DISPOSITION AND METABOLISM OF RIFAPENTINE, A RIFAMYCIN ANTIBIOTIC, IN MICE, RATS, AND MONKEYS

W. BART EMARY, PAUL C. TOREN, BRAD MATHEWS, AND KAY HUH

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ABSTRACT:

Rifapentine is a cyclopentyl derivative of rifampin under development for the treatment of Mycobacterium tuberculosis and Mycobacterium avium complex infections. These studies were designed to investigate the disposition and biotransformation of single iv and oral doses of 14C-rifapentine in mice, bile duct-cannulated and uncannulated rats, and monkeys. Mass balance studies included 14C analyses of urine, feces, bile, cage wash, carcasses, and cage air collected for up to 120 hr postdose. Separation of radioactive compounds extracted from urine, bile, and feces was conducted using high-performance liquid chromatography and radioisotope detection. The mass spectra of selected chromatographic peaks were obtained. Disposition results were similar for all three species. Less than 5% of the radioactive dose of 14C-rifapentine was recovered in urine, indicating that renal excretion is a minor route of elimination in these species. The major route of elimination of radioactivity was into the feces, where more than 75% of the radioactivity was recovered. Biliary excretion was the major route of elimination of radioactivity in bile duct-cannulated rats dosed either po or iv. Radiochromatograms were similar for fecal samples from animals dosed by iv or orally. Ten regions of radioactivity were observed in mouse and rat fecal sample radiochromatograms, and seven regions of radioactivity were observed in monkey fecal sample radiochromatograms. The most abundant compound identified in feces was usually intact rifapentine (27%–41% of dose in mouse, 3%–35% of dose in rat, and 17%–29% of dose in monkey). Other peaks identified or characterized in feces based on liquid chromatography/ultraviolet/14C and/or liquid chromatography/mass spectrometry methods included 25-desacetyl-rifapentine, 3-formyl-25-desacetyl-rifapentine, and 3-formyl-rifapentine. The compounds rifapentine, 25-desacetyl-rifapentine, and 3-formyl-rifapentine were present in rat bile samples. These studies show that the metabolism and disposition of rifapentine in mice, rats, and monkeys were similar.

Rifapentine is a semisynthetic, rifamycin-class antibiotic (Arioli et al., 1981) under development for the treatment of pulmonary tuberculosis and for the prophylaxis of Mycobacterium tuberculosis and Mycobacterium avium complex infections. The antimicrobial spectrum of rifapentine is similar to that of its homologues, rifampin and rifabutin (Arioli et al., 1981; Dickinson and Mitchison, 1987; Heifets et al., 1990; Yates and Collins, 1982); however, rifapentine has demonstrated greater therapeutic efficacy in experimental mycobacterium infections, compared with rifampin (Arioli et al., 1981; Pattyn, 1987; Truffot-Pernot et al., 1983). The structure of rifapentine is shown in fig. 1. Rifapentine differs from rifampin by the presence of a cyclopentyl ring instead of a methyl group at the piperazinyl moiety, which makes rifapentine more lipophilic. Pharmacokinetic studies of rats (Assandri et al., 1978) and healthy volunteers (Birmingham et al., 1978, Buniva et al., 1983) dosed with rifapentine indicate differences in the pharmacokinetic profiles between it and rifabutin. Peak serum concentrations obtained after oral or iv doses of rifapentine were comparable to those produced by rifampin, but the elimination half-lives of the two drugs were markedly different, with rifapentine persisting in serum about 4–5 times longer.

Previous studies in mice, rats, and monkeys have identified the pharmacokinetic profile of rifapentine and its metabolite, 25-desacetyl-rifapentine. In a study designed to assess disposition of radiolabeled-rifapentine in rats, Assandri and colleagues (Assandri et al., 1981) identified feces as the major route of elimination of intact rifapentine and metabolites. In a separate study (Assandri et al., 1984), the pharmacokinetics of 14C-rifapentine in rat, mouse, and rabbit were investigated. The mean bioavailability of rifapentine after a single oral dose of 3 mg/kg in rats was 84%. The primary route of elimination of radioactivity was through the feces (about 90%), with less than 10% of the radioactivity recovered in urine. The terminal elimination half-life of rifapentine was 14–21 hr in rats, 17–23 hr in mice, and only about 2 hr in rabbits. In these previous rifapentine studies, no attempts were made to determine mass balance or identify compounds.

