Bioavailability of levamisole after intramuscular and oral administration in sheep

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Abstract

Aims. To determine the bioavailability of levamisole in sheep.
Methods. Levamisole was administered to three groups of six Merino sheep orally and intramuscularly at three dose levels of 5, 7.5 and 10 mg/kg. There was a washout period of 1 week between treatments. Blood samples were collected by jugular venepuncture and plasma was separated immediately by centrifugation and stored at – 20 °C until analysed.The levamisole concentration in plasma was determined by high performance liquid chromatography with a u.v. detection method. Individual plasma levamisole concentration-time data were analysed using the compartmental method.
Results. The values obtained for k_a, C_{max}, t_{max} and F show a moderate rate and extent of absorption after oral administration of levamisole while, after intramuscular administration, these values demonstrate a high rate and extent of absorption of levamisole. The intramuscular bioavailability was higher than the oral bioavailability (rate of absorption three-fold faster, extent of absorption 25 -33% higher and C_{max} two-fold higher). The Friedman test involving dose and route of administration showed that the route of administration affects k_a, C_{max}, t_{max} and F; significant differences were found in these parameters.
Clinical relevance. On the basis of these data, the recommended routes for the administration of levamisole in sheep are oral for gastro-intestinal nematodiasis and intramuscular for extragastric nematodiasis.
Key words. Levamisole, bioavailability, sheep.

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Introduction

Levamisole (*l*-2,3,5,6-tetrahydro-6-phenylimidazo[2,1-b]tiazole) is a synthetic anthelmintic widely used for the control of gastro-intestinal worms and lungworms in sheep[1]. Levamisole also has immunostimulant effects[2][3]. It is normally administered to sheep as the hydrochloride salt orally or by the intramuscular or subcutaneous routes.

The pharmacokinetic behaviour of levamisole has been studied in cattle[4][5][6][7], in goats[8][9], in pigs[9][10], in rabbits[11][12], in dogs[4][13][14] and in human beings[15][16][17]. A limited amount of information on its pharmacokinetics in sheep has been published[8][18][19][20].

The objective of the present study was to determine both the oral and intramuscular bioavailability of levamisole in sheep. There are no published data on the bioavailability in this animal species.

Materials and Methods

Animals

The experiment was carried out in 18 healthy Merino sheep. All animals were males of about 6 months old and weighed from 22 to 30 kg. They were maintained indoors on a hay and concentrate diet and had free access to fresh water.

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Experimental design

The animals were individually weighed and randomly assigned to three groups of six sheep (A, B, C). Each group received levamisole chlorhydrate (Sigma, St. Louis, Mo.) at a dose of either 5 (group A), 7.5 (group B) or 10 (group C) mg of levamisole free base equivalents/kg, by two routes: intramuscular (i.m.) and oral. A washout period of 1 week was allowed between treatments. The i.m. injection site was about half-way between the upper and lower distal third of the neck, on the left side. For the oral dosing, levamisole was diluted in 3 ml of water and was given as an aqueous solution of about pH 4 using a gavage needle. Before drug administration, the sheep were fasted for 24 h. The sheep were returned to their normal diet 4 hours after treatment.

Blood sampling

Blood samples, 5 ml each, were collected by jugular venepuncture in heparinised vials (Venoject, Terumo, Leuven, Belgium) immediately before each treatment and at 5, 10, 15, 20, 25, 30, 45, 60, 90, 120, 180, 240, 360 and 480 minutes after i.m. administration; and 5, 10, 20, 30, 40, 50, 60, 75, 90, 120, 180, 240, 360, 480 and 600 minutes after oral administration. Plasma was separated immediately by centrifugation and stored at -20 °C until analysed.

Drug determination

The levamisole concentration in plasma was determined by high performance liquid chromatography with a u.v. detection method as previously described[21]. The lowest quantifiable concentration of levamisole in plasma was 0.08 μg/ml, with an accuracy of 90 ± 3% and coefficient of variation of 5.8 ± 0.2%.

Pharmacokinetic analysis

Individual plasma levamisole concentration v. time data were analysed using the compartmental method. For this analysis, the iterative weighted non-linear least-squares regression program PCNONLIN[22] was used and initial estimates of the parameters were determined by JANA[23]. The best pharmacokinetic model (one, two and three-compartment) was determined by application of Akaike's Information Criterion (AIC)[24] and graphical analysis of weighted residual. A bi-exponential equation was selected for all sheep and consequently the data were described by a two-compartment open model with first-order absorption. The values for α, β, A and B were obtained using initial estimates. The other parameters were calculated by standard methods [25]. The systemic availability (F) was calculated as AUC_{im} or AUC_o/AUC_{iv}, where AUC_{im}, AUC_o and AUC_{iv} are the AUC of the drug after intramuscular, oral (from this study) and intravenous[20] administration, respectively.

