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Bioavailability of levamisole administered by subcutaneous and oral routes in rabbits

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The bioavailability of levamisole in rabbits was determined after subcutaneous and oral administration at three dose levels of 12.5, 16.0 and 20.0 mg/kg. After non-compartmental analysis the mean values obtained were: C_{max} = 3.54, 4.51 and 5.39 μg/ml; t_{max} = 12.0, 22.0 and 20.0 min; F = 134.8, 105.4 and 124.1% after subcutaneous administration for each dose, respectively, and C_{max} = 0.71, 1.32 and 1.77 μg/ml; t_{max} = 46.0, 96.0 and 84.0 min; F = 53.0, 62.0 and 80.7% after oral administration. The extent and rate of absorption from the two routes differed significantly, except for t_{max} at the 12.5 mg/kg dose. After compartmental analysis the pharmacokinetics of levamisole was characteristic of a two-compartment open model in 13 rabbits and of a one-compartment open model in two rabbits after subcutaneous administration, while it was two compartmental in nine and one compartmental in six rabbits after oral administration. The k_a values were 0.321, 0.145 and 0.145 min^{-1} after subcutaneous administration and 0.054, 0.023 and 0.027 min^{-1} after oral administration. There were no significant differences between the values of C_{max}, t_{max} and *AUC* calculated by compartmental and non-compartmental analysis.

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INTRODUCTION

Levamisole, the levorotatory isomer of tetramisole [2,3,5,6-tetrahydro-6-phenylimidazo (2,1-b) thiazole], is a broad-spectrum anthelmintic active against most nematodes (Thienpont *et al.*, 1966) and therefore widely used in veterinary medicine. It also has an immunomodulating effect (Renoux & Renoux, 1977; Symoens & Rosenthal, 1977). Levamisole may be used against *Obeliscoides cuniculi* (Hayes & Mitrovic, 1974), *Graphidium strigosum* (Ghenne, 1969) and *Trichostrongylus spp.* (Herlich, 1976) in rabbits. Moreover, it may be used in several respiratory diseases as an immunomodulator (Espinasse, 1980). The drug is available as a formulation for both oral and subcutaneous administration in rabbits. Only limited data are available concerning the disposition kinetics of levamisole in rabbits (García *et al.*, 1992) and there are no data on bioavailability. The purpose of this study was to establish several pharmacokinetic parameters on bioavailability of levamisole in rabbits, a target species for this compound, after both oral and subcutaneous administrations.

MATERIALS AND METHODS

Animals

Thirty healthy male New Zealand white rabbits weighing between 2.10 and 3.26 kg were used. They were housed in individual cages. Environmental conditions consisted of a 12 h light-dark cycle at 22 ± 1°C room temperature. The rabbits were allowed water and Nanta rabbit chow *ad libitum*.

Treatment and blood sampling

The rabbits were randomly divided in two groups of 15 animals per group, which received levamisole HCl orally and subcutaneously, respectively. Levamisole HCl was dissolved in 2 ml of water and administered orally as an aqueous solution of pH about 4 using a gavage needle. Subcutaneous administration was carried out after dissolving levamisole HCl in 1 ml sterile water with a final pH of about 4 and injected under the skin over the back. Each group was divided into three subgroups of

five rabbits receiving 12.5, 16.0 and 20.0 mg/kg of drug, respectively.

The rabbits were anaesthetized with sodium pentobarbitone, 30 mg/kg intraperitoneally and the left carotid artery canulated with silastic medical-grade tubing 1.02 mm ID × 2.16 mm OD. Levamisole administration was carried out after total recovery from anaesthesia was achieved. Rabbits receiving the levamisole orally and subcutaneously were fasted for 12 h before dosing. Heparinized blood samples (3 ml) were collected from the left carotid artery at 5, 10, 20, 30 and 60 min and 2, 4, 6, 8, 24 and 48 h after drug administration for both routes of administration. Plasma was immediately separated and frozen at −20°C until analysed.

Analysis

The levamisole concentration in plasma was determined by high-performance liquid chromatography with a UV detection method as previously described (García *et al.*, 1990). The lowest detectable concentration of levamisole in plasma was 0.08 μg/ml.

Pharmacokinetic analysis

Compartmental analysis. Plasma levamisole concentration-time profiles were individually fitted to the following exponential equation:

$$C_p = \sum_{i=1}^{n} C_i e^{-\lambda_i t}$$

Where C_i is the y-intercept, λ_i is the slope of each of n first-order rate processes, e is the exponential function (base e) and t is time. The estimates of C_i and λ_i were calculated using a computer program based on non-linear iterative least-squares regression analysis PCNONLIN 3.0 (Statistical Consultants, Lexington, KY, USA). The equations were fitted to the data using a weighting factor 1/C and the optimum number of first-order rate processes was determined by residual analysis. The other compartmental parameters were calculated by standard methods (Gibaldi & Perrier, 1982).

