Effect of first-pass hepatic metabolism on the disposition of levamisole after intravenous administration in rabbits

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Objective—To evaluate the contribution of first-pass hepatic metabolism of levamisole on levamisole disposition in rabbits.

Animals—30 male New Zealand White rabbits.

Procedures—Rabbits were randomly placed into 2 groups. Rabbits in the first group received levamisole via the marginal ear vein at the following 3 doses: 12.5, 16, and 20 mg/kg (5 rabbits for each dose). Rabbits of the second group received levamisole via the jejunal vein at the same doses (5 rabbits each). During the following 240-minute period, plasma samples were obtained and quantified for levamisole concentrations by reversed-phase high-performance liquid chromatography.

Results—No significant differences were found between pharmacokinetic parameters calculated by compartmental or noncompartmental analysis. Mean hepatic extraction ratio ranged from −0.044 to 0.017 and from 0.020 to 0.081 when area under the plasma concentration-time curve values were obtained after compartmental or noncompartmental analysis, respectively. After compartmental analysis, plasma concentration decreased bi-exponentially. Mean pharmacokinetic parameter values were as follows for each dose (12.5, 16, and 20 mg/kg, respectively): after levamisole administration via the marginal ear vein, volume of distribution at steady state (V_{ss}) = 4.26, 4.33, and 3.20 L/kg; total body clearance (Cl) = 49.04, 43.77, and 39.26 mL/kg•min; and half-life associated with β-phase ($t_{1/2\beta}$) = 77.93, 85.39, and 69.79 minutes. After levamisole administration via the jejunal vein, V_{ss} = 4.38, 2.85, and 2.97 L/kg; Cl = 48.14, 42.40, and 39.69 mL/kg•min; and $t_{1/2b}$ = 101.9, 76.71, and 76.13 minutes.

Conclusions—Levamisole has a low degree of hepatic extraction in rabbits. (*Am J Vet Res* 2003;64: 1283–1287)

Levamisole is a broad-spectrum anthelmintic that has been used in livestock against a wide range of pulmonary and gastrointestinal nematodes.[1-3] It has also been shown to modulate immune functions[4-5] in humans and animals (cattle, dogs, and cats), and it is available for this use.

Levamisole may be used against *Obeliscoides cinuculi*, *Graphidium strigosum*, and *Trichostrongylus* spp in rabbits.[6-8] Moreover, it may be used in several respiratory tract diseases as immunomodulator.[9]

Data concerning pharmacokinetics for levamisole are available for several species,[10-23] including humans.[24-25] However, no information has been published on the first-pass hepatic metabolism of levamisole. Results of studies performed in several species indicate that after levamisole administration, the percentage of the dose excreted unchanged in urine ranges from 3% in humans[24,26] to 28% in dogs.[27] Differences between species can be attributed mainly to the different rates of hepatic biotransformation, making the study of first-pass hepatic metabolism of levamisole important. The purpose of the study reported here was to evaluate the contribution of first-pass hepatic metabolism of levamisole on levamisole disposition in rabbits.

Materials and Methods

Animals—Experiments were performed on 30 male New Zealand White rabbits weighing between 2.5 and 3.5 kg. Rabbits were housed in individual cages under controlled conditions of temperature (19 ± 2°C), humidity (55 ± 10%), and a light-dark cycle (12 hour) during the experimental period. Rabbits had free access to food (pelleted) and water.

Experimental design—Rabbits were randomly placed into 2 groups of 15 rabbits each. Levamisole hydrochloride[a] was dissolved in 1 mL of sterile water and administered by bolus injection to all rabbits. All group-1 rabbits received levamisole via the marginal ear vein at the following doses: 12.5 mg/kg (n = 5), 16 mg/kg (5), and 20 mg/kg (5). All 3 doses were within the therapeutic range for this species.

All group-2 rabbits received levamisole via the jejunal vein. This route of administration was used because the entire dose of levamisole passes through the liver before it reaches the systemic circulation. As with group-1 rabbits, group-2 rabbits were also placed in 3 subgroups (of 5 rabbits each) and received the 3 different doses of levamisole to determine whether first-pass hepatic metabolism of levamisole was dose dependent. If the first-pass hepatic metabolism of levamisole is substantial, high dose administration could saturate this process.

