Pharmacokinetics of levamisole in rabbits after intravenous administration

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A compartmental and non-compartmental pharmacokinetic study was carried out on rabbits after intravenous (i.v.) administration of levamisole at the three dose rates: 12.5, 16.0 and 20.0 mg/kg body weight. Using compartmental analysis, the disposition of levamisole best fitted a two-compartmental open model with mean values of α = 0.1278, 0.1019 and 0.1282 min^{-1}; β = 0.0139, 0.0126 and 0.0124 min^{-1}; A = 6.24, 5.27 and 10.58 μg/ml and B = 2.14, 3.83 and 5.08 μg/ml for each dose, respectively. The statistical moment theory was mainly used for non-compartmental analysis. Values for mean residence time (*MRT*) of 69.2, 71.7 and 73.1 min were obtained for each dose. The mean values for volume of distribution at steady state ($V_{d(ss)}$), determined by compartmental analysis, were 3879, 3279 and 2735 ml/kg for each dose, and values obtained using the statistical moment theory were 3760, 3015 and 2943 ml/kg; there were no statistically significant differences using Student's paired *t*-test. Identical conclusions were obtained using both methods when the parameters β, *AUC* and *Cl* were compared.

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INTRODUCTION

Levamisole, *l*-(2,3,5,6-tetrahydro-6-phenylimidazo(2,1-b)thiazole), is a broad-spectrum anthelmintic drug used mainly against gastrointestinal and pulmonary nematodes in several domestic animals (Raeymaekers *et al.*, 1966; Thienpont *et al.*, 1966; Janssen, 1976). In addition to its anthelmintic properties levamisole also has an immunomodulating effect (Renoux & Renoux, 1977; Symoens & Rosenthal, 1977) and it is available for this use (Anon., 1990). 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 purpose of the present work was to establish some pharmacokinetic parameters for levamisole in rabbits, a target species for this compound and for which there are no data at present.

MATERIALS AND METHODS

Experiments were carried out on fifteen healthy male New Zealand white rabbits, with a body weight range of 2.5–3.1 kg. The rabbits were prepared under anaesthesia (sodium pentobarbitone, 30 mg/kg intraperitoneal administration) with a left carotid artery cannula using silastic medical-grade tubing 1.02 mm ID × 2.16 mm OD. They were inserted before the trial commenced. The rabbits were randomly divided into three

groups which received 12.5, 16.0 and 20.0 mg/kg of levamisole, respectively, as levamisole HCl injection into the marginal ear vein. Heparinized blood samples (3 ml) were collected from the left carotid artery at the time intervals indicated in Table I for the three groups. Plasma was immediately separated and frozen at −20°C until analysed. The levamisole concentration in plasma was determined by high-performance liquid chromatography as previously described (García *et al.*, 1990).

Individual and mean plasma levamisole concentration–time data were analysed using both compartmental and non-compartmental methods. For compartmental analysis, the iterative weighted non-linear least-squares regression program PCNONLIN (Metzler & Weiner, 1986) was used and initial estimates of the parameters were determined by JANA (Dunne, 1985). The equation for the two-compartment model, used to describe levamisole pharmacokinetics, was

$$C = Ae^{-\alpha t} + Be^{-\beta t}.$$

Thus the values for α, β, A and B were obtained using initial estimates. The other parameters were calculated by standard methods (Gibaldi & Perrier, 1982). Several pharmacokinetic parameters were calculated using non-compartmental methods, based on statistical moments theory (Yamaoka *et al.*, 1978; Benet & Galeazzi, 1979; Gibaldi & Perrier, 1982). The linear terminal slope (β) was calculated using the method of least-squares. The half-life ($t_{1/2\beta}$) was calculated for the quotient 0.693/β. The area under the plasma concentration–time curve to the last time point (AUC_0^t) was calculated by the trapezoidal rule, and the total area under the plasma concentration–time curve (AUC) by adding AUC_0^t to the residual area AUC_t^∞ (calculated by the quotient of C_t, the last experimental plasma concentration, and the terminal slope, β). The area under the curve of the product of the time (t) and the plasma drug concentration (C) vs. (t) from time zero to infinity ($AUMC$) was calculated using the linear trapezoidal rule with extrapolation to infinity. The mean residence time (MRT) was determined by the equation

$$MRT = AUMC/AUC.$$

TABLE I. Mean plasma concentration of levamisole in five rabbits after intravenous administration

