Bioavailability of levamisole after intramuscular and oral administration in sheep

M. Fernández*, J.J. García*, M. Sierra*, M.J. Diez* and M.T. Terán†

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 $k_{e}$, $C_{max}$, $t_{max}$ 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 $k_{e}$, $C_{max}$, $t_{max}$ 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.

Introduction

Levamisole (1,2,3,5,6-tetrahydro-6-phenylimidazo[2,1-b]thiazole) is a synthetic anthelmintic widely used for the control of gastro-intestinal worms and lungworms in sheep[1]. Levamisole also has immunostimulant effects[20]. 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[48,16,7], in goats[8,9,10], in pigs[9,10,12], in rabbits[9,10,12], in dogs[10,13,14] and in human beings[15,16,17]. A limited amount of information on its pharmacokinetics in sheep has been published[8,10,19,20].

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

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Materials and Methods

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 AUCm or AUC/AUC, where AUCm, AUC, and AUC, 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 Kruskal-Wallis test(26). Differences in pharmacokinetic data between dosage and administration routes were analysed for statistical differences using the Friedman test(56).

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, tmax and F after i.m. administration were very similar for the three doses. Moreover, Cmax values increased with dose. The Kruskal-Wallis test showed no significant differences in k, tmax and F. However, significant differences were found in Cmax.

After oral administration the k, tmax 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. Cmax 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 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 values showed a slower absorption phase than intramuscular 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 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 Garcia 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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Dose</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>5 mg/kg</td>
</tr>
<tr>
<td>Intramuscular administration</td>
<td></td>
</tr>
<tr>
<td>AUC (µg.min/ml)*</td>
<td>201.2 ± 10.5</td>
</tr>
<tr>
<td>kₙ (min⁻¹)ᵇ</td>
<td>0.0591 ± 0.0200</td>
</tr>
<tr>
<td>t₀₂₀ (min)</td>
<td>21.78 ± 13.84</td>
</tr>
<tr>
<td>t½ (min)</td>
<td>123.2 ± 27.0</td>
</tr>
<tr>
<td>Cmax (µg/ml)*</td>
<td>1.613 ± 0.285</td>
</tr>
<tr>
<td>tmax (min)*</td>
<td>25.28 ± 1.97</td>
</tr>
<tr>
<td>F (%)</td>
<td>77.76 ± 10.21</td>
</tr>
<tr>
<td>Oral administration</td>
<td></td>
</tr>
<tr>
<td>AUC (µg.min/ml)**</td>
<td>160.0 ± 30.2</td>
</tr>
<tr>
<td>kₙ (min⁻¹)ᵇ</td>
<td>0.0414 ± 0.0307</td>
</tr>
<tr>
<td>t₀₂₀ (min)</td>
<td>25.94 ± 9.65</td>
</tr>
<tr>
<td>t½ (min)</td>
<td>243.2 ± 78.6</td>
</tr>
<tr>
<td>Cmax (µg/ml)**</td>
<td>0.7961 ± 0.2697</td>
</tr>
<tr>
<td>tmax (min)**</td>
<td>39.37 ± 13.80</td>
</tr>
<tr>
<td>F (%)</td>
<td>61.11 ± 9.32</td>
</tr>
</tbody>
</table>

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).
administration of 12.5, 16 and 20 mg/kg of levamisole, respectively, to rabbits). These differences between the \( k_2 \) values can be explained by species differences and different oral forms given.

\( C_{max} \) values obtained after intramuscular administration were higher than those obtained by Galtier \textit{et al.} \cite{2} in sheep (1.29 µg/ml), by Archambault \textit{et al.} \cite{3} in cattle (1.27 µg/ml), and in this same species by Nielsen \textit{et al.} \cite{4} (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 \textit{et al.} \cite{2} (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 \textit{et al.} \cite{2} 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\cite{5} 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\cite{6}.

The Friedman test involving dose and route of administration showed that route of administration affects \( C_{max} \), \( t_{max} \), \( F \), and \( k_2 \); 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.

**Acknowledgments**

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**References**

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