Anesthesia was induced in all rabbits by IV administration of sodium pentobarbital[b] (30 mg/kg) in order to cannulate the left carotid artery with a heparinized silastic catheter.[c] In group-2 rabbits, the jejunal vein was also cannulated with a heparinized silastic catheter.[d]

Body temperatures were maintained at 37.5 ± 0.5°C by placement of electric blankets under rabbits. Anaesthesia was maintained in all rabbits by sodium pentobarbital (2.5 mg/kg, IV) administration. A 30-minute stabilization period was allowed between the preparation of the rabbit and

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the initiation of the experiment. The Institutional Animal Care and Use Committee of the University of León approved the protocol followed in this study in advance.

Sample collection—Heparinized blood samples (3 mL) were collected from group-1 and -2 rabbits from the left carotid artery prior to drug administration and at 3, 5, 10, 15, 20, 25, 30, 60, 90, 120, 150, 180, 210, and 240 minutes after levamisole administration. Plasma was separated immediately by centrifugation and stored at –20°C until analysed. Every 30 minutes, 5 mL of isotonic saline (0.9% NaCl) solution was administered SC.

Analytic procedure—Levamisole concentrations in plasma were measured by reversed-phase high-performance liquid chromatography with ultraviolet detection following a method previously described.[28] The limit of quantitation was 0.8 µg/mL (corresponding to a plasma concentration of 0.16 µg/mL), the limit of detection was 0.25 µg/mL, and the extraction recovery was 83.38% for the entire range of concentrations of the assay. Interday and intraday accuracy and precision were within 10%.

Pharmacokinetic analysis—Pharmacokinetic analysis was performed on the basis of a compartmental as well as a noncompartmental description of the observed data. For compartmental analysis, plasma levamisole concentration-time profiles were individually fitted to the following experimental equation:

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

where C_i is the yintercept, 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 by use of a computer program based on the nonlinear iterative least-squares regression analysis.[29] Data were fitted to the equations by use of a weighting factor 1/C, and the initial estimates of the parameters were determined by use of a computer software program.[30]

The optimal pharmacokinetic model (1, 2, and 3 compartments) was determined by application of the Akaike information criterion[31] and graphic analysis of weighted residuals. Other compartmental parameters were calculated by standard methods.[32]

The model-independent pharmacokinetic parameters were calculated by use of expressions based on statistical moments theory[33] and formulas already mentioned.[32] The plasma elimination rate constant (λ) was estimated by least-squares regression of the logarithm of plasma concentration versus time curve over the terminal elimination phase, and maximum plasma concentration (C_{max}) and the time to reach maximum concentration (t_{max}) were determined by direct observation of data.

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 with extrapolation to infinity. The mean residence time (MRT) was defined as AUMC/AUC, the total body clearance (Cl) was determined by dividing the dose by AUC, the volume of distribution at steady state (V_{ss}) was calculated from the equation V_{ss} = MRT X Cl, and the apparent volume of distribution (V_a) was defined as Cl/λ.

The hepatic extraction ratio, which reflects the contribution of first-pass hepatic metabolism, was calculated as $E_H = 1 - (AUC_{IJ}/AUC_{IV})$,[34] where AUC_{IJ} and AUC_{IV} are the AUC when levamisole was administered via the jejunal and the ear marginal veins, respectively.

Statistical analysis—All pharmacokinetic parameters were calculated for each rabbit, and values are reported as means (± SD). Differences between data sets were determined by use of the Kruskal-Wallis test. A value of $P < 0.05$ was considered significant. When results were determined to be significant, they were evaluated by the Wilcoxon test with Bonferroni correction to assess differences between data sets.

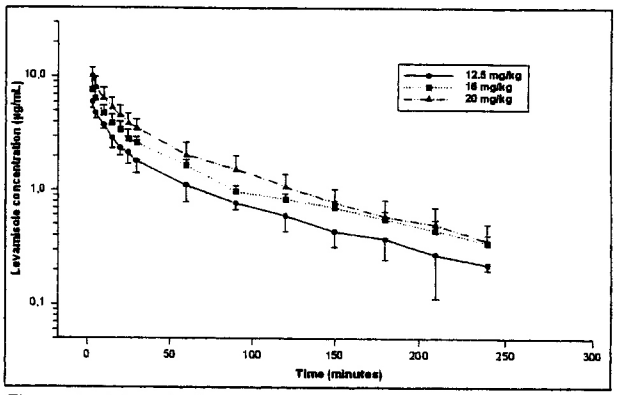

Figure 1—Mean (± SD) plasma concentrations of levamisole in rabbits after levamisole administration via the marginal ear vein at 3 different doses.

