.kg/min. The area

under the serum concentrations-time curve ($AUC_{0 \rightarrow \infty}$) integrated to infinity was 41.17±5.17 $\mu\text{g.h/ml}$ (Table 3).

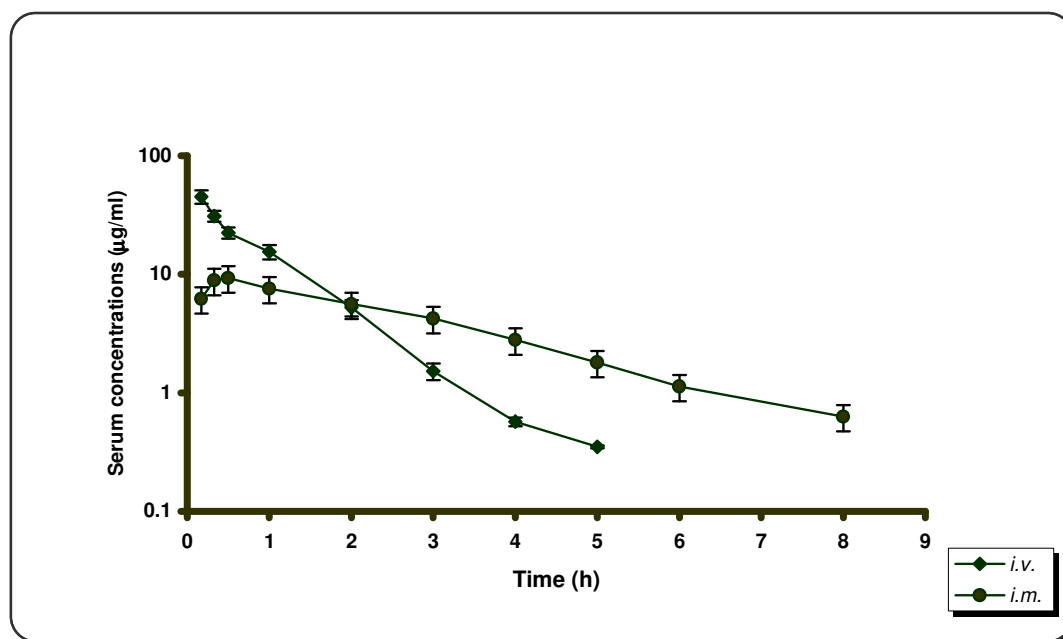


Figure 1. Serum concentrations of dicloxacillin after i.v. and i.m. administration of dicloxacillin sodium in sheep at a dose 25 mg/kg of body weight

When the antibiotic was administered i.m., the mean serum concentrations were 6.22±0.89 $\mu\text{g/ml}$ at 0.17 h. They increased to 9.33±1.02 $\mu\text{g/ml}$ at 0.5 h and gradually decreased to 0.63 ±0.12 $\mu\text{g/ml}$ at 8 hours (Table 1). The mean time to achieve peak serum concentrations of dicloxacillin (t_{max}) was 0.39±0.04 h and mean peak serum

concentration (C_{max}) was 9.81±0.86 $\mu\text{g/ml}$. The mean absorption time (MAT) after i.m. administration was 1.83±0.27 h, and $t_{1/2ab}$ was 1.27±0.19 h, respectively. The mean bioavailability (F) of the antibiotic following i.m. administration was relatively high ($F = 71.72 \pm 5.74 \%$) (Table 2).

Table 2. : Pharmacokinetic parameters (Mean±SEM) after i.v. bolus administration of dicloxacillin at a dose of 25 mg/kg of body weight in sheep (n=6)

Parameters	Units	S H E E P						Mean ±SEM
		1	2	3	4	5	6*	
B	$\mu\text{g/ml}$	24.45	60.74	53.62	37.51	38.81	34.32	41.58±5.94
β	h^{-1}	1.0917	1.1394	1.1717	1.0006	0.9678	0.9611	1.0554±0.04
$t_{1/2\beta}$	h	0.63	0.61	0.59	0.69	0.72	0.72	0.66±0.03
$V_{d_{(area)}}$	ml/kg	982.32	411.59	466.24	666.9	651.57	561.42	623.44±90.54
Cl_B	ml.kg/min	17.87	7.82	9.10	11.48	10.53	8.99	10.97±1.62
$AUC_{0 \rightarrow \infty}$	$\mu\text{g.h/ml}$	22.49	57.05	46.77	39.51	36.64	44.53	41.17±5.17

*The kinetic data for sheep 6 did not fit a one-compartment model. The data presented are for two-compartment model.

