Oral bioavailability of levamisole in goats

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Levamisole is a broad-spectrum anthelmintic available for veterinary medical use for over 30 years. It shows activity against a wide variety of gastrointestinal and pulmonary nematodes (Thienpont *et al.*, 1966; Walley, 1966; Janssen, 1976), and possesses an immunomodulating effect (Renoux & Renoux, 1977; Symoens & Rosenthal, 1977).

Although the oral pharmacokinetics of levamisole has been extensively investigated in several animal species (Graziani & De Martin, 1977; Galtier *et al.*, 1981, 1983; Bogan *et al.*, 1982; Nielsen *et al.*, 1983; Watson *et al.*, 1988; McKellar *et al.*, 1991; Garcia *et al.*, 1994; Fernández *et al.*, 1998) and in humans (Luyckx *et al.*, 1982; Kouassi *et al.*, 1986), there are only two studies regarding its disposition in goats after oral (p.o.) administration (Galtier *et al.*, 1981; Chartier *et al.*, 2000), and the pharmacokinetic parameters reported in both are scarce. The purpose of this study was to establish the bioavailability and other pharmacokinetic parameters of this drug following p.o. administration to goats, where pharmacokinetic data are currently extrapolated from those estimated in other ruminants, and it extends a previous work (Sahagún *et al.*, 2000) where we studied the pharmacokinetics of levamisole after intravenous (i.v.) and subcutaneous (s.c.) administration.

The experiment was carried out in eight healthy male crossbred goats (aged 6 months, weighing 15–21 kg). They were acclimatized for 15 days before the experiments, and were maintained indoors on a diet of mixed hay and pelleted feed concentrate and had free access to fresh water. Protocols and procedures were approved by the Institutional Animal Care and Use Committee of the University of León.

Each goat was weighed the day before the experiment and administered a single oral dose of 7.5 mg/kg levamisole HCl (Sigma Chemical Company, St. Louis, MO, USA). Levamisole was dissolved in 2.5 mL distilled water and given as an aqueous solution of about pH 4 using a gavage needle. After dosing, the gavage needle was flushed with 5 mL distilled water. Animals were fasted for 24 h and received their first fodder 4 h after drug administration. Blood samples (5 mL) were collected into heparinized vacuum tubes (Venoject,

Terumo Europe, Leuven, Belgium) by jugular venipuncture just prior to drug administration and at 5, 10, 20, 30, 40, 50, 60, 75, 90, 120, 150, 180, 210, 240, 300 and 360 min afterwards. Plasma was centrifuged, separated immediately and frozen at -20°C until assayed.

Plasma levamisole concentrations were determined by high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection (213 nm) following a method previously developed (García *et al.*, 1990) with minor modifications. The limit of detection was 0.016 $\mu\text{g/mL}$ and mean extraction recovery of levamisole from plasma was 82.8%.

Compartmental and non-compartmental methods were used for each goat in the pharmacokinetic analyses. Compartmental analysis was performed using the nonlinear regression analysis program PCNONLIN (Metzler & Weiner, 1989), with a weighting factor of $1/C$, and the initial estimates were determined by JANA (Dunne, 1985). The best fit was based on Akaike's information criterion (Yamaoka *et al.*, 1978a) and graphical analysis of weighted residuals. The other compartmental parameters were calculated by standard equations (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 plasma concentration vs. time curve over the terminal elimination phase. The area under the plasma concentration–time curve (AUC) and the area under the first moment curve (AUMC) were calculated by the linear trapezoidal rule and extrapolated to infinity. The mean residence time (MRT) was defined as $AUMC/AUC$, and the mean absorption time (MAT) as the difference between $MRT_{p.o.}$ and $MRT_{i.v.}$, where $MRT_{p.o.}$ and $MRT_{i.v.}$ are the MRT of the drug after p.o. and i.v. administration, respectively. Total body clearance (Cl) was defined as dose/AUC , the volume of distribution at steady state (V_{ss}) as $MRT \times Cl$, and the apparent volume of distribution (V_a) as Cl/λ . The maximum plasma concentration (C_{max}) and time of C_{max} (t_{max}) were read directly from the concentration vs. time curve, and the oral absorbed fraction (F) was calculated as $F = AUC_{p.o.}/AUC_{i.v.} \times 100$.

where $AUC_{i.v.}$ and $AUC_{p.o.}$ are the AUC after i.v. and p.o. administration, respectively.

All pharmacokinetic parameters were calculated for each animal and values are reported as mean $\pm$ standard deviation (SD). Differences between compartmental and non-compartmental data were determined by the paired *t*-test (Wayne, 1987) ($P \leq 0.05$). This test was also used to assess differences between oral data and those obtained after i.v. administration (Sahagún et al., 2000).

