Pharmacokinetics and Metabolism of $[^{14}\text{C}]$Rosaramicin in Dogs

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The pharmacokinetics and metabolism of $[^{14}\text{C}]$rosaramicin were studied in dogs after intravenous (i.v.; 10 mg/kg [bodyweight]) and oral (25 mg/kg) administration. After i.v. administration, rosaramicin levels in plasma declined rapidly, with half-lives of 0.22 h for the distribution phase and 0.97 h for the elimination phase. The apparent volume of distribution was 3.43 liters/kg, and the total body clearance was 106 mg/min·kg, indicating extensive distribution in tissue or metabolism or both. The absorption of oral solution was 58%, and the absolute bioavailability of rosaramicin was 35%. The plasma area under the curve of unchanged rosaramicin was only 5% of that of total radioactivity after oral administration and 8% after i.v. administration, indicating extensive metabolism of the drug. The total radioactivity excreted in urine accounted for only 24% of the i.v. dose and 17% of the oral dose. Fecal radioactivity accounted for 71% of the i.v. dose and 68% of the oral dose. Several metabolites were observed in the plasma and urine. The amount of unchanged rosaramicin in urine (1%) and 2% of the dose) was quite small after drug administration by either route.

Rosaramicin is a Micromonospora rosaria-produced macrolide antibiotic (10) with broad-spectrum activity against gram-positive aerobes and anaerobes. In addition, rosaramicin has been shown to be more active than erythromycin against a variety of gram-negative organisms and anaerobes, including Bacteroides and Mycoplasma species (11). Rosaramicin is a base with a pH of 8.7 and minimum water solubility but good lipid solubility. The antibiotic, which contains desosamine, is heat and pH stable.

After oral administration in dogs, rosaramicin is highly concentrated in urethral and vaginal secretions (2) and prostatic secretions and interstitial fluid (1, 8). In humans, rosaramicin has an elimination half-life ($t_{1/2}$) of 3.3 h after intravenous (i.v.) dosing, and oral bioavailability (BA) is 32 to 39% (6). However, oral absorbability of rosaramicin has not yet been evaluated in animals or humans.

This report describes the pharmacokinetics and metabolism of $[^{14}\text{C}]$rosaramicin in dogs after oral and i.v. administration. The oral absorbability and BA of rosaramicin in dogs are also evaluated.

MATERIALS AND METHODS

**Compound.** The compound, $[5,^{14}\text{C}]$rosaramicin (Fig. 1), was prepared biosynthetically with M. rosaria by using 1$^{14}$C-labeled sodium butyrate as precursor. The labeled antibiotic was isolated and extensively purified by column chromatography (XAD-2 and silica gel) and repetitive recrystallization. The chemical identity and purity were verified by mass spectrometry, thin-layer chromatography, and proton magnetic resonance spectrometry. The radioactivity (>95%) was confirmed by inverse isotope dilution and high-pressure liquid chromatography (HPLC).

$[^{14}\text{C}]$Rosaramicin administration. Six male beagle dogs weighing 12.9 to 13.9 kg were used in the two-way crossover study with a washout period of 1 week. After an overnight fast, each dog received either an i.v. bolus (10 mg/kg [body weight]) or oral intubation (25 mg/kg) dose of $[^{14}\text{C}]$rosaramicin solution followed by 100 ml of water.

The specific activities of rosaramicin solutions (100 mg/ml) for i.v. and oral administration were 1 and 0.4$\mu$Ci/mg, respectively. These solutions were obtained by mixing, in different ratios, the stock solution of $[^{14}\text{C}]$rosaramicin (solution A) and the stock solution of unlabeled rosaramicin (solution B). Solution A (100 mg/ml, 1.62$\mu$Ci/mg) was prepared by adding 0.6 g of $[^{14}\text{C}]$rosaramicin to 5.0 ml of H$_2$O and titrating with 10% H$_3$PO$_4$ (about 0.2 ml) to pH 7.0; the final volume was adjusted to 6.0 ml by the addition of H$_2$O. Solution B (100 mg/ml) was prepared by adding 1.2 g of unlabeled rosaramicin to 10 ml of H$_2$O and titrating with 10% H$_3$PO$_4$ (about 0.4 ml) to pH 7.0; the final volume was adjusted to 12 ml by the addition of H$_2$O.

**Collection of plasma, urine, and feces.** At 0, 10, 20, 30, and 45 min and 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 48, and 72 h after administration of the drug, blood samples were collected directly into heparinized VACUTAINER tubes and immediately centrifuged for 15 min to collect the plasma samples. During intervals of 0 to 2, 2 to 4, 4 to 6, 6 to 24, 24 to 48, 48 to 72, and 72 to 96 h, urine samples were collected in metabolic cages for analysis. The feces were collected in 24-h blocks for the estimation of radioactivity.