B - zero-time concentration intercept of the elimination curve; β - hybrid rate constant for the elimination phase; $t_{1/2\beta}$ - elimination phase half-life time; $V_{d_{(area)}}$ - volume of distribution; Cl_B - total body clearance; $AUC_{0 \rightarrow \infty}$ - area under the concentration vs. time curve.

DISCUSSION

As demonstrated in this study, the serum

concentration versus time curves fitted a one-compartment open model better than a two-

compartment model following i.v. injection of dicloxacillin in sheep. This coincides with the results reported in cats, dogs and cows (8; 9; 20).

In humans, this model was also most widely used for describing the pharmacokinetics of dicloxacillin after i.v. administration (4; 16).

Table 3: Pharmacokinetic parameters (Mean $\pm$ SEM) describing absorption and elimination of dicloxacillin i.m. administered (25 mg/kg of body weight) in sheep

Parameters	Units	S H E E P						Mean $\pm$ SEM
		1	2	3	4	5	6	
C _{max}	µg/ml	10.56	7.87	13.37	9.39	8.60	9.39	9.81 $\pm$ 0.86
t _{max}	h	0.50	0.50	0.33	0.33	0.33	0.33	0.39 $\pm$ 0.04
AUC _{0$\rightarrow$$\infty$}	µg.h/ml	20.99	34.91	35.35	24.29	28.47	27.41	28.57 $\pm$ 2.56
AUMC _{0$\rightarrow$$\infty$}	µg.h ² /ml	49.12	131.52	75.50	58.23	89.93	73.79	79.68 $\pm$ 13.02
MAT	h	1.42	2.88	1.28	1.47	2.15	1.78	1.83 $\pm$ 0.27
MRT _{i.m.}	h	2.34	3.77	2.14	2.40	3.16	2.69	2.75 $\pm$ 0.27
t _{1/2β}	h	1.62	2.61	1.48	1.66	2.19	1.86	1.90 $\pm$ 0.38
t _{1/2ab}	h	0.92	2.00	0.89	1.02	1.52	1.23	1.27 $\pm$ 0.19
F	%	93.00	61.00	75.58	61.48	77.70	61.55	71.72 $\pm$ 5.74

C_{max} – peak serum concentration; t_{max} – time to reach peak serum concentration; AUC_{0 $\rightarrow$ ∞} – area under the concentration vs. time curve; AUMC_{0 $\rightarrow$ ∞} – area under the first moment curve; MAT – mean absorption time; MRT – mean residence time; t_{1/2 β} – biological half-live; t_{1/2ab} – half-live of reabsorption; F – absolute bioavailability

The present study suggests that dicloxacillin sodium salt is rapidly distributed into the body. The volume of distribution after i.v. administration was comparable to that previously reported for lactating cows – 0.8 $\pm$ 0.22 l/kg body weight (20). This value probably was a consequence of the very high plasma protein binding of the antibiotic (78 % for cows; 92 % for dogs and 98,6 % for rabbits) (6; 9; 20). The elimination half-life after i.v. injection obtained in the present study was longer than that in cows (t_{1/2 β} = 10.1 $\pm$ 1.2 min) (20). Similarly, the terminal half-life values for the intramuscular route in this study were longer than the values (t_{1/2 β} = 8,2 $\pm$ 14,8 min) reported in (20) for lactating cows.

The minimum *in vitro* inhibitory concentration of dicloxacillin required for a large number of susceptible bacteria has been reported to be from 0.1 to 0.25 µg/ml of serum (5; 13; 14; 15; 17). The values of peak serum concentrations after its i.m. administration obtained in this study exceeded the MIC for non-penicillinase producer strains *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Streptococcus pneumoniae* and *Diplococcus pneumoniae* (2; 5; 7; 15; 17) usually by more than 20 and 10 times, respectively and the MIC for penicillinase-producer strains *Staphylococcus aureus* usually (2; 5; 7;) more than 10 and 5 times,

respectively.

Effective serum concentrations of dicloxacillin can be obtained after a single i.m. dose for 8 h for penicillinase-producing bacteria. In view of our findings, i.m. injection of dicloxacillin sodium salt at a dose 25 mg/kg body weight would be suitable for the systemic treatment of infections caused by both non-penicillinase and penicillinase-producing Gram-positive bacteria in sheep.

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