The purpose of our studies was to determine the disposition and biotransformation of 14C-rifapentine in mice, bile duct-cannulated and uncannulated rats, and monkeys after either a single oral or iv dose. Mass balance studies included 14C analysis of urine, feces, bile, cage wash, carcasses, and cage air collected for up to 120 hr postdose. Metabolite profiling of 14C in urine, bile, and feces was conducted using HPLC with radioisotope detection. Mass spectra of selected radioactive chromatographic peaks were obtained.

1 Abbreviations used are: HPLC, high-performance liquid chromatography; LC, liquid chromatography; MS, mass spectroscopy; LSC, liquid scintillation counting.
Materials and Methods

Chemicals and Dose Formulations. Standards of rifapentine, 25-desacetyl-rifapentine, and 3-formyl-rifapentine were synthesized at Hoechst Marion Roussel (Cincinnati, OH). The labeled drug was prepared with the imine carbon containing the $^{14}$C label (fig. 1). Unlabeled drug was coprecipitated with labeled drug to produce $^{14}$C-rifapentine and to limit the radioactivity exposure to less than 40 µCi/animal. The radiochemical purity was 98.41% and the chemical purity was 97.71%.

The iv dose (2 mg/ml solution for mice and 10 mg/ml solution for rats and monkeys) was prepared at 10°C by dissolving $^{14}$C-rifapentine in 0.1% sodium ascorbate/2% alcohol (95% ethanol)/20 mM sodium hydroxide. The specific activities of the oral doses were 24.0, 23.0, and 0.9 mCi/mg for mouse, rat, and monkey, respectively. After all of the test compound was dissolved, a small amount (about 3%) of 0.1 N HCl was added to adjust the pH to 10.0 ± 0.1. Immediately before administration, the iv dose was warmed to room temperature. The oral dose was prepared as a suspension (1 mg/ml for mice and rats, 10 mg/ml for rats and monkeys).

FIG. 1. Structures of compounds either identified (standard exists) or characterized (MS data only) in mouse, rat, and monkey feces after iv or oral doses or $^{14}$C-rifapentine.

All molecular weights are the monoisotope mass. The asterisk (*) denotes the position of the $^{14}$C label.
animals were thawed and mixed with a methanol solution (1 vials and 10 –15 ml of Permafluor® (Packard) was added as scintillant. With Soluene-350 (Packard), 10 ml of Hionic Fluor® (Packard) was added as scintillant.

**Animals. Mice.** Two groups, each consisting of six male NMRI mice weighing between 25 and 30 g, received either a single iv or oral 10 mg/kg dose of 14C-rifapentine. The mice were housed in glass Roth-type cages in a light- and temperature-controlled room. The animals fasted overnight before dosing, then had free access to food and water after dosing. The iv dose was administered into the tail vein and the oral doses were delivered by gavage.

**Rats.** Three parallel groups of four male Wistar rats weighing 250–300 g were administered 10 mg/kg 14C-rifapentine either orally (two groups) or by iv (one group). The rats were housed in glass Roth-type cages in a light- and temperature-controlled room. The animals fasted overnight before dosing, then had free access to food and water after dosing. The iv dose was administered into the tail vein and the oral doses were delivered by gavage.

The bile ducts of rats from two groups (oral and iv) were cannulated. Rats were anesthetized with isoflurane. A midline incision was made, and the common bile duct was isolated. A cannula, consisting of PE10 tubing attached to PE50 tubing, was inserted (PE10 end) into the bile duct slightly proximal to the small intestine. The cannula was exteriorized at the nape of the neck and was channeled through a rodent jacket and spring tether. The rats were housed in Roth-type metabolism cages. The rats were allowed to recover from the effects of the anesthetic before dosing.

**Monkeys.** One group of four male Cynomolgus monkeys weighing 3.0–5.0 kg was administered one of three treatments: 10 mg/kg 14C-rifapentine administered orally or by iv, or 40 mg/kg 14C-rifapentine administered orally. Monkeys were housed in individual stainless steel cages designed for the separation and collection of urine and feces. The treatments were administered sequentially, with a 3-week washout period between treatments. Before each dose, the animals fasted overnight and until about 4 hr postdose. The iv dose was administered via the saphenous vein, and the oral doses were administered via intragastric intubation.

All animals were housed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and all studies were conducted under protocols approved by the Hoechst Marion Roussel Animal Care and Use Committee. Anesthetic was not used during the dosing or sample collection for any of these studies.

