

BIOAVAILABILITY AND TISSUE ELIMINATION OF CHLORAMPHENICOL FOLLOWING PARENTERAL ADMINISTRATION IN THE PRERUMINANT CALF

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Résumé

BIODISPONIBILITÉ ET ÉLIMINATION TISSULAIRE DU CHLORAMPHÉNICOL APRÈS ADMINISTRATION PARENTALE CHEZ LE VEAU PRÉ-RUMINANT. — La biodisponibilité sérique d'une préparation à base de chloramphénicol dans du diméthylsulfoxyde et du glycérol formal a été déterminée chez le veau après injection intraveineuse (IV) et intramusculaire (IM) ainsi qu'après un rythme de traitement associant les deux voies d'administration. La posologie est de 30 mg/kg. Après l'application du traitement tel qu'il est généralement prescrit, la détermination des paramètres cinétiques et du taux résiduel tissulaire de chloramphénicol a été réalisée. Les taux sériques obtenus sont tels qu'une concentration thérapeutique de 5 µg/ml peut être maintenue durant plus de 12 heures après application par la voie IV et plus de 20 heures par la voie IM. La demi-vie biologique est de 6 heures et le volume de distribution d'environ 1,2 litre/kg. Aucun effet secondaire n'a été observé, ce qui démontre l'excellente tolérance de la préparation. Les taux résiduels tissulaires révèlent, tout d'abord, qu'il n'y a pas accumulation du produit dans un organe spécifique et, en second lieu, que le produit est rapidement éliminé après le traitement.

For economic and therapeutic purposes, chloramphenicol is a major antibiotic and is widely utilized in veterinary medicine.

The use of chloramphenicol in cattle has been substantiated in the publication of numerous pharmacological assays performed by either the parenteral route (Sisodia *et al* 1973, Pilloud 1973, De Corte-Baeten *et al* 1975, Archimbault *et al* 1980, Reiche *et al* 1980, Varma *et al* 1980 ab, Ziv *et al* 1982, Cole *et al* 1982, Burrows *et al* 1983 and 1984, Anderson *et al* 1983) or by the oral route (De Backer *et al* 1978 and 1979, Huffman *et al* 1981).

Research workers are all agreed that a minimal blood level of 5 µg/ml chloramphenicol is therapeutically effective against target microorganisms.

Accordingly, in adult cattle especially and at the usual dosage-levels prescribed, only local therapy can be achieved following oral administration. The compound is probably metabolized and/or degraded in the rumen. In young cattle with a monogastric structure of the digestive tract, an oral dose of 50 mg/kg can maintain blood levels higher than 5 µg/ml for 1 to 4 hours only (De Backer *et al* 1978 and 1979) with the exception, however, of some application during the post-natal period; but in this latter case, repeated doses result in severe adverse effects (Huffman *et al* 1981).

Therefore, in cattle, the interest of a parenteral utilization of chloramphenicol seems preponderant. With the dosage-level used in the last decades (10 mg/kg) it is not possible to reach and/or to potentially maintain therapeutic levels. The aim of this work in cattle was to improve the galenic forms, so as to achieve a few rational criteria in the field of the veterinary therapy based on chloramphenicol:

- potentially effective dosage-level on the base of 5 µg/ml;
- compatible treatment schedule with breeding practice;
- choice of one or more target species for which chloramphenicol is definitely important;
- within the same species, the pharmacological differences observed should be considered;
- equivalent bioavailability between the intravenous and intramuscular routes.

Accordingly, we had to study the blood bioavailability after intravenous and intramuscular administration, and the tissue elimination of this antibiotic following single and/or repeated applications of a chloramphenicol preparation.

Materials and Methods

1 — Animals

A total number of 22 calves of Montbeliard x Abondance crossbreed were used in the experiment, the animals were 5 to 9 week-old at the various times of treatment. They were divided into 2 groups of 6 calves (bioavailability) and a group of 10 calves (tissue residues). The calves were kept in the cow-shed and were exclusively fed with a milk replacer. Their approximate body weight ranged from 55 to 80 kg.

2 — Product

The chloramphenicol base was administered in the form of a 30 g/100 ml solution in dimethylsulfoxide and glycerol formal (Cloralon ND, Gifavet 06516 Carros).

3 — Experimental groups

The first group (n : 6) received a single dose of chloramphenicol of 30 mg/kg by the intravenous route, then, two weeks later the same dose was injected by the intramuscular route. The slow intravenous injection was made in the jugular vein. The intramuscular injection was performed in the neck muscle (in two injection sites at least).

The second group (n : 6) received the following treatment : one intravenous injection of 30 mg/kg followed by one intramuscular injection 12 hours later, then two intramuscular doses at 24 hour intervals.

The third group (n : 10) received the same treatment applied to the second group.

