# INTRA-ARTERIAL PHARMACOKINETICS AND PULMONARY FIRST-PASS OF LEVAMISOLE IN RABBITS

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The pharmacokinetics of levamisole after intra-arterial administration of 12.5, 16 and 20 mg kg$^{-1}$ was investigated in rabbits. After compartmental analysis, the disposition of levamisole was well described by a two-compartment open model with mean values $\pm$ SD of: $\alpha = 0.1650 \pm 0.0839, 0.1611 \pm 0.0298, 0.2312 \pm 0.0540$ min$^{-1}$, and $\beta = 0.0118 \pm 0.0022, 0.0125 \pm 0.0026, 0.0120 \pm 0.0024$ min$^{-1}$, for the three doses studied, respectively. There were no dose-related differences (one-way analyses of variance (ANOVA), $P \leq 0.05$) in $\alpha$, $\beta$, total body clearance ($Cl$) and volume of distribution at steady state ($V_{ss}$). The AUC increased significantly with the doses (249.7, 376.7 and 562.5 $\mu$g min ml$^{-1}$). After non-compartmental analysis there were no significant differences in plasma elimination rate constant ($\lambda$), MRT and $V_{ss}$ as a function of dose, but these differences were significant for $Cl$, between 16 and 20 mg kg$^{-1}$, and AUC (one-way ANOVA, $P \leq 0.05$). The two-way ANOVA showed no significant differences between the values obtained for the three doses when $\lambda$–$\beta$, $Cl$, $V_{ss}$ and $V_a$ were compared while AUC showed significant changes. On the other hand, the pharmacokinetic analysis (compartmental and non-compartmental) showed significant differences in AUC, $Cl$, $V_{ss}$ and $V_a$, but there were no significant differences when $\lambda$–$\beta$ were compared. The slow clearance of levamisole by rabbit lung compared to a high pulmonary blood flow rate makes the possibility of significant first-pass lung metabolism unlikely in this animal species. © 2002 Elsevier Science Ltd. All rights reserved.

KEY WORDS: levamisole, pulmonary first-pass, rabbits.

## INTRODUCTION

Levamisole (l-2,3,5,6-tetrahydro-6-phenylimidazo-[2,1-b]thiazole) is a broad-spectrum anthelmintic active against most gastrointestinal and pulmonary nematodes which parasitize man and domestic animals [1, 2].

Besides its anthelmintic activity, levamisole also possesses immunomodulatory properties [2, 3]. On the basis of these properties this drug has been used to improve vaccine therapy in numerous species [4, 5] and for treatment of different diseases affecting domestic animals [6, 7]. Its effectiveness as an adjuvant in cancer therapy and its immunomodulating effects have also been proved in men and animals [8, 9].

Organs other than liver possess ability to eliminate drugs, though their metabolic capacity is low [10]. Lungs, in addition to their role in gas exchange, have been shown to perform non-respiratory functions such as the uptake, accumulation and metabolism of numerous chemicals [11, 12], particularly some basic and volatile drugs, environmental toxicants and endogenous substances [10].

The purpose of this study is to establish several pharmacokinetic parameters of levamisole after intra-arterial administration in the rabbit, a target species for this drug and estimate the pulmonary clearance and extraction ratio during the first-passage through the lung by comparison of plasma levamisole concentrations after intravenous [13] and intra-arterial administration at 12.5, 16 and 20 mg kg$^{-1}$ in rabbits.

## METHODS

### Animals

Fifteen healthy male New Zealand white rabbits weighing between 2.500 and 3.400 kg were used. Animals were housed in individual cages. Environmental

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conditions were as follows: a 12 h light–dark cycle at $19 \pm 2\,°C$ room temperature and $55 \pm 10\%$ relative humidity. Rabbits were allowed water and rabbit chow *ad libitum*.

### Treatment and blood sampling

Rabbits were randomly distributed in three groups of five animals each depending on the administered dose (12.5, 16 and 20 mg kg$^{-1}$).

The animals were anaesthetized with sodium pentobarbital (30 mg kg$^{-1}$, intravenous administration in the ear marginal vein), and the left carotid artery was canulated with a Silastic Medical Grade Tubing$^{®}$ catheter 1.02 mm ID $\times$ 2.16 OD.

Levamisole HCl administration was carried out after total recovery from anaesthesia was achieved. Each animal received the corresponding dose dissolved in 0.3 ml sterile water through the arterial catheter.