**Measurement of radioactivity.** The radioactivity in samples of plasma (0.5 ml) and urine (0.2 ml) was measured directly by liquid scintillation spectrometry (Packard Tri-Carb model 3255). Feces samples (0.1 to 0.2 g, lyophilized and homogenized) were combusted in a tissue sample oxidizer (Packard Tri-Carb model 306) and $^{14}$CO$_2$ was trapped in a scintillation medium composed of 1 ml of Carbosorb and 3 ml of Permafluor (Packard Instrument Co., Inc., Rockville, Md.); the radioactivity was then determined by liquid scintillation. All samples were placed in a refrigerated liquid scintillation counter for at least 1 h to permit sufficient time for decay chemiluminescence. All counts of radioactivity were corrected for efficiency by the channel ratio method.

**Analytical HPLC method for the determination of rosaramicin in plasma.** The HPLC procedure involved the addition of an internal standard, adjustment to alkaline pH, treatment with potassium carbonate, ether extraction, and a reverse-phase column separation with acetonitrile-acetate buffer mixture as the mobile phase. This technique produced a good linear relationship between the peak height ratio and the rosaramicin concentration. This method has proven to be quite specific for rosaramicin, because many of the deriva-
tives of rosaramicin do not interfere with the assay. The method is accurate and reproducible, with a sensitivity of 0.01 µg of rosaramicin per ml of serum. The details of this HPLC method were described previously (5).

Semipreparative HPLC procedure for studying the metabolic profiles of the drug in plasma and urine. Plasma samples (1.5 ml) were mixed with an equal volume of acetonitrile. The mixtures were then centrifuged, and the supernatant was filtered with 0.5-µm organic membrane filters (type FH; Millipore Corp., Bedford, Mass.). A 2-ml sample of the filtrate was injected into the HPLC apparatus through a loop injector (Rheodyne model 70-10).

Urine samples (0.3 ml) were added to 0.7 ml of water and 1.0 ml of acetonitrile. The mixtures were shaken and then centrifuged, and 1.5 ml of the supernatant was injected into the HPLC apparatus.

An HPLC apparatus (model 6000-A; Waters Associates, Inc., Milford, Mass.) equipped with a stainless steel M9-ODS-2 (preparative Partisil-10 ODS-2; Whatman, Inc., Clifton, N.J.) column (9.4-mm inner diameter, 50-cm length) was used. The solvent mixture, acetonitrile-0.01 M acetic acid buffer (pH 4)-tetrahydrofuran (3:1:0.4), was delivered at 2.4 ml/min. Fractions (3.6 ml) of the eluent were collected by a Unifrac apparatus (Savant Instruments, Inc., Hicksville, N.Y.), and the radioactivity in each fraction was determined by liquid scintillation counting.

Pharmacokinetic analysis. Pharmacokinetic analysis of the rosaramicin concentrations (Cp) and radioactivity levels in the plasma (Cp) after oral and i.v. administration was done by the triexponential equation (equation 1) and biexponential equation (equation 2), respectively, and the nonlinear least-squares computer program, NONLIN (9). The equations are as follows.

\[ C_p = A_1 e^{-\alpha t} + A_2 e^{-\beta t} - A_3 e^{-\kappa t} \]  
\[ C_p = A_1 e^{-\alpha t} + A_2 e^{-\beta t} \]

in which \( \alpha \) and \( \beta \) are the first-order disposition rate constants for the distribution and elimination phases, \( \kappa \) is the apparent first-order absorption rate constant, and \( A_1 \), \( A_2 \), and \( A_3 \) are the coefficients for the exponential terms of the rate constants \( \alpha \), \( \beta \), and \( \kappa \), respectively.

The area under the plasma rosaramicin time curve from 0 to 6 h (AUC0-6) and the area under the radioactivity levels in the plasma with the time curve from 0 to 72 h (AUC0-72) were calculated by the trapezoidal rule. The total AUC from 0 h to infinity (AUC0-\( \infty \)) was calculated by equation 3 for rosaramicin and by equation 4 for radioactivity in the plasma:

\[ \text{AUC}_{0-\infty} = \text{AUC}_{0-6} + C_p(6 \text{ h})/\beta \]  
\[ \text{AUC}_{0-\infty} = \text{AUC}_{0-72} + C_p(72 \text{ h})/\beta \]

in which \( C_p(6 \text{ h}) \) and \( C_p(72 \text{ h}) \) are the estimated concentrations in plasma at 6 and 72 h, respectively, and \( \beta \) is the first-order disposition rate constant for the elimination phase.