Statistical analysis

The pharmacokinetic parameters were compared for statistical significance by using the Kruskall-Wallis test[26]. Differences in pharmacokinetic data between dosage and administration routes were analysed for statistical differences using the Friedman test[26].

Results

The mean (s.d.) levamisole plasma concentration-time profiles for the 5, 7.5 and 10 mg/kg doses after intramuscular and oral administration are presented in Figures 1 and 2, respectively. Values of the pharmacokinetic parameters following intramuscular and oral administration are given in Table I.

After both intramuscular and oral administration, the pharmacokinetics were described by a two-compartment open model in all sheep. The values obtained for k_a, t_{max} and F after i.m. administration were very similar for the three doses. Moreover, C_{max} values increased with dose. The Kruskal-Wallis test showed no significant differences in k_a, t_{max} and F. However, significant differences were found in C_{max}.

After oral administration the k_a, t_{max} and F values were also very similar for the three doses and no statistically significant differences were found between them in the Kruskal-Wallis test. C_{max} values also increased with dose after oral administration, and statistically significant differences were found between them in the Kruskal-Wallis test.

Discussion

The pharmacokinetics of levamisole were best described by a two-compartment open model in sheep after intramuscular and oral administration. This was also reported by Galtier *et al.*[8] in sheep after the intramuscular (7.5 mg/kg) and oral (10 mg/kg) administration of levamisole.

The values obtained for k_a indicated a rapid process of absorption after intramuscular administration and the present values were similar to those obtained by Galtier *et al.*[8] in sheep (0.0923 min^{-1}). After oral administration, k_a values showed a slower absorption phase than intramuscularly and the values were lower than those obtained by Galtier *et al.*[8] in this species (0.6086 min^{-1}).

On the other hand, other authors have obtained k_a values after the oral administration of levamisole lower than those reported by Galtier *et al.*[8] (0.072 min^{-1} reported by Watson *et al.*[14] in dogs; 0.0210 min^{-1} obtained by Kouassi *et al.*[17] in humans; and 0.0535 ± 0.023, 0.0234 ± 0.0035, 0.0273 ± 0.0189 min^{-1} obtained by García *et al.*[12] after the oral

Table I. Pharmacokinetic parameters (mean ± s.d.) in three groups of sheep (n = 6) after both intramuscular and oral administration of levamisole

Parameter	Dose		
	5 mg/kg	7.5 mg/kg	10 mg/kg
Intramuscular administration			
AUC (μg.min/ml)[a]	201.2 ± 10.5	233.7 ± 54.2	501.7 ± 102.2
k_a (min^{-1})[b]	0.0591 ± 0.0200	0.0745 ± 0.0253	0.0982 ± 0.0503
$t_{1/2\alpha}$ (min)	21.78 ± 13.84	27.38 ± 20.53	31.69 ± 21.51
$t_{1/2\beta}$ (min)	123.2 ± 27.0	128.2 ± 37.4	140.3 ± 34.5
C_{max} (μg/ml)[a]	1.613 ± 0.285	2.145 ± 0.509	3.434 ± 0.304
t_{max} (min)[b]	25.28 ± 1.97	23.07 ± 7.29	24.68 ± 9.38
F (%)[b]	77.76 ± 10.21	75.59 ± 9.15	82.81 ± 12.45
Oral administration			
AUC (μg.min/ml)[a,c]	160.0 ± 30.2	187.7 ± 44.4	403.8 ± 116.8
k_a (min^{-1})[b,c]	0.0414 ± 0.0307	0.0305 ± 0.0098	0.0312 ± 0.0117
$t_{1/2\alpha}$ (min)	25.94 ± 9.65	28.66 ± 10.12	38.98 ± 11.19
$t_{1/2\beta}$ (min)	243.2 ± 78.6	166.1 ± 38.7	326.6 ± 175.6
C_{max} (μg/ml)[b,c]	0.7961 ± 0.2697	1.131 ± 0.254	1.648 ± 0.160
t_{max} (min)[b,c]	39.37 ± 13.80	42.81 ± 13.89	48.20 ± 7.19
F (%)[b,c]	61.11 ± 9.32	60.41 ± 3.91	66.60 ± 16.37

a Significant differences between doses (Kruskal-Wallis test).

b No significant differences between doses (Kruskal-Wallis test).

c Significant differences between administration routes (Friedman test).