Heparinized blood samples (3 ml) were collected from the carotid artery at 0, 5, 10, 15, 20, 25, 30, 60, 90, 120, 150, 180, 210 and 240 min after drug administration. Immediately after collection, plasma was separated by centrifugation and stored at $-20\,°C$ until analysed.

### Analysis

Levamisole plasma concentrations were determined by high-performance liquid chromatography with UV detection, following the method previously described by García *et al.* 1990 [14]. The lowest detectable concentration of levamisole in plasma was 0.08 $\mu$g ml$^{-1}$.

### Pharmacokinetic analysis

Pharmacokinetic analysis was performed based on a compartmental as well as on a non-compartmental description of the data observed.

*Compartmental analysis.* Individual plasma concentration–time profiles of levamisole after intra-arterial (i.a.) administration were analysed using the PCNONLIN computer program [15] with reciprocal concentration weights ($1/C$). Initial estimates of the parameters were determined by the JANA program [16]. The pharmacokinetic model (one, two or three compartments) best describing the experimental data was determined by application of Akaike's information criterion (AIC) [17] and graphical analysis of weighted residuals. The equation for the two-compartment open model, used to describe levamisole pharmacokinetics was:

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

where $\alpha$ and $\beta$ are the disposition constants, and $A$ and $B$ are their respective zero time intercepts. The other compartmental parameters were calculated by standard methods [18].

*Non-compartmental analysis.* The model-independent pharmacokinetic parameters were calculated using expressions based on statistical moments theory [19] and

formulae described by Gibaldi and Perrier, 1982 [18]. The plasma elimination rate constant ($\lambda$) was calculated by least square regression of the logarithm of plasma concentration versus time curve over the terminal elimination phase.

The area under the plasma concentration–time curve from time zero to the last determined sample time (AUC$_{0-t}$) was calculated by the trapezoidal rule, and the total area under the plasma concentration versus time curve (AUC) by adding AUC$_{0-t}$ to the residual area AUC$_{t-\infty}$ (calculated from $C_t$, the last experimental plasma concentration, divided by the terminal slope, $\lambda$). The total area under the first moment curve (AUMC) from time zero to time infinite was calculated by adding AUMC$_{0-t}$ to AUMC$_{t-\infty}$. The area from time 0 to $t$ was determined using the trapezoidal rule, and the area from time $t$ to $\infty$ was given by: AUMC$_{t-\infty} = t \cdot C_t/\lambda + C_t/\lambda^2$.

The mean residence time (MRT) was determined by using the equation: MRT $=$ AUMC/AUC.

Total body clearance was calculated from the quotient of the dose ($D$) and AUC. The terminal volume of distribution ($V_a$) was calculated from the ratio of the total body clearance ($Cl$) and the terminal slope ($\lambda$). The volume of distribution at steady state ($V_{ss}$) was determined by using the equation: $V_{ss} = $ MRT $\times$ dose/AUC.

*Pulmonary first-pass effect studies.* In order to determine if levamisole undergoes pulmonary first-pass effect, data obtained after intra-arterial administration were compared to those previously obtained after intravenous administration [13].

Pulmonary clearance ($Cl_p$) and extraction ratio ($E_p$) were calculated by using the equations: $Cl_p = (Cl_{tot})_{i.v.} - (Cl_{tot})_{i.a.}$ and $E_p = 1 - (\text{AUC})_{i.v.}/(\text{AUC})_{i.a.}$, respectively, where the subscripts i.v. and i.a. stand for the intravenous and intra-arterial administration, respectively [12].

### Statistical evaluation

All pharmacokinetic parameters were calculated for each animal and the data presented as mean $\pm$ SD. The data obtained from the three groups were compared for statistical significance by using the one-way and two-way analyses of variance (ANOVA). When results were significant, Duncan 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. One-way analysis of variance was used to assess differences with dose and two-way ANOVA was used to test the effect of dose and pharmacokinetic analysis (compartmental and non-compartmental).

## RESULTS

The values of the pharmacokinetic parameters determined by both compartmental and non-compartmental analysis for each dose after i.a. administration are given in tables I and II, respectively.