Time	Dose		
(min)	12.5 mg/kg ($\bar{x}$ ± SD)	16.0 mg/kg ($\bar{x}$ ± SD)	20.0 mg/kg ($\bar{x}$ ± SD)
3	6.29 ± 1.69	7.62 ± 0.43	12.16 ± 1.31
5	4.69 ± 1.06	6.90 ± 0.34	9.53 ± 0.13
10	3.70 ± 0.66	5.21 ± 0.50	7.59 ± 0.88
15	2.68 ± 0.46	4.34 ± 0.22	6.52 ± 1.52
20	2.42 ± 0.48	3.86 ± 0.30	5.27 ± 0.90
25	1.83 ± 0.25	3.37 ± 0.44	4.41 ± 1.18
30	1.81 ± 0.28	3.17 ± 0.37	3.84 ± 1.08
60	1.14 ± 0.37	1.77 ± 0.44	2.47 ± 0.48
90	0.74 ± 0.18	1.34 ± 0.30	1.66 ± 0.57
120	0.42 ± 0.18	0.93 ± 0.42	1.12 ± 0.73
150	0.35 ± 0.07	0.64 ± 0.18	0.91 ± 0.51
180	0.24 ± 0.03	0.38 ± 0.07	0.66 ± 0.52
210	0.19 ± 0.04	0.35 ± 0.12	0.48 ± 0.31
240	0.19 ± 0.02	0.25 ± 0.11	0.33 ± 0.32

$\bar{x}$ = mean plasma concentration (μg/ml); SD = standard deviation.

The total body clearance (Cl) was calculated from the quotient of the dose (D) and AUC. The terminal volume of distribution ($V_{d\beta}$) was calculated from the ratio of the total body clearance (Cl) and the terminal slope (β). The volume of distribution at steady state ($V_{d(ss)}$) was determined by the equation

$$V_{d(ss)} = D.AUMC/(AUC)^2.$$

The pharmacokinetic parameters determined by compartmental and non-compartmental models were compared using the paired Student's t-test. Significance was tested at the level of $P \leq 0.05$.

RESULTS

Table I shows the mean plasma concentration values of levamisole obtained after the i.v. administration of 12.5, 16.0 and 20.0 mg/kg body weight. The mean levamisole plasma concentration–time profiles and the best fitted curves for the three doses are shown in Fig. 1. The values of the pharmacokinetic parameters determined by compartmental analysis for each dose are shown in Table II, and those calculated by non-compartmental analysis in Table III. The pharmacokinetic disposition of levamisole both in the fifteen rabbits and in the three mean plasma concentration–time data sets was better described by a two-compartmental open model. The pharmacokinetics followed by levamisole in the dose interval studied, as the AUC/dose indicates, was linear. In addition, no significant statistical differences were found when the compartmental parameters α, β, Cl and $V_{d(ss)}$ were compared, although important inter-individual variations were observed. Thus, the clearance range was from 26.4 to 77.5 ml/(kg.min). Levamisole was also shown to have a high distribution volume at the steady state ($V_{d(ss)}$) with a range of 2369 to 5095 ml/kg.