Table 1—Mean (± SD) values for pharmacokinetic parameters obtained by compartmental analysis in rabbits after levamisole administration via the marginal ear vein

Parameters	Dose (mg/kg)		
	12.5	16	20
A (µg/mL)[c]	5.37 ± 1.21	6.73 ± 3.06	9.80 ± 1.36
B (µg/mL)[c]	1.78 ± 0.39	2.37 ± 0.35	4.41 ± 1.63
α (1/min)	0.0943 ± 0.0361	0.0785 ± 0.0237	0.1153 ± 0.0617
β (1/min)	0.0091 ± 0.0014	0.0083 ± 0.00[illegible]12	0.0103 ± 0.0021
k_{12} (1/min)	0.0449 ± 0.0239	0.0360 ± 0.0158	0.0535 ± 0.0339
k_{21} (1/min)	0.0307 ± 0.0126	0.0267 ± 0.0045	0.0448 ± 0.0276
k_{10} (1/min)	0.0277 ± 0.0049	0.0241 ± 0.0066	0.0273 ± 0.0041
AUC (µg•min/mL)[a,b,c]	261.3 ± 44.4	371.0 ± 49.2	521.9 ± 87.1
Cl (mL/kg•min)	49.04 ± 8.92	43.77 ± 6.14	39.26 ± 7.00
V_c (L/kg)	1.79 ± 0.31	1.98 ± 0.82	1.46 ± 0.33
V_p (L/kg)	2.47 ± 0.67	2.34 ± 0.56	1.74 ± 0.41
V_{ss} (L/kg)	4.26 ± 0.42	4.33 ± 0.84	3.20 ± 0.74
V_a (L/kg)	5.44 ± 0.74	5.37 ± 0.89	3.98 ± 1.30
$t_{1/2\alpha}$ (min)	8.35 ± 3.49	9.72 ± 3.77	7.46 ± 3.59
$t_{1/2\beta}$ (min)	77.93 ± 11.57	85.39 ± 12.74	69.79 ± 15.20
$t_{1/2k10}$ (min)	25.64 ± 4.61	30.95 ± 10.52	25.84 ± 4.05
C_0 (µg/mL)[c]	7.15 ± 1.16	9.09 ± 3.30	14.21 ± 2.83
AUC/dose (g•min/mL)	20.90 ± 3.55	23.19 ± 3.07	26.09 ± 4.35

Different superscript letters indicate parameters that differ significantly (Kruskal-Wallis test, $P < 0.05$) between doses. Wilcoxon test with Bonferroni correction: [a]Significant differences between 12.5 and 16 mg/kg, [b]significant differences between 16 and 20 mg/kg, [c]significant differences between 12.5- and 20-mg/kg doses.

A = α zero-time intercept. B = β zero-time intercept. α and β = Apparent first-order disposition rate constants. k_{12} = Apparent first-order transfer rate constant from the central compartment to the peripheral compartment. k_{21} = Apparent first-order transfer rate constant from the peripheral compartment to the central compartment. k_{10} = Apparent first-order elimination rate constant from the central compartment. AUC = Area under the plasma concentration-time curve. Cl = Total body clearance. V_c = Apparent volume of distribution in the central compartment. V_p = Apparent volume of distribution in the peripheral compartment. V_{ss} = Volume of distribution at steady state. V_a = Apparent volume of distribution. $t_{1/2\alpha}$ = Half-life associated with α phase. $t_{1/2\beta}$ = Half-life associated with β phase. $t_{1/2k10}$ = Elimination from the central compartment half-life. C_0 = Addition of the α and β zero-time intercepts.

Results

Administration via the marginal ear vein—Following administration of levamisole via the marginal ear vein, mean plasma levamisole concentrations-time profiles for the 3 doses (12.5, 16, and 20 mg/kg; Fig 1) were best described in all rabbits by a 2-compartment open model. The compartmental pharmacokinetic parameters were calculated (Table 1). The steady-state volume of distribution ranged from 3.20 to 4.33 L/kg, and clearance ranged from 39.26 to 49.04 mL/kg•min. Values obtained for AUC increased significantly with dose (261.3, 371.0, and 521.9 μg•min/mL, respectively). No significant differences were found for the 3 doses studied when the apparent first-order disposition rate constants (distribution [α] and elimination [β]), Cl, and V_{ss} were compared.