RESULTS

Levels of rosaramicin in plasma. After i.v. administration (10 mg/kg), the disposition kinetics of rosaramicin were biphasic (Fig. 2), with a distribution phase (\( t_{1/2p} = 0.22 \) h) and an elimination phase (\( t_{1/2g} = 0.97 \) h) (Table 1). The mean volume of distribution in the central compartment was 3.43 liters/kg, and the mean total body clearance of rosaramicin was 106 ml/min·kg.

To achieve a similar plasma AUC for unchanged rosaramicin, dogs were given a higher oral dose (25 mg/kg). After oral administration, rosaramicin was rapidly absorbed with an absorption (ABS) half-life of 0.28 h, and the maximum concentration of the drug in plasma (1.0 mg/ml) was reached at 0.7 h (Table 1). The \( t_{1/2g} \) and \( t_{1/2g} \) after oral administration were 0.40 and 1.25 h, respectively, which were slightly longer than the corresponding half-lives after i.v. administration. The plasma AUC0-\( \infty \) were 1.34 µg·h/ml after an oral dose of 25 mg/kg. This is similar to the 1.59 µg·h/ml obtained after an i.v. dose of 10 mg/kg. Based on these data, the absolute BA of rosaramicin was calculated to be 35% for the oral solution (Table 1).

Radioactivity level in plasma. After i.v. administration of \(^{14}C\)-rosaramicin, the levels of radioactivity in plasma fell rapidly with time (Fig. 3). The radioactivity \( t_{1/2a} \) and \( t_{1/2g} \) after i.v. dosing were 0.79 and 8.7 h, respectively.

After oral administration of the \(^{14}C\)-labeled drug, an ABS


![FIG. 2. Levels in plasma of rosaramic (micrograms per milliliter) in dogs (n = 6) after i.v. and oral administration. The solid lines represent the NONLIN (9) best fit. The \( r^2 \) was 1.0 and 0.998 for i.v. and oral administration, respectively.)](http://aac.asm.org/DownloadedFrom/10.1128/AAC.0128-0502.2006)
TABLE 1. Pharmacokinetic parameters of rosaramicin and total radioactivity

<table>
<thead>
<tr>
<th>Parametera</th>
<th>Oral dose (25 mg/kg)</th>
<th>i.v. dose (10 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rosaramicin</td>
<td></td>
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</tr>
<tr>
<td>$t_{1/2a}$</td>
<td>h</td>
<td>0.28 ± 0.10</td>
</tr>
<tr>
<td>$t_{1/2b}$</td>
<td>h</td>
<td>0.40 ± 0.10</td>
</tr>
<tr>
<td>$V_{1}$</td>
<td>h</td>
<td>1.23 ± 0.24</td>
</tr>
<tr>
<td>$V_{ss}$</td>
<td>liters/kg</td>
<td>3.43 ± 0.47</td>
</tr>
<tr>
<td>TBC</td>
<td>ml/min/kg</td>
<td>8.80 ± 0.93</td>
</tr>
<tr>
<td>$C_{max}$</td>
<td>µg/ml</td>
<td>1.0 ± 0.3</td>
</tr>
<tr>
<td>$T_{max}$</td>
<td>h</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td>AUC$_{0-ss}$</td>
<td>h · µg/ml</td>
<td>1.34 ± 0.31</td>
</tr>
<tr>
<td>BA</td>
<td>%</td>
<td>35 ± 11</td>
</tr>
<tr>
<td>Radioactivity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$t_{1/2a}$</td>
<td>h</td>
<td>0.37 ± 0.09</td>
</tr>
<tr>
<td>$t_{1/2b}$</td>
<td>h</td>
<td>0.94 ± 0.19</td>
</tr>
<tr>
<td>$T_{1/2b}$</td>
<td>h</td>
<td>8.94 ± 1.2</td>
</tr>
<tr>
<td>AUC$_{0-ss}$</td>
<td>h · µg/ml</td>
<td>29.0 ± 5.1</td>
</tr>
<tr>
<td>ABS</td>
<td>%</td>
<td>0.58 ± 0.11</td>
</tr>
</tbody>
</table>

*a n = 6.

The $t_{1/2a}$ for radioactivity (0.94 h) and the $t_{1/2b}$ (8.9 h) were comparable to those obtained after i.v. administration (Table 1).