The two first groups served to collect the blood samples required for the bioavailability study, while the third group was used for the tissue residue study only.

Blood samples were taken with dry Venoject^R (Terumo, Polylabo, 67023 Strasbourg Cedex) by puncture in the jugular vein. They were allowed to clot at room temperature. Serum was collected by centrifuga-

tion of clotted blood and was immediately frozen pending analysis.

Tissue samples (liver, kidney, perirenal fat, thigh muscle, muscle in the injection site) were collected at slaughter, immediately frozen and stored pending analysis.

Blood samples were obtained at the following times after administration :

IV : 0, 10, 20, 30, 45 min and 1, 1.5, 2, 3, 4, 5, 7, 9, 12 and 24 hours.

IM : 0, 0.5, 1, 1.5, 2, 3, 4, 5, 7, 9, 12 and 24 hours.

IV + IM : 0, 1, 4, 6, 8, 10, 12 (IM administration), 13, 24, 28, 36 (IM administration), 48, 52, 60 (IM administration), 72 and 84 hours.

Tissue samples were taken at 12 hours (D0.5), 3 days (D3), and 8 days (D8) after the last administration (IV then, 3 IM) on three calves at each time.

4 — Assay of chloramphenicol

Serum samples were analysed according to a conventional microbiological method using *Sarcina lutea* ATCC 9341 as the test organism, medium 11 (FDA) as the culture medium, and the agar plate diffusion method (Arret *et al* 1971). The detection limit of the method was 1.7 µg/ml.

Tissues were analysed after extraction by the high performance liquid chromatography method (HPLC) described by Bories *et al* (1983). The apparatus used included a dual pump high-performance liquid chromatography system with controller (Waters 510 and 680), an injector (Rheodyne^R 7125) with 50 µl loop, a variable wavelength detector (Waters 481) set at 280 nm, and a calculator-integrator (SP 4270).

The analysis was performed by reverse phase chromatography using a C18 5 µm (Nova-Pak^R, Waters) and a C18 pre-column. The mobile phase was a mixture of water and methanol (55:45) with 0.5 ml/min flow rate. The detection limit of the method was lower than 0.002 µg chloramphenicol per gramme of tissue.

Table 1. — Serum chloramphenicol concentrations in calves following intravenous administrations of 30 mg/ kg

Time (h)	Chloramphenicol (µg/ml)						Mean	SD
	Calf Number							
	1	2	3	4	5	6		
0	0	0	0	0	0	0	0	0
0.17	31.5	53.7	36.9	36.9	37.9	35.9	38.8	7.6
0.34	27.5	41.2	49.5	32.3	33.2	29.8	35.6	8.3
0.50	25.4	35.9	35.0	29.8	28.3	26.1	30.1	4.5
0.75	22.8	33.2	29.0	27.5	26.1	23.5	27.0	3.8
1.00	22.2	26.1	26.1	24.4	24.7	20.5	24.0	2.2
1.50	19.4	27.5	24.7	19.4	22.2	20.0	22.2	3.3
2.00	17.5	19.4	22.2	19.4	22.2	16.6	19.6	2.3
3.00	14.5	17.0	19.4	17.5	19.4	15.7	17.3	2.0
4.00	13.7	14.5	14.9	17.0	17.5	13.4	15.2	1.7
5.00	12.7	14.5	14.5	13.0	15.3	10.2	13.4	1.8
7.00	10.0	10.0	11.7	11.4	11.7	7.6	10.4	1.8
9.00	8.3	8.1	9.0	10.2	10.5	6.1	8.7	1.6
12.00	5.9	6.1	6.3	7.6	7.6	5.0	6.4	1.0
24.00	<1.7	<1.7	<1.7	<1.7	<1.9	<1.7	<1.7	<1.7

5 — Pharmacokinetic analysis

It was performed on the serum concentrations obtained after IV administration. The parameters were calculated by fitting data to nonlinear models (PC NONLIN).

The bioavailability of chloramphenicol administered by the IM route (defined by $F =$ the absorbed fraction) was obtained by the ratio of the areas under the curves ($AUC\ 0 \rightarrow \infty$) between the IV and IM routes.

Results

After IV administration of chloramphenicol at a dosage level of 30 mg/kg, the serum concentrations obtained appear in table 1.

The parameters calculated according to a two-compartment open model are shown in table 2 (values have been determined from the mean curve). The persistence of serum levels higher than 5 µg/ml was obtained for over 12 hours.

Following IM administration at a dosage-level of 30 mg/kg, the serum levels obtained are shown in table 3. With this formulation, it appears that levels higher or equal to 5 µg/ml were reached approximately one hour after the IM injection. The theoretical peak serum of 11.0 µg/ml was achieved after a mean time of 6 hours.

In fact, the presence of a level plateau of 10 µg/ml was observed between 5 to 12 hours following treatment. Also, serum concentrations higher than 5 µg/ml were sustained for a period of 20 hours after the injection.