**Table I**
**Pharmacokinetic parameters obtained by compartmental analysis in rabbits after intra-arterial administration of levamisole[a]**

| Parameters | Dose (mg kg$^{-1}$) | | |
|---|---|---|---|
| | 12.5 | 16 | 20 |
| $A (\mu g\ ml^{-1})^{b}$ | $13.02 \pm 7.99$ | $16.23 \pm 6.65$ | $50.93 \pm 44.78$ |
| $B (\mu g\ ml^{-1})^{c,d}$ | $2.037 \pm 0.436$ | $3.475 \pm 1.088$ | $4.301 \pm 0.852$ |
| $\alpha\ (min^{-1})^{b}$ | $0.1650 \pm 0.0839$ | $0.1611 \pm 0.0298$ | $0.2312 \pm 0.0540$ |
| $\beta\ (min^{-1})^{b}$ | $0.0118 \pm 0.0022$ | $0.0125 \pm 0.0026$ | $0.0120 \pm 0.0024$ |
| $k_{12}\ (min^{-1})^{b}$ | $0.0824 \pm 0.0549$ | $0.0816 \pm 0.0133$ | $0.1187 \pm 0.0297$ |
| $k_{21}\ (min^{-1})^{b}$ | $0.0344 \pm 0.0099$ | $0.0395 \pm 0.0064$ | $0.0377 \pm 0.0121$ |
| $k_{10}\ (min^{-1})^{b}$ | $0.0601 \pm 0.0311$ | $0.0525 \pm 0.0196$ | $0.0869 \pm 0.0518$ |
| $AUC (\mu g\ min\ ml^{-1})^{d,e}$ | $249.7 \pm 30.0$ | $376.7 \pm 63.9$ | $562.5 \pm 171.1$ |
| $Cl (ml\ kg^{-1}\ min^{-1})^{b}$ | $50.66 \pm 6.32$ | $43.29 \pm 6.00$ | $38.43 \pm 12.08$ |
| $V_{c}\ (l\ kg^{-1})^{b}$ | $1.089 \pm 0.673$ | $0.916 \pm 0.360$ | $0.6739 \pm 0.5193$ |
| $V_{p}\ (l\ kg^{-1})^{b}$ | $2.042 \pm 0.267$ | $1.855 \pm 0.598$ | $1.820 \pm 0.922$ |
| $V_{ss}\ (l\ kg^{-1})^{b}$ | $3.130 \pm 0.627$ | $2.771 \pm 0.952$ | $2.494 \pm 1.430$ |
| $V_{a}\ (l\ kg^{-1})^{b}$ | $4.352 \pm 0.612$ | $3.626 \pm 1.063$ | $3.355 \pm 1.374$ |
| $t_{1/2\alpha} (min)^{b}$ | $4.945 \pm 1.935$ | $4.434 \pm 0.887$ | $3.155 \pm 0.850$ |
| $t_{1/2\beta}\ (min)^{b}$ | $60.34 \pm 12.10$ | $57.58 \pm 12.53$ | $59.54 \pm 12.15$ |
| $t_{1/2k10}\ (min)^{b}$ | $14.70 \pm 8.05$ | $14.63 \pm 4.97$ | $10.94 \pm 6.51$ |
| $C_{0} (\mu g\ ml^{-1})^{b}$ | $15.06 \pm 8.04$ | $19.70 \pm 7.24$ | $55.23 \pm 45.41$ |
| $AUC/dose (kg\ min\ ml^{-1})^{b}$ | $0.0200 \pm 0.0024$ | $0.0235 \pm 0.0040$ | $0.0281 \pm 0.086$ |

[a] Values are the mean $\pm$ standard deviation for five rabbits. One-way ANOVA results: [b] no significant differences ($P \leq 0.05$); significant differences (Duncan test, $P \leq 0.05$) between: [c] 12.5 and 16 mg kg$^{-1}$; [d] 12.5 and 20 mg kg$^{-1}$; [e] 16 and 20 mg kg$^{-1}$.

**Table II**
**Pharmacokinetic parameters obtained by non-compartmental analysis in rabbits after intra-arterial administration of levamisole[a]**

| Parameters | Dose (mg kg$^{-1}$) | | |
|---|---|---|---|
| | 12.5 | 16 | 20 |
| $\lambda\ (min^{-1})^{b,f}$ | $0.0105 \pm 0.0012$ | $0.0128 \pm 0.0025$ | $0.0114 \pm 0.0018$ |
| $AUC (\mu g\ min\ ml^{-1})^{c,d,e,g,h,i,j}$ | $205.6 \pm 27.5$ | $322.3 \pm 58.7$ | $431.3 \pm 82.1$ |
| $AUMC (\mu g\ min^2\ ml^{-1})^{d}$ | $14977.9 \pm 3211.7$ | $22151.9 \pm 5440.4$ | $30568.5 \pm 8637.1$ |
| $MRT (min)^{b}$ | $72.32 \pm 7.88$ | $68.60 \pm 12.34$ | $71.38 \pm 15.90$ |
| $Cl (ml\ kg^{-1}\ min^{-1})^{e,g}$ | $61.60 \pm 7.66$ | $50.77 \pm 7.84$ | $71.87 \pm 15.30$ |
| $V_{ss}\ (l\ kg^{-1})^{b,g}$ | $4.424 \pm 0.477$ | $3.469 \pm 0.745$ | $5.167 \pm 1.647$ |
| $V_{a}\ (l\ kg^{-1})^{c,e,g}$ | $5.958 \pm 1.285$ | $4.099 \pm 1.057$ | $6.419 \pm 1.496$ |
| $AUC/dose (kg\ min\ ml^{-1})^{b,g,i}$ | $0.0165 \pm 0.0022$ | $0.0201 \pm 0.0037$ | $0.0216 \pm 0.0041$ |