In the same way as in the compartmental analysis, some non-compartmental parameters were compared and there were no

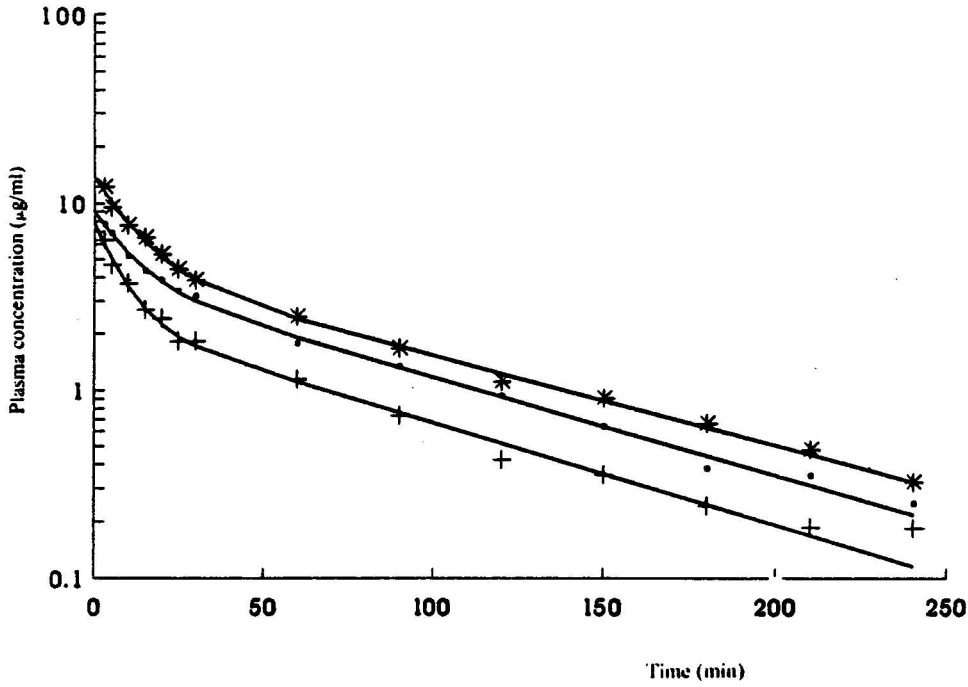

FIG. 1. Mean plasma concentration in five rabbits after i.v. administration of levamisole at doses of 12.5 (+), 16.0 (■) and 20.0 (*) mg/kg body weight. Continuous lines are fitted by the PCNONLIN program.

TABLE II. Pharmacokinetic parameters obtained by compartmental analysis in five rabbits after intravenous administration of levamisole

Parameter	Dose		
	12.5 mg/kg ($\bar{x}$ ± SD)	16.0 mg/kg ($\bar{x}$ ± SD)	20.0 mg/kg ($\bar{x}$ ± SD)
A (μg/ml)	6.24 ± 2.73	5.27 ± 1.01	10.58 ± 2.35
B (μg/ml)	2.14 ± 0.54	3.83 ± 9.66	5.08 ± 1.02
α (min $^{-1}$)	0.1278 ± 0.0592*	0.1019 ± 0.0431*	0.1282 ± 0.0536*
β (min $^{-1}$)	0.0114 ± 0.0019*	0.0126 ± 0.0014*	0.0124 ± 0.0021*
$t_{1/2\alpha}$ (min)	6.30 ± 2.39	7.66 ± 2.73	6.10 ± 2.19
$t_{1/2\beta}$ (min)	62.70 ± 13.36	55.92 ± 6.01	57.54 ± 11.61
K_{12} (min $^{-1}$)	0.0644 ± 0.0427	0.0390 ± 0.0232	0.0596 ± 0.0380
K_{21} (min $^{-1}$)	0.0411 ± 0.0117	0.0500 ± 0.0183	0.0494 ± 0.0159
K_{13} (min $^{-1}$)	0.1156 ± 0.1898	0.0254 ± 0.0048	0.0316 ± 0.0079
V_c (ml/kg)	1705.4 ± 724.4	1782.8 ± 224.6	1305.7 ± 214.9
V_p (ml/kg)	1813.0 ± 960.9	1296.4 ± 274.6	1429.5 ± 327.3
AUC (μg.min/ml)	244.34 ± 68.77	362.61 ± 49.625	520.54 ± 162.20
Cl (ml/kg/min)	54.61 ± 15.75*	44.74 ± 5.63*	41.22 ± 11.35*
$V_{d\beta}$ (ml/kg)	4782.4 ± 964.6	3572.4 ± 404.2	3284.9 ± 463.3
V_{dss} (ml/kg)	3878.5 ± 770.3*	3279.3 ± 372.6*	2735.2 ± 247.9*
AUC/dose (kg.min/ml)	0.0195 ± 0.0055*	0.0237 ± 0.0035*	0.0260 ± 0.0081*

$\bar{x}$ = mean; SD = standard deviation; * = not significantly different ($P \leq 0.05$).