Plasma concentration–time profiles following 7.5 mg/kg oral administration (Fig. 1) were best described by a two-compartment open model. Levamisole was detected in plasma for up to 6 h post-administration. Compartmental and non-compartmental pharmacokinetic parameters are summarized in Tables 1 and 2, respectively.

Levamisole plasma concentration decreased rapidly, with a mean half-life ($t_{1/2\beta}$) of 86.57 ± 24.62 min. The mean AUC was 74.30 ± 15.23 $\mu\text{g}\cdot\text{min}/\text{mL}$; V_{ss} , 7.867 ± 1.789 L/kg and Cl , 105.3 ± 24.76 mL/kg·min. The pharmacokinetic parameters describing the rate and extent of absorption exhibited mean values of 0.0515 ± 0.0145 min^{-1} for k_a , 0.703 ± 0.123 $\mu\text{g}/\text{mL}$ for C_{max} and 29.49 ± 6.31 min for t_{max} , whereas F was $63.22 \pm 6.34\%$.

The paired *t*-test revealed significant differences between compartmental and non-compartmental values for V_{ss} , C_{max} , t_{max} and F . Significant differences were also found for AUC and Cl when both i.v. and oral routes were compared.

Levamisole plasma concentrations vs. time data after p.o. administration were best described by a two-compartment open model, which is in agreement with previous studies reported in goats (Galtier et al., 1981) and sheep (Galtier et al., 1981; Fernández et al., 1998). By contrast, pigs (Galtier et al., 1983) and dogs (Watson et al., 1988) followed a one-compartment

open model, whereas in humans (Luyckx et al., 1982; Kouassi et al., 1986) and rabbits (García et al., 1994) both models have been utilized. In previous work (Sahagún et al., 2000), levamisole was also described in goats by a two-compartment open model after i.v. and s.c. administration.

Neither the oral disposition rate constant β nor its $t_{1/2\beta}$ showed significant differences with the values determined after i.v. administration (Sahagún et al., 2000). Beta (β) was twofold higher than that determined after p.o. administration in goats and sheep by Galtier et al. (1981), and in pigs by Galtier et al. (1983). The relatively fast elimination of the drug from the body would reduce the presence of residues in goat tissues. The oral clearance rate (Cl) was also higher than values previously reported in goats (Galtier et al., 1981), sheep (Galtier et al., 1981) and humans (Luyckx et al., 1982; Kouassi et al., 1986).

On the other hand, k_a showed a moderately rapid absorption from the gastrointestinal tract, with a mean value 12-fold lower than in sheep and four-fold lower than in goats and pigs (Galtier et al., 1981, 1983), similar to those reported in humans (Luyckx et al., 1982), rabbits (García et al., 1994) and sheep (Fernández et al., 1998), and somewhat higher than those described in humans by Kouassi et al. (1986) and in dogs (Watson et al., 1988).

The maximum plasma concentration (C_{max}) was similar to those previously found in the same animal species (Galtier et al., 1981) with a higher dose (10 mg/kg), and in sheep (Bogan et al., 1982; McKellar et al., 1991) and cattle (Nielsen et al., 1983) with the same oral dose (7.5 mg/kg). However, Chartier et al. (2000) in parasitized goats (12 mg/kg), and Galtier et al. (1981) (10 mg/kg) and Fernández et al. (1998) (7.5 mg/kg) in sheep obtained values higher than that of ours.

Levamisole was absorbed more slowly in the present study, with a t_{max} of 29.49 min, compared with 10 min indicated by

Fig. 1. Mean plasma $\pm$ SD concentrations of levamisole after oral administration of 7.5 mg/kg b.w. to goats.

Table 1. Pharmacokinetic parameters determined by compartmental analysis in goats ($n = 8$) after oral administration of 7.5 mg/kg levamisole

Parameter	Mean $\pm$ SD	Parameter	Mean $\pm$ SD
A ($\mu\text{g/mL}$)	4.539 $\pm$ 3.301	V_p (L/kg)	2.305 $\pm$ 1.139
B ($\mu\text{g/mL}$)	0.519 $\pm$ 0.455	V_{ss} (L/kg)	7.867 $\pm$ 1.789
α (min^{-1})	0.0335 $\pm$ 0.0106	V_a (L/kg)	13.35 $\pm$ 5.309
β (min^{-1})	0.0086 $\pm$ 0.0025	$t_{1/2\alpha}$ (min)	22.46 $\pm$ 6.85
k_a (min^{-1})	0.0515 $\pm$ 0.0145	$t_{1/2\beta}$ (min)	86.57 $\pm$ 24.62
k_{12} (min^{-1})	0.0062 $\pm$ 0.0038	$t_{1/2k_a}$ (min)	14.48 $\pm$ 4.297
k_{21} (min^{-1})	0.0167 $\pm$ 0.0105	$t_{1/2k_{10}}$ (min)	37.42 $\pm$ 7.269
k_{10} (min^{-1})	0.0193 $\pm$ 0.0044	$C_{\max}$ ($\mu\text{g/mL}$)	0.703 $\pm$ 0.123
AUC ($\mu\text{g}\cdot\text{min/mL}$)	74.30 $\pm$ 15.23	$t_{\max}$ (min)	29.49 $\pm$ 6.31
Cl (mL/kg·min)	105.3 $\pm$ 24.76	F (%)	63.22 $\pm$ 6.336
V_c (L/kg)	5.562 $\pm$ 1.111		