[a] Values are the mean $\pm$ standard deviation for five rabbits. One-way ANOVA results: [b] no significant differences ($P \leq 0.05$); significant differences (Duncan test, $P \leq 0.05$) between: [c] 12.5 and 16 mg kg$^{-1}$; [d] 12.5 and 20 mg kg$^{-1}$; [e] 16 and 20 mg kg$^{-1}$. Two-way ANOVA results: [f] no significant differences ($P \leq 0.05$); [g] significant differences with compartmental parameter ($P \leq 0.05$); significant differences between: [h] 12.5 and 16 mg kg$^{-1}$; [i] 12.5 and 20 mg kg$^{-1}$; [j] 16 and 20 mg kg$^{-1}$.

After compartmental analysis, each individual plasma concentration–time curve was better described by a two-compartment open model following intra-arterial administration in all rabbits. Plasma levamisole concentrations after each dose declined rapidly in a biexponential fashion with a rapid early $\alpha$ phase (ranging from 0.1611 to 0.2312 min$^{-1}$) and a terminal $\beta$ phase about 15-fold lower (ranging from 0.0118 to 0.0125 min$^{-1}$). The AUC of levamisole increased significantly with dose. The clearance values were similar (50.66, 43.29 and 38.43 ml kg$^{-1}$ min$^{-1}$) and the steady state volume of distribution ranged from 2.494 to 3.130 l kg$^{-1}$. No significant differences were found when the compartmental parameters $\alpha$, $\beta$, $Cl$, $V_{ss}$ and AUC/dose were compared. Therefore, the pharmacokinetics followed by levamisole in the dose interval studied was linear, as the quotient AUC/dose indicates.

In the same way as in the compartmental analysis, some non-compartmental parameters were compared and there were no significant differences in $\lambda$, MRT and $V_{ss}$ for the three doses studied. The AUC of levamisole increased significantly with dose, while $Cl$ values were found to be statistically different between the 16 and 20 mg kg$^{-1}$ groups. We think that this difference may be due to interindividual variations in the $\lambda$ values used to calculate $Cl$.

The two-way ANOVA showed no significant differences between the values obtained for the three doses when $\lambda$–$\beta$, $Cl$, $V_{ss}$ and $V_{a}$ were compared while AUC revealed significant changes. Finally, this statistical test showed significant differences between
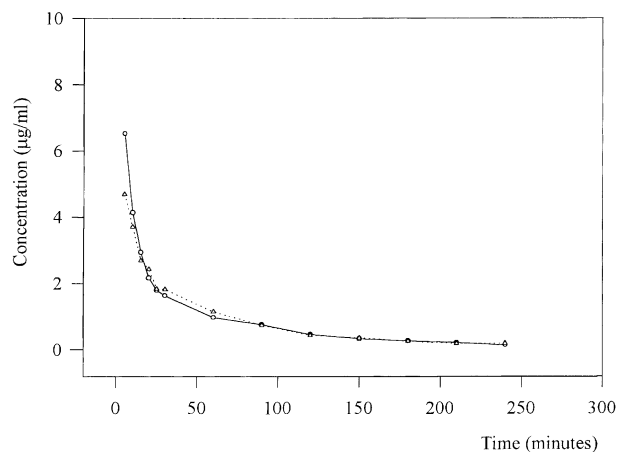
**Fig. 1.** Mean plasma concentrations of levamisole in rabbits after intra-arterial (—O—) and intravenous (--△--) administration of 12.5 mg kg$^{-1}$.
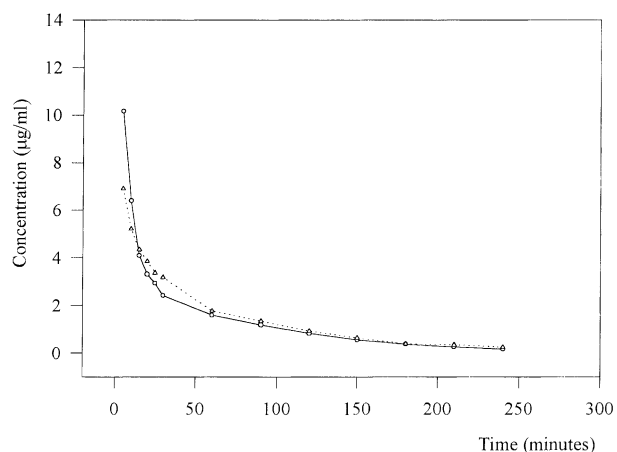


