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Subcutaneous bioavailability of levamisole in goats

A. M. SAHAGÚN*
J. J. GARCÍA
M. SIERRA
N. FERNÁNDEZ
M. J. DIEZ &
M. T. TERÁN

*Department of Pharmacology, Toxicology and Nursing, Veterinary Faculty, University of León, Campus de Vegazana s/n, 24071 León, Spain; *Correspondence*

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Levamisole (*l*-(2,3,5,6-tetrahydro-6-phenyl-imidazo-[2,1-b]-thiazole) is a broad-spectrum anthelmintic active against gastrointestinal and pulmonary nematodes (Thienpont *et al.*, 1966; Walley, 1966; Janssen, 1976) widely used in veterinary medicine. It also has an immunomodulating effect (Renoux & Renoux, 1977; Symoens & Rosenthal, 1977).

The pharmacokinetic behaviour of levamisole is well documented in several animal species and in man (Bogan *et al.*, 1982; Luyckx *et al.*, 1982; Kouassi *et al.*, 1986; Watson *et al.*, 1988; McKellar *et al.*, 1991; Garcia *et al.*, 1992, 1994; Fernandez *et al.*, 1997), but only limited data are available in goats (Galtier *et al.*, 1981; Nielsen & Rasmussen, 1983). The therapeutic usefulness of levamisole in goats relates in part to its disposition and the purpose of this study was to establish the bioavailability and pharmacokinetic parameters of levamisole following intravenous (i.v.) and subcutaneous (s.c.) administration to goats.

Eight healthy male crossbred goats (aged 6 months, weighing between 15 and 21 kg) were used. Goats were acclimatised for 15 days before the experiments and were maintained indoors on a diet of hay and pelleted feed concentrate, with water available *ad libitum*. The protocol followed in this experiment was approved in advance by the Institutional Animal Care and Use Committee of the University of León.

Levamisole HCl (Sigma Chemical Company, St. Louis, MO, USA) was dissolved in 1 mL of sterile water and administered by i.v. (left jugular vein) and s.c. (under the skin in the thoracic region) injection at 7.5 mg/kg to the same animals with a 1-week washout interval between experiments. Blood samples (5 mL) were collected into heparinized vacuum tubes (Venoject, Terumo Europe, Leuven, Belgium) by jugular venipuncture just before drug administration and at 5, 10, 20, 30, 60, 90, 120, 150, 180, 240, 300, 360 and 480 min after i.v. dosing, and before and at 5, 10, 15, 20, 25, 30, 45, 60, 90, 120, 150, 180, 210, 240, 300 and 360 min following s.c. administration. After centrifugation, plasma was separated immediately and frozen at −20 °C until analysed.

Levamisole concentration was determined in plasma by high-performance liquid chromatography (Garcia *et al.*, 1990) with UV detection (213 nm). The lowest quantifiable concentration of levamisole in plasma was 0.016 μg/mL, with an accuracy of 100.3 ± 3.34% and a coefficient of variation of 4.52 ± 1.21%. The extraction recovery of levamisole from plasma was 82.8 ± 6.12%.

Pharmacokinetic analyses were performed for each goat by both compartmental and non-compartmental methods. Compartmental analysis was performed using the nonlinear regression analysis program PCNONLIN (Metzler & Weiner, 1989), with a weighting factor of $1/C$. The initial estimates of the parameters were determined by JANA (Dunne, 1985). Selection of the appropriate compartment was based on Akaike's criterion (Yamaoka *et al.*, 1978a) and graphical analysis of weighted residuals. The other compartmental parameters were calculated by standard methods (Gibaldi & Perrier, 1982).

Non-compartmental parameters were calculated using expressions based on statistical moments theory (Yamaoka *et al.*, 1978b; Gibaldi & Perrier, 1982). The plasma elimination rate constant (λ) was estimated by least squares regression of the logarithm of the plasma concentration vs. time over the terminal elimination phase, and maximum plasma concentration (C_{max}) and time of C_{max} (t_{max}) were determined by direct observation of the data. The area under the plasma concentration–time curve (AUC) and the area under the moment curve ($AUMC$) were calculated by the linear trapezoidal rule with extrapolation to infinity. The mean residence time (MRT) was defined as $AUMC/AUC$, and the mean absorption time (MAT) as $MRT_{s.c.} - MRT_{i.v.}$. Total body clearance (Cl) was determined by dividing the dose by AUC, the volume of distribution at steady-state (V_{ss}) from the equation $V_{ss} = MRT \times Cl$, and the apparent volume of distribution (V_a) as Cl/λ. Subcutaneous absorbed fraction (F) was calculated as $F = AUC_{s.c.}/AUC_{i.v.} \times 100$.