A, B = Intercept terms; α , β = hybrid rate constants of distribution and elimination phases; k_{12} , k_{21} = transfer rate constant between central and peripheral compartment and vice versa; k_{10} = elimination rate constant from central compartment; $t_{1/2\alpha}$, $t_{1/2\beta}$ = distribution and elimination half-lives; $t_{1/2k_{10}}$ = elimination from central compartment half-life.

Table 2. Pharmacokinetic parameters determined by non-compartmental analysis in goats ($n = 8$) after oral administration of 7.5 mg/kg levamisole

Parameter	Mean $\pm$ SD	Parameter	Mean $\pm$ SD
λ (min^{-1})	0.0088 $\pm$ 0.0023	Cl (mL/kg min)	105.4 $\pm$ 24.82
$AUC_{t-\infty}$ ($\mu\text{g}\cdot\text{min/mL}$)	2.40 $\pm$ 0.64	V_{ss} (L/kg)*	9.429 $\pm$ 1.935
AUC ($\mu\text{g}\cdot\text{min/mL}$)	74.28 $\pm$ 15.49	V_a (L/kg)	12.92 $\pm$ 5.271
$AUMC_{t-\infty}$ ($\mu\text{g}\cdot\text{min}^2/\text{mL}$)	576.0 $\pm$ 1534.0	$C_{\max}$ ($\mu\text{g/mL}$)*	0.743 $\pm$ 0.141
AUMC ($\mu\text{g}\cdot\text{min}^2/\text{mL}$)	6748.6 $\pm$ 1664.0	$t_{\max}$ (min)*	33.75 $\pm$ 7.440
MRT (min)	90.27 $\pm$ 9.40	F (%)*	66.44 $\pm$ 6.580
MAT (min)	47.44 $\pm$ 7.062		

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

Galtier *et al.* (1981) and Chartier *et al.* (2000) in goats or with 5 min determined by Galtier *et al.* (1981) in sheep. Other authors obtained higher values in sheep (Bogan *et al.*, 1982; Fernández *et al.*, 1998), cattle (Nielsen *et al.*, 1983), dogs (Watson *et al.*, 1988) and rabbits (García *et al.*, 1994) than those described by us.

The reported differences on kinetic behaviour could be attributed to differences in experimental conditions, sex, inter- or intraspecies variations, the presence of nematodes or to the different preparations used. For example, Galtier *et al.* (1981) and Chartier *et al.* (2000) administered commercial formulations to female goats only.

The absorbed fraction was about 66%, suggesting that levamisole was relatively well absorbed by this route. There is no data on oral bioavailability in goats, but this is consistent with the results obtained after p.o. administration in sheep (Fernández *et al.*, 1998), pigs (Galtier *et al.*, 1983), dogs (Watson *et al.*, 1988), rabbits (García *et al.*, 1994) and humans (Luyckx *et al.*, 1982; Kouassi *et al.*, 1986). A higher bioavailability is achieved by s.c. route (78%) (Sahagún *et al.*, 2000). Thus, it would be expected that s.c. administration provides better efficacy than p.o. administration against lungworms.

Although caution should be maintained when this anthelmintic is administered to goats (Smith & Bell, 1971), a narrow

therapeutic index has not been shown for levamisole in this animal species. While adverse effects such as hyperexcitability, deep respiratory movements, sialosis and muscle tremors after levamisole i.v. administration (Sahagún *et al.*, 2000) and tremors and hyperesthesia in i.m. route (Galtier *et al.*, 1981) have been reported in goats, there are no data on toxicosis concerning s.c. administration. On the other hand, only mild signs of transitory intoxication have been described in goats with an oral dose of 16 mg/kg, and no adverse reactions have been seen at oral dose levels of 12 mg/kg (Babish *et al.*, 1990; Chartier *et al.*, 2000) and with a dose of 7.5 mg/kg in this study or in Galtier *et al.* (1981) (10 mg/kg).

Understanding of the pharmacokinetic behaviour of anthelmintic drugs can contribute to improve parasite control in livestock. From the relatively high bioavailability observed in the current study, we recommend the oral route as an effective way to administer levamisole in goats for treating gastrointestinal nematodes.

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