The bioavailability of the intramuscular form was calculated by comparison of the mean areas under the curves obtained by the IV and IM routes. This $F \sim 1$ ratio is significant of some bioavailability equivalence between the IV and IM applications of the 30 g/100 ml chloramphenicol formulation.

Repeated administrations according to the following treatment-schedule of one IV then three

IM injections resulted in the serum levels shown in table 4. It appears that the concentrations measured were always higher than 5 µg/ml at the time of the following injection. This treatment-schedule makes it possible to provide the animal with a 84-hour therapeutic protection.

The study on chloramphenicol tissue elimination was performed after application of the sche-

Table 2. — Pharmacokinetic parameters in the calf of about 5 weeks of age

Parameters	Values
A	21.71 µg/ml
B	24.21 µg/ml
α	1.983 h ⁻¹
t1/2 (α)	0.349 h
β	0.115 h ⁻¹
t1/2 (β)	6.003 h ⁻¹
K12	0.791 h ⁻¹
K21	1.100 h ⁻¹
K10	0.208 h ⁻¹
t1/2 (Kel)	3.330 h ⁻¹
Vc	39.2 l
Vd <i>extrapol.</i>	1.239 l/kg
AUC 0 — ∞	220.6 (µg/ml)×h

A : interception of distribution slope α with ordinate
 B : interception of back-extrapolated monoexponential elimination slope β with ordinate
 α : distribution half-life
 t1/2 α : distribution half-life
 β : overall elimination slope
 t1/2 β : elimination half-life
 K12 : distribution rate constant for transfer from central to peripheral compartment
 K21 : distribution rate constant for transfer from peripheral to central compartment
 K10 : elimination rate constant from central compartment
 T1/2 (Kel) : elimination half-life
 Vc : volume of central compartment
 Vd *extrapol.* : volume of distribution
 AUC 0 — ∞ : area under the curve

Table 3. — Serum chloramphenicol concentrations in calves following intramuscular administrations of 30 mg/kg

Time (h)	Chloramphenicol (µg/ml)						Mean	SD
	Calf Number							
	1	2	3	4	5	6		
0	0	0	0	0	0	0	0	0
0.50	3.8	3.9	6.4	5.6	2.6	4.5	4.5	1.5
1.00	4.7	4.9	7.4	5.3	3.5	5.1	5.2	1.3
1.50	6.4	6.4	8.2	8.0	4.2	6.1	6.6	1.5
2.00	5.9	6.2	9.2	11.5	5.6	9.2	7.9	2.4
3.00	9.7	10.9	11.8	11.1	6.2	9.7	9.9	2.0
4.00	8.9	7.8	10.6	9.7	5.7	10.3	8.8	1.8
5.00	11.1	10.9	10.9	16.0	6.1	10.6	10.9	3.1
7.00	9.2	9.2	10.0	15.6	7.8	10.3	10.4	2.7
9.00	8.7	7.8	11.5	17.4	7.4	11.8	10.8	3.7
12.00	10.2	10.3	10.9	14.7	7.0	8.2	10.3	2.7
24.00	2.8	2.3	1.9	3.4	5.9	1.7	3.0	1.6

Table 4. – Serum chloramphenicol concentrations in calves following IV and IM administrations of 30 mg/kg

Time (h)	Chloramphenicol (µg/ml)						Mean	SD
	Calf Number							
	1	2	3	4	5	6		
0	0	0	0	0	0	0	0	0
<i>Intravenous administration</i>								
1.0	28.1	23.4	22.5	20.4	28.7	20.8	23.9	3.7
4.0	20.0	17.0	17.7	14.2	16.3	16.0	16.9	1.9
6.0	18.9	15.1	15.4	12.3	14.2	12.3	14.7	2.5
8.0	13.3	10.7	9.3	7.6	10.1	10.1	10.2	1.9
10.0	10.1	8.8	9.7	6.5	10.6	10.0	9.3	1.5
12.0	9.4	7.2	7.4	5.5	8.8	7.9	7.7	1.4
<i>Intramuscular administration</i>								
13.0	13.3	12.9	11.9	11.7	12.9	12.5	12.5	0.6
24.0	11.3	12.3	10.5	7.0	13.1	11.7	11.0	2.1
28.0	10.3	9.7	9.4	5.9	11.0	9.8	9.3	1.8
36.0	6.8	5.9	6.2	4.5	7.6	7.6	6.4	1.2
<i>Intramuscular administration</i>								
48.0	10.2	10.8	7.2	8.6	9.4	9.2	1.4
52.0	8.9	7.9	6.1	8.2	7.2	7.7	1.1
60.0	6.5	4.1	4.9	6.5	5.4	5.5	1.0
<i>Intramuscular administration</i>								
72.0	10.3	5.3	6.9	6.3	6.1	10.3	7.5	2.2
76.0	8.8	4.7	5.4	4.9	5.4	9.1	6.4	2.0
84.0	6.5	3.8	4.3	4.3	5.6	8.2	5.4	1.7