TABLE III. Pharmacokinetic parameters obtained by non-compartmental analysis in five rabbits after intravenous administration of levamisole

Parameter	Dose		
	12.5 mg/kg ($\bar{x}$ ± SD)	16.0 mg/kg ($\bar{x}$ ± SD)	20.0 mg/kg ($\bar{x}$ ± SD)
β (min $^{-1}$)	0.0120 ± 0.0023*†	0.0120 ± 0.0015*†	0.0125 ± 0.0025*†
$t_{1/2\beta}$ (min)	59.27 ± 10.43	58.79 ± 8.16	57.76 ± 13.55
AUC (μg.min/ml)	238.72 ± 37.48†	381.28 ± 49.84‡	520.60 ± 179.41†
$AUMC$ (μg.min^2/ml)	16 122.9 ± 2509.2	27 605.0 ± 7126.1	40 239.2 ± 24 924.9
MRT (min)	69.24 ± 12.98*	71.67 ± 11.85*	73.06 ± 19.64*
Cl (ml/kg/min)	53.48 ± 8.97*†	42.52 ± 5.39*‡	42.21 ± 14.15*†
$V_{d\beta}$ (ml/kg)	4645.4 ± 1497.5	3578.1 ± 527.6	3328.7 ± 567.6
V_{dss} (ml/kg)	3759.6 ± 1279.8*†	3014.9 ± 392.3*†	2943.3 ± 765.6*†

$\bar{x}$ = mean; SD = standard deviation; * = not significantly different ($P \leq 0.05$); † = not significantly different ($P \leq 0.05$) from the values found in Table II; ‡ = not significantly different ($P \leq 0.02$) from the values found in Table II.

significant differences in the values for the three studied doses, although they also showed inter-individual variation. Thus, the range for the mean residence time (*MRT*) was from 54.6 to 107.3 min. Finally, there were no statistically significant differences between the values obtained using the compartmental and non-compartmental analysis when β, *AUC*, *Cl* and $V_{d(ss)}$ were compared. In the 16.0-mg/kg dose there were some differences found in the *AUC* and *Cl* values, but they were not statistically significant at the level of $P \leq 0.02$.

DISCUSSION

Levamisole showed a two-compartmental disposition in rabbits. This was also reported by Galtier *et al.* (1983) in pigs and by Nielsen & Rasmussen (1983) in pigs and goats, after the i.v. administration of 5 mg/kg. However, Watson *et al.* (1988) showed that dogs followed a one-compartmental pattern after the i.v. administration of 10 mg/kg. This may be due to the fact that the authors did not have enough experimental points during the first half-hour, and also to the fact that levamisole presents a rapidly declining initial stage. The biexponential character of the curve is not immediately obvious.

The mean values of the K_{12}/K_{21}, V_c/V_p and K_{12}/K_{13} for each dose, as well as the volume of the central (V_c) and peripheral (V_p) compartment values, show that levamisole has a wide distribution within the rabbit, with ready access to the peripheral compartment where it is not appreciably retained. However, it is possible that it attaches to a tissue in the central compartment, in view of the fact that the distribution volume is higher than the total volume of the organism and that the K_{12} and K_{21} values are similar. Analogously, Graziani & De Martin (1977) showed that levamisole, in pigs and cattle, accumulates principally in liver and kidney after oral administration, and that the levels were considerably lower in fat and brain. Other authors have also found very high values of $V_{d(ss)}$ for levamisole in humans and different animal species; for instance, Galtier *et al.* (1981) in sheep and goats, Luyckx *et al.* (1982) in humans, Nielsen & Rasmussen (1983) in pigs and Watson *et al.* (1988) in dogs.

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