**Sample Collection.** The collection times for urine, bile (rats only), and fecal samples were intervals of 0 – 6, 6 –12, 12–24, 24–48, 48–72, 72–96, and 96–120 hr after the dose was administered. The samples were collected in tared glass containers for mice and rats and in plastic containers for monkeys. Cage washes were conducted at the end of each urine-collection interval (mice and rats) or after each 24-hr fecal collection (monkeys). In addition, for mice and the uncannulated rats, cage air was scrubbed through a CO2 trapping solution (5 M ethanolamine in 2-methoxyethanol, 3:7 v/v) to trap any 14C-labeled CO2 at 12-hr intervals. At the end of the mass balance study, carcasses of mice and rats were analyzed for radioactivity content.

Rifapentine is known to degrade chemically by hydrolysis and oxidation (G. Beck, unpublished data, 1994). Therefore, all samples were collected over dry ice to minimize degradation, frozen, and stored at −20°C until analysis. In addition, 10 μl of 250 μg/ml ascorbic acid was added to all urine and bile samples immediately upon collection to stabilize the samples. Fecal samples were homogenized in methanol/water (1:1, v:v) containing 2.5 μg/ml ascorbic acid.

**Fecal Sample Preparation for LC/UV/14C or LC/MS.** Fecal samples were thawed and mixed with a methanol solution (1 μg/ml ascorbic acid) in a ratio of about 2 ml/g of fecal homogenate. Samples were centrifuged at 3500 rpm for 2 min after mixing; the supernatant was then transferred to a test tube. This extraction procedure was repeated two more times and the extracts were combined and dried in a vacuum centrifuge. The dry residue was reconstituted in a methanol/water solution, filtered, and injected onto the HPLC system.

**Radioactivity Analysis.** Concentrations of 14C in urine, bile, cage wash, carcass, and expired air were measured by direct liquid scintillation counting (LSC) with external standardization for quench correction. Weighed aliquots of urine, bile, and cage wash were transferred to vials, and 5–15 ml of Ultima Gold® (Packard, Meriden, CT) scintillant added. After incubation of carcass with Soluene-350 (Packard), 10 ml of Hionic Fluor® (Packard) was added as scintillator. The CO2 trapping solution from expired air was aliquoted into LSC vials and 10–15 ml of Permafluor V® (Packard) was added as scintillator.
summarized in table 1. After iv administration, radioactivity excreted in urine and feces during the 120-hr collection period was 4.9% and 73.0%, respectively. The cage wash contained 3.7% of the dose, and 3.4% of the dose remained in the carcass. Expired air did not contain any radioactivity over the first 24 hr postdose. The mass balance averaged 86.2% of dose. Radioactivity recovery results with oral administration were quite similar to the iv dose. During the 120-hr postdose collection period, 4.5% and 75.5% of the oral dose was recovered in urine and feces, respectively. The mass balance averaged 88.5% of dose.

Rats. After iv administration of 14C-rifapentine to bile-duct-cannulated rats, radioactivity excreted in urine, feces, and bile over the 96 hr postdose was 4.8%, 23.2%, and 30.2% of the dose, respectively; 35.8% remained in the carcass (table 1). The total recovery of radioactivity was 94.6%. Similar to the findings with mice, disposition results after oral administration of 14C-rifapentine to bile-duct-cannulated rats were comparable to iv dosing results. During the 96-hr postdose collection period, 4.2%, 15.4%, and 21.5% of the oral dose was recovered in urine, feces, and bile, respectively. The amount of radioactivity in the carcass was 41.7% of the oral dose and the mass balance was 84.2%. After oral administration in uncannulated rats, radioactivity excreted in urine and feces was 4.4% and 100.2% of the dose, respectively, over 144 hr postdose; only 3.1% of the radioactive dose remained in the carcass and the mass balance was 108.4%. No radioactivity was detected in expired air in any of the treatment groups.

Monkeys. After iv administration of 10 mg/kg 14C-rifapentine, the radioactivity excreted in urine and feces over 120 hr postdose was 2.8% and 88.1% of the dose, respectively (table 1). After oral administration, the total recovery of radioactivity in urine and feces was 3.3% and 92.1% of the 10 mg/kg dose, respectively, and 4.5% and 85.1% of the 40 mg/kg dose, respectively. The mass balances after dosing of 10 mg/kg IV, 10 mg/kg oral, and 40 mg/kg oral were 91.8%, 95.8%, and 89.9% of dose respectively.