**Fig. 2.** Mean plasma concentrations of levamisole in rabbits after intra-arterial (—O—) and intravenous (--△--) administration of 16 mg kg$^{-1}$.



**Fig. 3.** Mean plasma concentrations of levamisole in rabbits after intra-arterial (—O—) and intravenous (--△--) administration of 20 mg kg$^{-1}$.

**Table III**
**Clearance ($Cl_p$) and extraction ratio ($E_p$) for levamisole in the rabbit lung**

| Parameter | Dose (mg kg$^{-1}$) | | |
|---|---|---|---|
| | *12.5* | *16* | *20* |
| $Cl_p$ (ml kg$^{-1}$ min$^{-1}$) | 3.95 | 1.45 | 2.79 |
| $E_p$ | 0.0215 | 0.0374 | 0.0746 |

the values obtained using the compartmental and non-compartmental analysis when AUC, $Cl$, $V_{ss}$ and $V_a$, were compared, but here were no significant differences between $\lambda$ and $\beta$.

Plasma levamisole concentration–time profiles following intra-arterial and intravenous [13] routes at 12.5, 16 and 20 mg kg$^{-1}$ are shown in Figs 1–3, respectively. In these figures it can be observed that the terminal elimination phase seemed to decline in an almost parallel fashion after i.v. and i.a. dosing.

Effect of dose on pulmonary first-pass clearance ($Cl_p$) and extraction ratio ($E_p$) are summarized in table III. Each parameter fluctuated slightly with dose. In the absence of dispersion data, we have employed an approach using as compartmental reference value $Cl_{i.v.}$ [13], to calculate $Cl_p$ and $E_p$ for each animal. Under these conditions, these parameters were not dose dependent (one-way ANOVA).
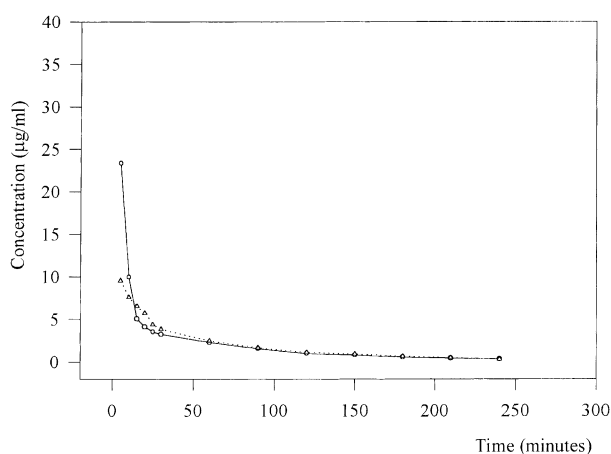
## DISCUSSION

Levamisole showed a two-compartment disposition in rabbits. This was also reported [13] in a previous study carried out after intravenous administration at the same doses to this species. The pharmacokinetics followed by levamisole in the dose interval studied, as the quotient AUC/dose indicates, was linear.

The mean values of the $k_{12}/k_{21}$, $V_c/V_p$ And $k_{12}/k_{10}$ 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.

It is clear that in rabbits the intact lung acts upon many compounds of diverse structure, such as imipramine [20, 21], amphetamine [20], pentobarbital [22] and chlorpromazine [23].

According to organ clearance concept [24, 25], $E_p = Cl_{int}/(Cl_{int} + Q)$, where $Cl_{int}$ is the intrinsic clearance and $Q$ the blood flow. $Cl_{int}$ for the pulmonary elimination of levamisole at 12.5–20 mg kg$^{-1}$ was estimated as about 2.15–7.66% of $Q$. In this way, the slow clearance of levamisole by rabbit lung compared to a high pulmonary blood flow rate makes unlikely the possibility of significant first-pass lung metabolism in this animal species.

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