Non-compartmental analysis. The pharmacokinetic parameters were determined for each animal using standard formulae (Gibaldi & Perrier, 1982) and formulae based on statistical moment theory (Yamoaka *et al.*, 1978; Benet & Galeazzi, 1979). Calculated values included: area under the curve from time 0 to infinity (*AUC*), area under the first moment curve (*AUMC*), mean residence time (*MRT*), mean absorption time (*MAT*), the maximum observed drug concentration in plasma (C_{max}), the time to reach maximum concentration (t_{max}), fraction absorbed (F) and relative oral-subcutaneous fraction absorbed (FR p.o./s.c.). *AUC* and *AUMC* were calculated by the trapezoidal rule up to the last measurable concentration (C_t) and from that point on to infinity (AUC_t^{∞} and $AUMC_t^{\infty}$) by the equations

$$AUC_t^{\infty} = \frac{C_t}{\lambda}$$

$$AUCM_t^{\infty} = \frac{C_t t}{\lambda} + \frac{C_t}{\lambda^2}$$

where the terminal slope λ was obtained by linear regression analysis of the log-transformed plasma concentration data. *MRT* was determined as *AUMC/AUC*. *MAT* was computed as $MAT = MRT - MRT_{i.v.}$ where $MRT_{i.v.}$ is the mean residence time obtained after i.v. administration. F was calculated as $F = AUC/AUC_{i.v.}$ where $AUC_{i.v.}$ is the area under the curve obtained after i.v. administration. $FR_{p.o./s.c.}$ was determined as $FR_{p.o./s.c.} = AUC_{p.o.}/AUC_{s.c.}$. C_{max} and t_{max} were read directly from the individual plasma concentration-time curves.

Statistical analysis

All pharmacokinetic parameters were calculated for each animal and the data presented as arithmetic mean ± standard deviation (mean ± SD). Data were analysed by analyses of variance (ANOVA) and when the results were significant, the t-test was used to evaluate differences between data sets and a $P \leq 0.05$ was taken as the level of significance for all analyses.

RESULTS

The mean (± SD) levamisole plasma concentration-time profiles for the 12.5, 16.0 and 20.0 mg/kg doses after subcutaneous and oral administrations are shown in Figs 1 and 2, respectively. Values of the pharmacokinetic parameters determined by both compartmental and non-compartmental analyses following subcutaneous administration are given in Tables 1 and 2 respectively. Those after oral administration are shown in Tables 3 and 4.

Compartmental analysis

After subcutaneous administration, pharmacokinetics was better described by a two-compartment open model for all rabbits except for one animal at a dose of 12.5 mg/kg and another one at a dose of 16.0 mg/kg which were better adjusted to a one-compartment open model. After oral administration, nine rabbits fitted better to a two-compartment model while three animals at a dose of 12.5 mg/kg and three animals at a dose of 20.0 mg/kg adjusted better to a one-compartment model. The values obtained for k_a after subcutaneous administration were practically the same for the two higher doses and these values were slightly lower than those for 12.5 mg/kg dose, but no statistically significant differences were found at $P \leq 0.05$. The values obtained in the rabbits exhibiting one-compartment kinetics (0.6730 min^{-1} at a dose of 12.5 mg/kg and 0.1705 at a dose of 16 mg/kg) were among the highest obtained. After oral adminis-

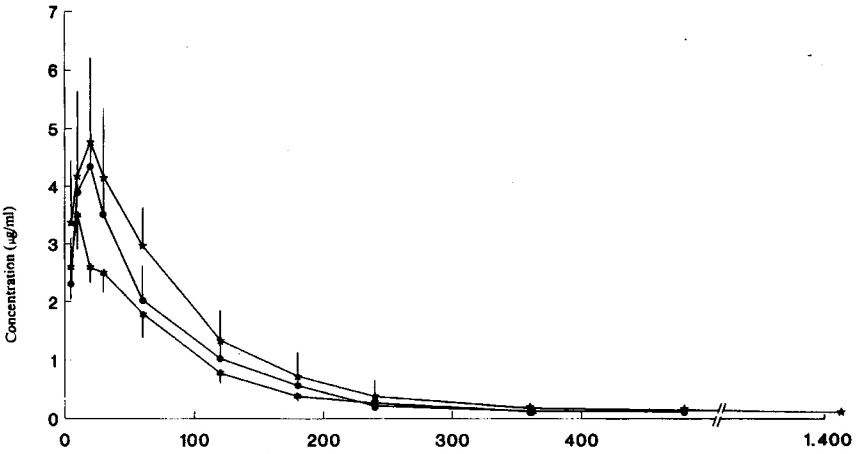

Fig. 1. Mean plasma concentration ± SD in five rabbits after subcutaneous administration of levamisole at doses of 12.5 (*) 16.0 (●) and 20.0 (★) mg/kg body weight