Tableau 5. – Tissue concentration levels in calves after IV and 3 IM administrations of 30 mg/kg

Sample	Withdrawal time			
	control	D0.5	D3	D8
	(chloramphenicol, µg/g)			
<i>Muscle (injection site)</i>				
	0	13500	0	0.014
		690	0	0.002
		1410	0.009	0
<i>Muscle (near injection site)</i>				
	0	125.0	0	0
		44.7	0	0
		91.0	0.008	0
<i>Thigh muscle</i>				
	0	22.8	0	0.014
		16.6	0	0
		26.0	0.032	0
<i>Liver</i>				
	0	0.54	0.002	0
		0.25	0	0
		0.38	0	0
<i>Kidneys</i>				
	0	0.51	0	0
		0.07	0	0
		0.69	0	0
<i>Fat</i>				
	0	3.57	0.002	0
		0.95	0	0
		2.99	0	0

duled injections such as it has been described above. The values of the tissue concentrations assayed after the last injections are given in table 5.

The persistence of chloramphenicol was found primarily in the samples concerning the intramuscular injection site. Impregnation of the other tissues was low, with the exception of the thigh muscles. However, elimination was rapid, since three days after withdrawal of the treatment, the levels evaluated were either lower or approached the detection limit of the HPLC method used. It should also be underlined that at time D3, due to the absence of tissue necrosis, the injection site was difficult to locate exactly.

Discussion

The pharmacokinetic studies undertaken in calves, in the first few weeks of life, show the evidence of a different metabolic picture for chloramphenicol compared with that of adult cattle.

Reiche *et al* (1980), indicate that the elimination behaviour in the 10 to 12 week-old calf is the same as that observed for the adult animal. Burrows *et al* (1983) confirm the previous results. Therefore, it seems possible, at a time when economically the risk potentialities for the animal's life are high, to adopt a sensible therapy involving the use of chloramphenicol in the calf.

However, on the galenic level, it seems necessary to produce a preparation in which the vehicle drug is not propylene glycol. Indeed, the risk of intolerance — which is sometimes severe — for this excipient is well known (Burrows *et al* 1984), especially by the intravenous route.

The present vehicle utilized enables us to obtain the proper 30 % solubilization (W/V) of chloram-

phenicol and also the galenic stability of the preparation for both IM and IV administrations.

The results of the bioavailability studies show that in 5 to 9-week old calves, the injection of the preparation at a dose of 30 mg/kg by the intravenous route makes it possible to maintain serum levels of chloramphenicol higher than 5 µg/ml for more than 12 hours. Repeated intramuscular injections every 24 hours result in no accumulation of chloramphenicol of chloramphenicol in the blood. The evaluation of the pharmacokinetic parameters following IV administration agrees with that established by Burrows *et al* (1983).

The bioavailability of the preparation reaches 100 % by the intramuscular route. The tolerance in the muscle mass at the injection site seems quite satisfactory in the absence of necrosis more than 12 hours after interruption of the treatment since no traces can be found indicating the exact location of any intramuscular deposit site of the chloramphenicol solution.

The results of the tissue residue study are significant not only because of the absence of storage of the compound in edible tissues but also of the rapid rate of elimination of chloramphenicol. The bioavailability of the preparation, reliability of the HPLC method, and detection limit (2 parts per billion), enable us to propose a withdrawal time preventing all risks of contamination for the edible product intended for human consumption and for which the economic incidence is quite negligible (Milhaud 1985).

In conclusion, the preparation studied here and containing 30 g/100 ml chloramphenicol in an organic excipient, is of special therapeutic interest in the calf during the first few weeks of life. Therefore, this establishes the rationalised use of a broad spectrum antibiotic such as chloramphenicol in a target species.

Summary

The serum bioavailability of a preparation based on chloramphenicol in dimethylsulfoxide and glycerol formal was determined after intravenous (IV) and intramuscular (IM) injection, and a treatment schedule combining both routes of administration in calves. The dosage-level applied was 30 mg/kg. Pharmacokinetic parameters after administration were determined and a residue study was performed for chloramphenicol in the tissues after the treatment such as it is normally prescribed. Serum concentrations obtained were such that the potential therapeutic level of 5 µg/ml was maintained for more than 12 hours after IV administration and for more than 20 hours after IM application. The biological half-life was 6 hours and the volume of distribution was approximately 1.2 l/kg. No adverse reactions were observed, thus, demonstrating the perfect tolerance of the preparation. The tissue residue evaluation shows that, first, the compound does not accumulate in any organ in particular and, secondly, that it is rapidly eliminated after the treatment.

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