All pharmacokinetic parameters were calculated for each animal and values are reported as mean ± standard deviation (mean ± SD). Differences between compartmental and non-com-

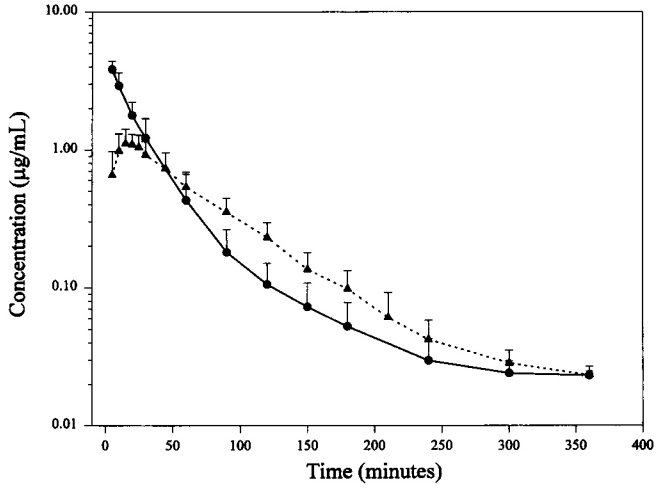

Fig. 1. Levamisole mean plasma concentrations in goats after intravenous (●) and subcutaneous (▲) administration of 7.5 mg/kg.

partmental data were determined by the paired *t* test ($P \leq 0.05$).

Adverse effects (hyperexcitability, deep respiratory movements, sialosis and tremors) were observed only after i.v. administration and disappeared after 3–4 min.

Plasma concentration vs. time curves after i.v. and s.c. routes were best fitted to a two-compartment open model. Plasma levamisole concentration–time profiles following i.v. and s.c. routes are shown in Fig. 1. Compartmental and non-compartmental pharmacokinetic parameters after i.v. administration are shown in Table 1 and s.c. data are listed in Table 2.

After i.v. administration, V_{ss} was 2.75 L/kg and *MRT* 42.82 min. Statistical analysis revealed significant differences for *AUC* and *Cl* between compartmental and non-compartmental values.

After s.c. injection, levamisole showed a fast rate and a high extent of absorption, with an absorption rate constant (k_a) of 0.1095 min^{-1}, a C_{max} of 1.168 μg/mL reached at t_{max} of 18.75 min, and *F* of 77.83%. The paired *t* test showed significant differences for *F* calculated using compartmental and non-compartmental analysis.

The finding of a two-compartment open model was similar to those observed in goats and pigs (Nielsen & Rasmussen, 1983), rabbits (Garcia *et al.*, 1992) and sheep (Fernandez *et al.*, 1997) after i.v. administration, and in goats (Galtier *et al.*, 1981), rabbits (Garcia *et al.*, 1994) and ewes (Galtier *et al.*, 1981) following s.c. administration. In dogs, levamisole followed a one-compartment model after i.v. administration (Watson *et al.*, 1988).

The high V_{ss} obtained after i.v. administration show that levamisole has a wide distribution within the body. On the other hand, k_{12}/k_{21} (transfer rate constants for drug distribution between central and peripheral compartments and vice versa, respectively), k_{12}/k_{10} (k_{10} = elimination rate constant of drug from central compartment) and V_c/V_p (apparent volumes of distribution in the central and peripheral compartments, respectively) values indicate that even with ready access to the peripheral compartment, it is not appreciably retained in this compartment. High values of V_{ss} have also been found in pigs and goats (Nielsen & Rasmussen, 1983), sheep (Fernandez *et al.*, 1997), dogs (Watson *et al.*, 1988) and rabbits (Garcia *et al.*, 1992).

Table 1. Pharmacokinetic parameters (mean ± SD) obtained by compartmental and non-compartmental analysis in goats after intravenous administration of 7.5 mg/kg levamisole

Compartmental parameters		Non-compartmental parameters	
A (μg/mL)	4.621 ± 0.705	λ (min^{-1})	0.0091 ± 0.0023
B (μg/mL)	0.281 ± 0.174	*AUC* (μg·min/mL)*	113.78 ± 30.85
α (min^{-1})	0.0555 ± 0.0160	*AUMC* (μg·min^2/mL)	4949.0 ± 1841.1
β (min^{-1})	0.0091 ± 0.0017	*MRT* (min)	42.82 ± 8.69
k_{12} (min^{-1})	0.0094 ± 0.0065	*Cl* (mL/kg·min)*	70.66 ± 20.45
k_{21} (min^{-1})	0.0118 ± 0.0035	V_{ss} (L/kg)	2.972 ± 0.878
k_{10} (min^{-1})	0.0433 ± 0.0121	V_a (L/kg)	8.140 ± 2.876
AUC (μg·min/mL)	119.2 ± 30.65		
Cl (mL/kg·min)	66.85 ± 17.77		
V_c (L/kg)	1.556 ± 0.209		
V_p (L/kg)	1.190 ± 0.597		
V_{ss} (L/kg)	2.746 ± 0.733		
V_a (L/kg)	7.536 ± 1.983		
$t_{1/2\alpha}$ (min)	13.36 ± 3.462		
$t_{1/2\beta}$ (min)	79.38 ± 16.93		
$t_{1/2k10}$ (min)	16.92 ± 3.770		
C_0 (μg/mL)	4.902 ± 0.697		

* Significantly different from corresponding compartmental parameters (paired *t* test, at $P \leq 0.05$).