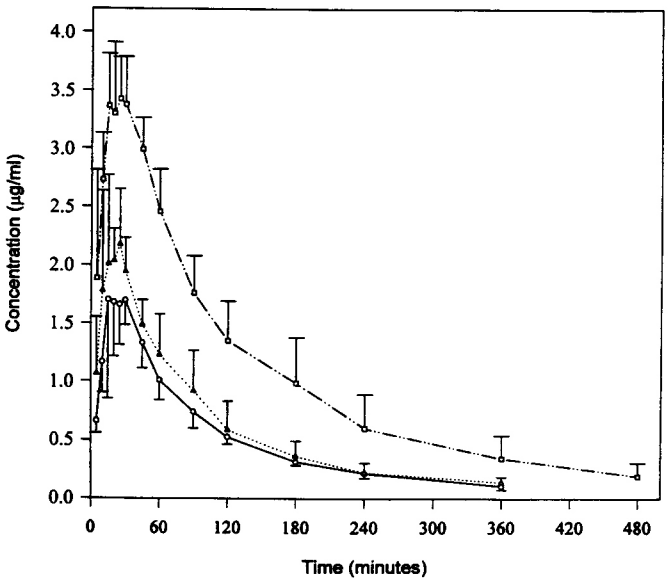

Figure 1. Mean plasma concentration ± s.d. in six sheep after intramuscular administration of levamisole at doses of 5 (-○-), 7.5 (-△-) and 10 (-□-) mg/kg body weight.

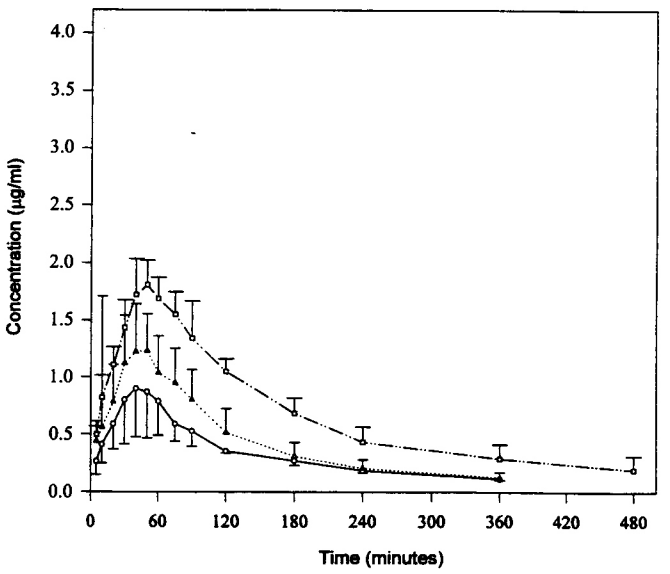

Figure 2. Mean plasma concentration ± s.d. in six sheep after oral administration of levamisole at doses of 5 (-○-), 7.5 (-△-) and 10 (-□-) mg/kg body weight.

administration of 12.5, 16 and 20 mg/kg of levamisole, respectively, to rabbits). These differences between the k_a values can be explained by species differences and different oral dosage forms administered.

C_{max} values obtained after intramuscular administration were higher than those obtained by Galtier *et al.*[8] in sheep (1.29 µg/ml), by Archambault *et al.*[5] in cattle (1.27 µg/ml) and in this same species by Nielsen *et al.*[6] (1.1 µg/ml), when the data by these authors are normalised for dose.

After oral administration, C_{max} values were about two-fold lower than those obtained intramuscularly but were also higher than those obtained by Galtier *et al.*[8] (1.06 µg/ml). On the other hand, the time to reach maximum concentration after intramuscular administration was lower than that obtained orally. However, Galtier *et al.*[8] in sheep obtained t_{max} values lower (5 min) after oral administration than those obtained intramuscularly (10 min).

On the basis of these data, the intramuscular bioavailability was higher than the oral (rate of absorption three-fold faster, extent of absorption 25 - 33% higher and C_{max} two-fold higher). Similarly to our results, Symoens and Shuermans[27] have shown that, also in man, peak blood levels were about twice as high after intramuscular administration than after oral administration at the same dosage.

The lower bioavailability of levamisole after oral administration than after intramuscular administration could be due to the metabolism of levamisole by gastro-intestinal micro-organisms, probably in the rumen, or adsorption of levamisole on to solids in the gastro-intestinal tract. A first-pass effect is unlikely as levamisole is not metabolised sufficiently rapidly[17].

The Friedman test involving dose and route of administration showed that route of administration affects C_{max}, t_{max}, F, and k_a; significant differences were found in these parameters.

On the basis of these data, oral administration of levamisole for gastro-intestinal nematodiasis and intramuscular administration for extragastric nematodiasis are recommended in sheep.

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