tration, k_a values were similar for the doses of 16.0 and 20.0 mg/kg and lower than those for the dose of 12.5 mg/kg, with statistically significant differences. The k_a one-compartment and two-compartment values were similar. With regard to the most representative parameter values of bioavailability: *AUC*, C_{max} and t_{max} (McGilveray *et al.*, 1990; Ritchel, 1987) ANOVA multifactor analysis involving dose, route of administration and pharmacokinetic analysis showed that pharmacokinetic analysis did not affect these parameters. However, they were affected by dose and route. For this reason, only the non-compartmental values are considered (see Discussion).

Non-compartmental analysis

After i.v. administration of levamisole in rabbits (García *et al.*, 1992) at 12.5, 16.0 and 20.0 mg/kg doses, the following average values ± SDs (n = 5) for non-compartmental analysis of the pharmacokinetic parameters were obtained: *AUC* = 238.7 ± 37.5, 381.3 ± 49.8 and 520.6 ± 179.4 in µg.min/ml; *MRT* = 69.2 ± 13.0, 71.7 ± 11.9 and 73.1 ± 19.6 min. *MRT* and *MAT* values were similar for the doses 12.5 and 16.0 mg/kg and these values were lower than those for the 20 mg/kg dose. However, no statistically significant differences were found in ANOVA, both after subcutaneous administration and after oral administration.

C_{max} values increased with dose after subcutaneous administration; t_{max} values were similar for the two larger doses and both were slightly larger than the values for a dose of 12.5 mg/kg. However, t-test analysis indicated statistically significant differences only between t_{max} values for the 12.5 and 16.0 mg/kg dose rates. C_{max} values also increased with dose after oral administration, while t_{max} values were very similar for the three doses, and no statistically significant differences were found between them in ANOVA. These values demonstrate a moderate rate and extent of absorption lower than those obtained by the subcutaneous route. The fraction of dose absorbed (F), was greater than 100% for the three doses after subcutaneous administration. On the other hand, the fraction of absorbed dose increased with the dose and was always less than 100% after oral administration. The relative absorbed oral-subcutaneous fraction ($FR_{p.o./s.c.}$) was determined in order to provide a comparison between both routes without taking into account the variation due to the i.v. route; FR also increased with the dose and was less than 100.

DISCUSSION

In a previous study (García *et al.*, 1992) the pharmacokinetics of

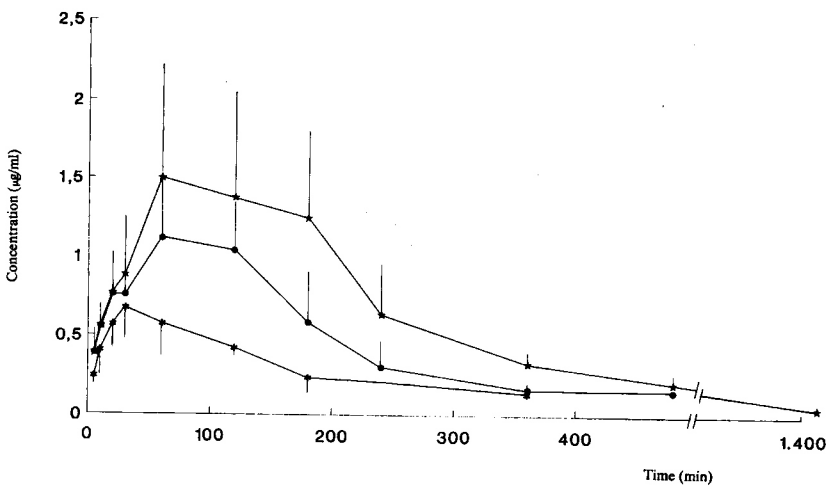

Fig. 2. Mean plasma concentration ± SD in five rabbits after oral administration of levamisole at doses of 12.5 (*), 16.0 (●) and 20.0 (★) mg/kg body weight

Table 1. Pharmacokinetic parameters (mean ± SD) determined by compartmental analysis in three groups of rabbits ($n = 5$) after subcutaneous administration of levamisole