A, *B* = intercept terms; α, β = hybrid rate constants of distribution and elimination phases; $t_{1/2\alpha}$, $t_{1/2\beta}$ = distribution and elimination half-lives; $t_{1/2k10}$ = elimination from the central compartment half-life; C_0 = zero-time plasma drug concentration; λ = slope of the terminal phase of the plasma concentration–time curve. Other terms are fully cited in the text.

Table 2. Pharmacokinetic parameters (mean ± SD) obtained by compartmental and non-compartmental analysis in goats after subcutaneous administration of 7.5 mg/kg levamisole

Compartmental parameters		Non-compartmental parameters	
A (μg/mL)	4.684 ± 3.085	λ (min^{-1})	0.0114 ± 0.0027
B (μg/mL)	0.781 ± 0.387	AUC (μg·min/mL)*	87.62 ± 21.54
α (min^{-1})	0.0497 ± 0.0217	$AUMC$ (μg·min^2/mL)	6358.8 ± 1976.7
β (min^{-1})	0.0119 ± 0.0029	MRT (min)	72.14 ± 7.942
k_a (min^{-1})	0.1095 ± 0.0786	MAT (min)	29.32 ± 11.17
k_{12} (min^{-1})	0.0133 ± 0.0109	Cl (mL/kg·min)*	90.25 ± 22.41
k_{21} (min^{-1})	0.0248 ± 0.0094	V_{ss} (L/kg)*	6.493 ± 1.732
k_{10} (min^{-1})	0.0234 ± 0.0054	V_a (L/kg)	8.337 ± 2.892
AUC (μg·min/mL)	86.73 ± 21.49	C_{max} (μg/mL)	1.168 ± 0.254
Cl (mL/kg·min)	91.26 ± 22.81	t_{max} (min)	18.75 ± 4.432
V_c (L/kg)	3.981 ± 0.862	F (%)*	77.83 ± 6.300
V_p (L/kg)	1.894 ± 1.443		
V_{ss} (L/kg)	5.875 ± 1.797		
V_a (L/kg)	8.209 ± 3.035		
$t_{1/2\alpha}$ (min)	17.64 ± 10.41		
$t_{1/2\beta}$ (min)	63.24 ± 23.13		
$t_{1/2a}$ (min)	7.848 ± 2.574		
$t_{1/2k10}$ (min)	31.15 ± 7.652		
C_{max} (μg/mL)	1.110 ± 0.282		
t_{max} (min)	18.16 ± 5.073		
F (%)	73.14 ± 4.910		

* Significantly different from compartmental parameters (paired t test, at $P \leq 0.05$).
See text and Table 1 for full unabbreviated terms.

Following s.c. administration, levamisole absorption was rapid ($k_a = 0.1095 min^{-1}$) and high ($F = 73.14\%$). In our study k_a was two-fold higher than that obtained in goats and ewes (Galtier *et al.*, 1981). Maximum concentration values were also twice as high as values reported by these authors in goats and ewes, but were similar to concentrations in lambs (McKellar *et al.*, 1991) and lower than concentrations in sheep (Bogan *et al.*, 1982), where levamisole was administered at 7.5 mg/kg by the s.c. route. Time to C_{max} values were lower than in goats (Galtier *et al.*, 1981), sheep (Bogan *et al.*, 1982) and lambs (McKellar *et al.*, 1991), although Galtier *et al.* (1981) found a t_{max} of 3 min in ewes, that is considerably lower than our value. These differences could be attributed to inter- or intra-species variations or to differences in the drug formulations used. In the study of Galtier *et al.* (1981) the goats were Alpine. Moreover, all the authors mentioned above have used commercial formulations. There is no data available regarding s.c. bioavailability apart from values determined by Garcia *et al.* (1994), who obtained a complete absorption in rabbits.

Signs of temporary toxicosis at oral dose levels as small as 16 mg/kg have been observed in this animal species (Smith & Bell, 1971), but a narrow therapeutic index has not been shown for levamisole in goats. So, and based on the high bioavailability and good absorption obtained in this study, the s.c. route appeared to be a practical option for levamisole administration to goats.

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