Noncompartmental pharmacokinetic parameters were also determined (Table 2). The V_{ss} ranged from 3.06 to 4.25 L/kg, and clearance ranged from 40.29 to 51.96 mL/kg•min. Values obtained for AUC increased significantly with dose (249.3, 371.0, and 509.1 μg•min/mL, respectively). No significant differences were found between doses in λ, MRT, Cl, and V_{ss}. In addition, no significant differences were found between values obtained by compartmental and noncompartmental methods for the apparent first-order disposition rate constants (λ - β), AUC, Cl, and V_{ss}.

Table 2—Mean (± SD) values for pharmacokinetic parameters obtained by noncompartmental analysis in rabbits after levamisole administration via the marginal ear vein

Parameters	Dose (mg/kg) 12.5	16	20
λ (1/min)	0.0089 ± 0.0017	0.0075 ± 0.0010	0.0100 ± 0.0016
AUC (μg•min/mL)[a,b,c]	249.3 ± 52.2	371.0 ± 43.8	509.1 ± 87.4
AUMC (μg•min/mL)[a,c]	21,741.8 ± 5,895.9	33,277.3 ± 5,180.1	39,081.0 ± 9,251.2
MRT (min)	82.49 ± 14.26	89.63 ± 9.28	76.28 ± 7.40
Cl (mL/kg•min)	51.96 ± 10.94	43.65 ± 5.61	40.29 ± 7.35
V_{ss} (L/kg)	4.26 ± 1.12	3.91 ± 0.57	3.06 ± 0.52
AUC/dose (g•min/mL)	19.94 ± 4.17	23.19 ± 2.73	25.45 ± 4.37

λ = Elimination rate constant. AUMC = Area under the first moment curve. MRT = Mean residence time.
See Table 1 for remainder of key.

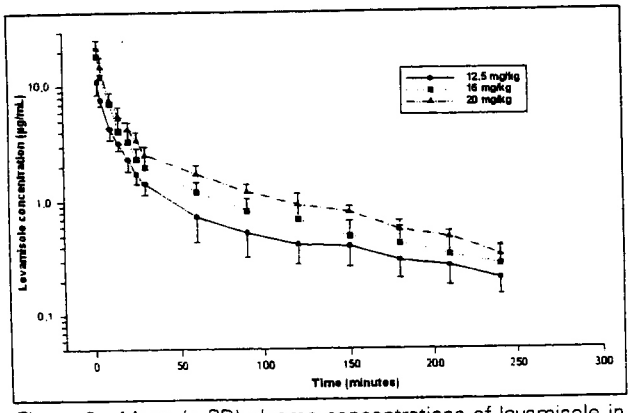

Figure 2—Mean (± SD) plasma concentrations of levamisole in rabbits after levamisole administration via the jejunal vein at 3 different doses.

Administration via the jejunal vein—The development of the 3 mean concentration-time curves after levamisole administration via the jejunal vein also followed a 2-compartment open model (Fig 2). After compartmental analysis, V_{ss} ranged from 2.85 to 4.38 L/kg (Table 3), and Cl ranged from 39.69 to 48.14 mL/kg•min. A significant increase in AUC values (267.1, 389.5, and 512.9 μg•min/mL) was observed with increasing doses. Results of a Kruskall-Wallis test indicated that the dose did not affect any other pharmacokinetic parameter than AUC.