Parameter	Dose 12.5 mg/kg	16.0 mg/kg	20.0 mg/kg
AUC, μg.min/ml	374.41 ± 151.38	382.94 ± 102.84	579.14 ± 99.17*,†
C_{max}, μg/ml	3.16 ± 0.09	4.31 ± 1.44	4.82 ± 0.84*
t_{max}, min‡	11.51 ± 3.94	18.75 ± 9.18	19.56 ± 7.64
K_a, min^{-1}‡	0.3206 ± 0.2021	0.1451 ± 0.0642	0.1453 ± 0.0697

*Significantly different from 12.5 mg/kg (*t*-test, at $P \leq 0.05$); †significantly different from 16.0 mg/kg (*t*-test, at $P \leq 0.05$); ‡no significant differences between dose (ANOVA, at $P \leq 0.05$).

Table 2. Pharmacokinetic parameters (mean ± SD) determined by non-compartmental analysis in three groups of rabbits ($n = 5$) after subcutaneous administration of levamisole

Parameter	Dose 12.5 mg/kg	16.0 mg/kg	20.0 mg/kg
AUC, μg.min/ml§	321.73 ± 48.09	401.87 ± 103.37	646.02 ± 115.75*,†
AUMC, μg.min^2/ml	39708.6 ± 17789.8	63370.4 ± 57528.8	351742.5± 372649.4
MRT, min‡	120.95 ± 40.15	148.56 ± 108.96	496.22 ± 471.07
MAT, min‡	51.71 ± 40.15	76.89 ± 108.96	423.16 ± 471.07
C_{max}, μg/ml§	3.54 ± 0.58	4.51 ± 1.28	5.39 ± 0.98*
t_{max}, min§	12.00 ± 4.47	22.00 ± 4.47*	20.00 ± 7.07
F, %‡	134.77 ± 20.15	105.40 ± 27.22	124.09 ± 22.23

*Significantly different from 12.5 mg/kg (*t*-test, at $P \leq 0.05$); †significantly different from 16.0 mg/kg (*t*-test, at $P \leq 0.05$); ‡no significant differences between dose (ANOVA, at $P \leq 0.05$); §not significantly different from the values found in Table 1 (ANOVA, at $P \leq 0.05$).

Table 3. Pharmacokinetic parameters (mean ± SD) determined by compartmental analysis in three groups of rabbits ($n = 5$) after oral administration of levamisole

Parameter	Dose 12.5 mg/kg	16.0 mg/kg	20.0 mg/kg
AUC, μg.min/ml	120.65 ± 25.45†	269.75 ± 93.96*,‡	389.20 ± 154.19*,†
C_{max}, $\mu g/ml^{-1}$	0.66 ± 0.15†	1.16 ± 0.24*,†	1.40 ± 0.48*,†
t_{max}, min	38.78 ± 9.74†	64.73 ± 19.25*,†	73.12 ± 24.54*,†
K_a, min^{-1}	0.0535 ± 0.0235†	0.0234 ± 0.0035*,†	0.0273 ± 0.0189†

*Significantly different from 12.5 mg/kg (*t*-test, at $P \leq 0.05$); †significantly different from the value found in Table 1 (*t*-test, at $P \leq 0.05$); ‡not significantly different from the value found in Table 1 (*t*-test, at $P \leq 0.05$).

Table 4. Pharmacokinetic parameters (mean ± SD) determined by non-compartmental analysis in three groups of rabbits ($n = 5$) after oral administration of levamisole

Parameter	Dose 12.5 mg/kg	16.0 mg/kg	20.0 mg/kg
AUC, μg.min/ml‡	126.43 ± 27.9§	236.36 ± 66.49*,§	420.36 ± 175.10*,§
AUMC, μg.min^2/ml	27125.3 ± 20953.8	41810.8 ± 19906.1	168502.8 ± 217215.8
MRT, min†	195.34 ± 116.55	170.06 ± 41.04	325.81 ± 328.86
MAT, min†	126.10 ± 116.55	98.39 ± 41.04	252.75 ± 328.86
C_{max}, μg/ml‡	0.71 ± 0.16§	1.32 ± 0.20*,§	1.77 ± 0.40*,§
t_{max}, min‡	46.00 ± 41.59¶	96.00 ± 32.86§	84.00 ± 32.86§
F, %†	52.96 ± 11.69§	61.99 ± 17.44§	80.74 ± 33.64§
$FR_{p.o./s.c.}$, %	39.30	58.82	65.07