Comparison of the plasma radioactivity AUC$_{0-ss}$ after an oral dose of 25 mg/kg (29.0 µg · h/ml) and that after an i.v. dose of 10 mg/kg (20.6 µg · h/ml) showed the ABS of oral rosaramicin solution to be 58% (Table 1).

The AUC$_{0-ss}$ of unchanged rosaramicin was only 5% that of total radioactivity after oral administration and 8% after i.v. administration, indicating extensive metabolism of the drug (Table 1).

Excretion of radioactivity in urine and feces. The cumulative excretion data for radioactivity in urine and feces are shown in Table 2. Over a 96-h period, 23.8% of the i.v. dose and 16.9% of the oral dose were excreted in the urine. In the feces, 71.4% of the i.v. dose and 67.9% of the oral dose were excreted. These data demonstrate a significant biliary excretion of the drug; radioactivity was excreted primarily into the feces.

By comparing the amount (percentage of dose) of radioactivity excreted in the urine after an oral dose and that excreted after an i.v. dose, the ABS of oral rosaramicin solution was estimated to be 71%, which is slightly higher than the ABS calculated from the plasma radioactivity AUC$_{0-ss}$ obtained after i.v. and oral administration.

Metabolic profile of [14C]rosaramicin in plasma and urine. The metabolic profile of [14C]rosaramicin in dog plasma and urine is shown in Table 3. The radioactivity in plasma consisted of fractions A, C, and rosaramicin, and the radioactivity in urine consisted of fractions A, B, C, D, and rosaramicin. Fraction D has been identified as 20-bis-ureidoro-rosaramicin (7). The relative amount of unchanged rosaramicin decreased with time in both plasma and urine. The total amount of rosaramicin excreted in urine accounted for only 1.8% of the i.v. dose and 0.6% of the oral dose.

DISCUSSION

Rosaramicin was rapidly absorbed in dogs after oral administration with an absorption half-life of 0.28 h. The extent of ABS was estimated to be 58% based on an assessment from the AUC$_{0-ss}$ of plasma total radioactivity and 71% based on an assessment from cumulative urinary excretion of total radioactivity. The BA of rosaramicin for the oral solution was 35% as estimated from the plasma AUC$_{0-ss}$ of unchanged rosaramicin. This agrees with the results (34%) estimated from cumulative urinary excretion. This BA of rosaramicin in dogs (34 to 35%) was also in good agreement with that reported (6) in humans (32 to 39%).

After i.v. administration in dogs, rosaramicin was rapidly distributed with a $t_{1/2a}$ of 0.22 h. The volume of distribution in the central compartment for rosaramicin was large (3.43
liters/kg) and similar to that reported (6) in humans (3.78 liters/kg). The total body clearance in dogs (106 ml/min · kg) was much larger than the glomerular filtration rate in dogs (5 ml/min · kg). These results suggest that rosaramicin is highly distributed in tissues or extensively metabolized or both. The $t_{1/2}$ for rosaramicin in dogs (0.97 h) was much shorter than that reported (6) in humans (3.3 h), suggesting a rapid and extensive metabolism of the drug in dogs. This agrees with the data for AUC$_{0-\infty}$ for rosaramicin in dog plasma, which is only 7.7% of the AUC$_{0-\infty}$ for total radioactivity in plasma. This is also in good agreement with the observation that the cumulative urinary excretion of unchanged rosaramicin in dogs was only 8% of the cumulative urinary excretion of total radioactivity.

The amount of unchanged rosaramicin excreted in the urine accounted for 1.8% of the i.v. dose and 0.6% of the oral dose. Similar results were obtained for erythromycin, a well-known macrolide antibiotic with a structure closely related to that of rosaramicin. Lee et al. (3) reported that 2.25% of doses are excreted in urine as unchanged erythromycin in dogs after oral administration.

After administration of [14C]rosaramicin in dogs, fecal excretion of radioactivity accounted for 71% of the i.v. dose and 68% of the oral dose. In humans, fecal excretion of radioactivity accounts for 87% of the oral dose. The high recovery of radioactivity in dog feces after i.v. administration suggests significant biliary excretion of the drug. However, it could also be due to secretion of drug or metabolites or both into the gastrointestinal lumen. Lee et al. (4) also showed that in the duodenal fistula of dogs after i.v. administration of radiolabeled erythromycin, 33.6% of the radioactivity is recovered in the bile, confirming the important role of the liver in the transport of macrolide antibiotics. This significant biliary excretion may in part explain the rapid decline in the concentration in the serum or plasma of both erythromycin and rosaramicin.

### LITERATURE CITED