Regarding noncompartmental pharmacokinetic parameters for the 3 doses (Table 4), V_{ss} values ranged from 3.09 to 3.99 L/kg, and Cl ranged from 43.55 to 50.67 mL/kg•min. Values obtained for AUC increased significantly with dose (254.2, 357.1, and 467.8 μg•min/mL, respectively). Significant differences were found between doses for AUC when noncompartmental parameters were compared. No signifi-

Table 3—Mean (± SD) values for pharmacokinetic parameters obtained by compartmental analysis in rabbits after levamisole administration via the jejunal vein

Parameters	Dose (mg/kg) 12.5	16	20
A (μg/mL)[a,c,d]	13.62 ± 3.57	25.39 ± 5.81	28.10 ± 6.38
B (μg/mL)[c]	1.25 ± 0.60	2.20 ± 0.46	3.19 ± 0.96
α (1/min)[d]	0.1325 ± 0.0402	0.1690 ± 0.0497	0.1676 ± 0.0349
β (1/min)	0.0076 ± 0.0027	0.0098 ± 0.0031	0.0093 ± 0.0015
k_{12} (1/min)[d]	0.0655 ± 0.0247	0.0827 ± 0.0289	0.0896 ± 0.0252
k_{21} (1/min)[d]	0.0182 ± 0.0075	0.0226 ± 0.0064	0.0251 ± 0.0062
k_{10} (1/min)[d]	0.0565 ± 0.0176	0.0735 ± 0.0219	0.0622 ± 0.0065
AUC (μg•min/mL)[a,b,c]	267.1 ± 49.6	389.5 ± 77.2	512.9 ± 75.4
Cl (mL/kg•min)	48.14 ± 9.07	42.40 ± 8.46	39.69 ± 6.01
V_c (L/kg)[d]	0.9064 ± 0.3069	0.6079 ± 0.1620	0.6494 ± 0.1557
V_p (L/kg)[d]	3.48 ± 1.67	2.24 ± 0.72	2.32 ± 0.59
V_{ss} (L/kg)	4.38 ± 1.98	2.85 ± 0.85	2.97 ± 0.74
V_a (L/kg)	7.16 ± 3.42	4.54 ± 1.15	4.40 ± 1.17
$t_{1/2\alpha}$ (min)[d]	5.65 ± 1.75	4.37 ± 1.17	4.27 ± 0.80
$t_{1/2\beta}$ (min)	101.90 ± 38.90	76.71 ± 24.81	76.13 ± 11.90
$t_{1/2k10}$ (min)[d]	13.12 ± 3.49	10.37 ± 4.06	11.24 ± 1.24
C_0 (μg/mL)[a,c,d]	14.87 ± 4.05	27.59 ± 6.07	32.18 ± 7.25
AUC/dose (g•min/mL)	21.37 ± 3.97	24.34 ± 4.82	25.65 ± 3.77

[d]Significantly (Kruskal-Wallis test, $P < 0.05$) different from values obtained following administration of levamisole via the marginal ear vein.
See Table 1 for remainder of key.

Table 4—Mean (± SD) values for pharmacokinetic parameters obtained by noncompartmental analysis in rabbits after levamisole administration via the jejunal vein

Parameters	Dose (mg/kg) 12.5	16	20
λ (1/min)	0.0064 ± 0.0018	0.0084 ± 0.0016	0.0089 ± 0.0009
AUC (μg•min/mL)[a,b,c]	254.2 ± 47.9	357.1 ± 55.2	467.8 ± 69.2
AUMC (μg•min²/mL)[c]	20,453.3 ± 5,558.7	29,928.0 ± 6,808.2	33,101.3 ± 5197.0
MRT (min)	79.76 ± 15.69	83.24 ± 8.46	70.86 ± 6.64
Cl (mL/kg•min)	50.67 ± 10.08	45.68 ± 7.02	43.55 ± 6.73
V_{ss} (L/kg)	3.99 ± 0.94	3.78 ± 0.47	3.09 ± 0.56
AUC/dose (g•min/mL)	20.34 ± 3.83	22.32 ± 3.45	23.39 ± 3.46

See Table 1 and 2 for key.

cant differences were found between the values obtained by compartmental and noncompartmental methods for (λ - β), AUC, Cl, and V_{ss}. Values obtained for AUC per dose indicated that the pharmacokinetics were linear in the range of doses studied.

To determine the effect of first-pass hepatic metabolism, an analysis was performed to investigate the influence of location of IV administration (ie, marginal ear vein vs jejunal vein). Results indicate that the following compartmental parameters are affected by location of IV administration: α zero-time intercept (A), α, apparent first-order transfer rate constant from the central compartment to the peripheral compartment (k_{12}), apparent first-order transfer rate constant from the peripheral compartment to the central compartment (k_{21}), apparent first-order elimination rate constant from the central compartment (k_{10}), apparent volume of distribution in the central compartment (V_c), half-life associated with α phase ($t_{1/2\alpha}$), elimination from the central compartment half-life ($t_{1/2k10}$), and the addition of the α and β zero-time intercepts (C_0). No significant differences were found for noncompartmental parameters. Mean hepatic extraction ratio ranged from –0.044 to 0.017 and from 0.020 to 0.081 when AUC values were obtained after compartmental or noncompartmental analysis, respectively.