*Significantly different from 12.5 mg/kg (*t*-test, at $P \leq 0.05$); †no significant differences between dose (ANOVA, at $P \leq 0.05$); ‡not significantly different from the values found in Table 3 (ANOVA, at $P \leq 0.05$); §significantly different from the value found in Table 2 (*t*-test, at $P \leq 0.05$); ¶significantly different from the value found in Table 2 (*t*-test, at $P \leq 0.05$).

levamisole was best described by a two-compartment open model in all rabbits after i.v. administration at the same dose rates used in the present investigation. In this study a one-compartment model applied to some animals presumably due to individual differences, since the same animals were not used for the three routes of administration and a phenomenon of vanishing exponential terms may have been produced (Gibaldi & Perrier, 1982; Firsov & Piotrovskii, 1986). Watson *et al.* (1988) have shown that levamisole follows a one-compartment model in dogs after both i.v. and p.o. administration. However, Galtier *et al.* (1983) showed that levamisole follows a two-compartment model in pigs after i.v. administration and a one-compartment model after both i.m. and p.o. administration. However, Kouassi *et al.* (1986) in humans and Galtier *et al.* (1981) in ewes and goats obtained a two-compartment model while Luyckx *et al.* (1982) showed a one-compartment model in humans after p.o. administration.

The values obtained for k_a indicated a rapid process of absorption after subcutaneous administration and the present values were higher than those obtained by Galtier *et al.* (1981) in ewes and goats. After oral administration k_a values showed a slower absorption phase than subcutaneously and values were similar to those obtained by Luyckx *et al.* (1982) and Kouassi *et al.* (1986) in humans and Watson *et al.* (1988) in dogs. However, they were lower than those obtained by Galtier *et al.* (1981) in ewes and goats and Galtier *et al.* (1983) in pigs. These differences are not unexpected, because ewes and goats are ruminants and pigs were treated with a different dosage form.

C_{max} values obtained after subcutaneous administration were similar to those reported by Bogan *et al.* (1982) in sheep and higher than those obtained by Galtier *et al.* (1981) in goats and ewes and by McKellar *et al.* (1991) in lambs, when the data by these authors are normalized to dose. On the other hand, t_{max} values were lower than those reported by the same authors. Only Galtier *et al.* (1981) obtained a t_{max} of 3 min in ewes which is lower than the present value. After oral administration C_{max} and t_{max} values described in other studies in several animal species show a wide range as a consequence of species differences and different oral dosage forms administered (Baggot, 1977; Bogan *et al.*, 1983).

Thus, C_{max} values in this study were similar to those reported by Galtier *et al.* (1981) in ewes and goats, Bogan *et al.* (1982) in sheep, Nielsen and Rasmussen (1983) in heifers and McKellar *et al.* (1991) in lambs and lower than those obtained by Luyckx *et al.* (1982) and Kouassi *et al.* (1986) in humans and Watson *et al.* (1988) in dogs after all values have been normalized to dose. Our t_{max} values are similar to those described by Luyckx *et al.* (1982), Kouassi *et al.* (1986), Watson *et al.* (1988) and McKellar *et al.* (1991), lower than those reported by Bogan *et al.* (1982) and Nielsen *et al.* (1983) and higher than those by Galtier *et al.* (1981).

The fraction of absorbed dose (F) after subcutaneous administration indicates complete absorption by this route. Since values greater than 100% were obtained this may have been due to the fact that the last measured concentration was not the same for each animal and this led to inter-individual variations in AUC_t^{∞}. Another factor may be the existence of a lung first-pass effect which might be more pronounced after i.v. dosing than by subcutaneous and oral administration routes. On the other hand, the fraction of absorbed dose after oral administration was less than 100% and this fact may be due to the existence of a hepatic saturable first-pass effect and to excretion in the faeces of part of the administered dose. Both aspects are currently being investigated. Values in this study were similar to those obtained by other authors which range from 49% in dogs (Watson *et al.*, 1988) to 68% in humans (Luyckx *et al.*, 1982). Comparing the data with the modified $FR_{p.o.}/_{s.c.}$ values obtained by other authors, it can be seen that they were similar to those reported by Galtier *et al.* (1981) in ewes and Bogan *et al.* (1982) in sheep but are lower than those obtained by Galtier *et al.* (1981) in goats and McKellar *et al.* (1991) in lambs. The values of AUC, C_{max} and t_{max} obtained by ANOVA multifactor analysis show statistically significant variations which are due to the different doses and routes.

On the basis of those data oral levamisole administration for gastric nematodosis treatment and subcutaneous administration for extragastric nematodosis are recommended.

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