Discussion

Levamisole is extensively metabolized in most species. The metabolites of levamisole are numerous, although oxidation and hydrolysis are considered to be the main metabolic reactions.[18,35]

It has been reported that in the rat,[14] 91 to 94% of the levamisole administered is metabolized. These authors also found that p-hydroxylation was not major metabolic route. However, results of other studies[27,36] indicate that this metabolic route is important in levamisole biotransformation.

Results of studies[36,37] performed in dogs reveal that the degree of metabolism of levamisole in dogs is lower than in rats. These authors reported that 28[36] and a 20%[37] of the dose of levamisole administered was excreted unchanged in urine of dogs. When levamisole was administered to pigs, sheep, and goats, the percentage of the dose excreted unchanged in urine ranged from 5 to 10%.[38] Results obtained for humans were even lower, with 3% of levamisole excreted unaltered in urine.[24,26] Taking into account all these findings, the possibility of a first-pass hepatic effect for levamisole metabolism was suggested.[25]

As a rule, it is difficult to predict whether a particular compound is a good candidate for hepatic uptake.[39] In our study, we estimated the first-pass metabolism of levamisole in the liver. In all cases, mean values of the hepatic extraction ratio were near zero, indicating that levamisole is a drug with a low hepatic extraction in rabbits. Therefore, other organs and tissues should participate in levamisole clearance. It must be pointed out that the difference between AUC values obtained after levamisole administration via the jejunal versus the marginal ear vein was 8.1%, which was observed with the dose of 20 mg/kg. It is therefore possible that hepatic clearance mechanisms are more efficient when presented with a high dose. This finding is in agreement with the possibility that levamisole acts as an enzymatic inductor.[40] In addition, as a result of the variability in levamisole metabolism, we believe that hepatic extraction could be higher in species other than rabbits.

In addition to the study of the first-pass effect, we have also evaluated other pharmacokinetic parameters of levamisole after levamisole administration via jejunal and the marginal ear veins. Levamisole plasma concentration versus time data was best described by a 2-compartment open model in both situations, which was also reported for nonanaesthetized rabbits,[16] pigs,[15,21] sheep,[14] and goats,[21,22] whereas Watson et al[23] reported that metabolism of levamisole in dogs followed a 1-compartment open model.

Mean values of k_{12}/k_{21} and k_{12}/k_{10} after administration via the marginal ear vein revealed the tendency of levamisole to distribute within the peripheral compartment rather than to be eliminated. The high V_{ss} values obtained confirm the wide distribution of levamisole. High values of V_{ss} for levamisole have also been found in pigs and goats,[20] sheep,[14] dogs,[23] and rabbits.[16]

Following administration via the jejunal vein, levamisole also has a wide distribution within the body, with ready access to the peripheral compartment, where it is appreciably retained. Clearance values were similar to those obtained when the drug was administered via the marginal ear vein, which would explain the fast elimination of levamisole from the body.

With regard to the influence of location of IV administration (ie, marginal ear vein vs jejunal vein) on the pharmacokinetics of levamisole in rabbits, results of a Kruskall-Wallis test revealed that noncompartmental parameters calculated after administration via the marginal ear vein or jejunal vein are kinetically equivalent. For compartmental parameters, the location of IV administration only influences the initial phase of distribution and elimination, whereas those parameters that characterize the terminal phase (β zero-time intercept, β, and half-life associated with β phase) or the whole process (AUC, Cl, V_{ss}, and AUC per dose) remain invariable.

[a]Levamisole hydrochloride, Sigma Chemical Co, St Louis, Mo.
[b]Roig Farma, Terrasa, Barcelona, Spain.
[c]0.8-mm internal diameter, 2.16-mm outer diameter, 15 cm in length, Dow Corning Co, Midland, Mich.
[d]0.51-mm internal diameter, 10-cm in length, Dow Corning Co, Midland, Mich.

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