Metabolism. None of the mouse, rat, or monkey urine samples contained more than 5% of the radiolabeled dose of rifapentine. Therefore, urine samples were not analyzed except for the one that contained the most radioactivity (range, 0.1% to 0.7% of dose). Fecal samples containing radioactivity greater than 5% of dose were subjected to LC/UV/14C. For all three species, the oral and iv routes of rifapentine administration produced similar metabolite profiles in feces samples.

Metabolites were identified by comparison of the LC/UV/14C chromatographic peak retention time of an authentic standard to that resulting from injection of a fecal or bile extract. Supporting identification data was produced using LC/MS. Standards of rifapentine and analogs gave a poor response by either atmospheric pressure chemical ionization or electrospray ionization LC/MS. No (M+H)+ ions were observed after injection of standards using positive atmospheric pressure chemical ionization, and the electrospray ionization (M+H)^+ ion response of rifapentine was approximately 100 times less than the typical pharmaceutical compounds we study. This is possibly the result of factors including poor ionization efficiency and poorer transmission of higher mass ions through the quadrupole. In an effort to improve MS response, different mobile phase buffers and pH conditions were tested but did not yield any improvements. Thus, LC/MS could only be utilized to confirm the presence of some of the metabolites that were identified with the LC/UV/14C system.

Mice. A summary of identified metabolites and their abundance in fecal samples is presented in table 2. Representative LC/14C chromatograms (fig. 2) resulting from injection of a mouse fecal extract contained 10 regions of significant radioactivity. The radiochromatograms were similar after either intravenous or oral dosing. One of the abundant regions of radioactivity (8–18% of dose) eluted form the column as a broad peak with retention time from 9 to 17 min. Occasionally, small peaks were observed on top of the broad peak. Unsuccessful attempts were made to resolve the broad peaks into individual components using other columns and mobile phase components and conditions. The entire region was summed for the purpose of calculating percentage of dose.

Four of the peaks in each radiochromatogram were assigned as 25-desacetyl-rifapentine, rifapentine, 3-formyl-25-desacetyl-rifapentine, and 3-formyl-rifapentine. Initial assignments were made by matching LC/14C/UV retention times with standards. Separate LC/MS analyses discussed below were subsequently performed to verify some of these assignments.

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**TABLE 1**

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Excreted or Recovered Radioactivity as Percentage of Dose (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mouse (N = 4)</td>
</tr>
<tr>
<td></td>
<td>10 mg/kg iv</td>
</tr>
<tr>
<td></td>
<td>(cannulated, N = 4)</td>
</tr>
<tr>
<td>Urine</td>
<td></td>
</tr>
<tr>
<td>0–24 hr</td>
<td>2.4 ± 0.4</td>
</tr>
<tr>
<td>24–72 hr</td>
<td>2.0 ± 0.3</td>
</tr>
<tr>
<td>72-end hr</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>Feces</td>
<td></td>
</tr>
<tr>
<td>0–24 hr</td>
<td>26.6 ± 7.6</td>
</tr>
<tr>
<td>24–72 hr</td>
<td>35.7 ± 4.5</td>
</tr>
<tr>
<td>72-end hr</td>
<td>10.7 ± 1.9</td>
</tr>
<tr>
<td>Bile</td>
<td></td>
</tr>
<tr>
<td>0–24 hr</td>
<td>NA</td>
</tr>
<tr>
<td>24–72 hr</td>
<td>NA</td>
</tr>
<tr>
<td>72-end hr</td>
<td>NA</td>
</tr>
<tr>
<td>Carcass</td>
<td>3.4 ± 1.8</td>
</tr>
<tr>
<td>Mass balance</td>
<td>86.2 ± 3.9</td>
</tr>
<tr>
<td></td>
<td>91.8 ± 11.2</td>
</tr>
</tbody>
</table>
The most abundant compound identified in mouse feces was usually rifapentine (27–41% of the dose). The other three compounds identified, 25-desacetyl-rifapentine, 3-formyl-25-desacetyl-rifapentine, and 3-formyl-rifapentine, accounted for 7–11%, 0–1%, and 3–6% of the dose in mice, respectively. Together, these four compounds constituted approximately 65% of the radioactivity in feces.

3-Formyl-rifapentine is a known hydrolysis product of rifapentine in an aqueous environment (G. Beck, unpublished data, 1994). To ensure that processing did not degrade rifapentine in samples, an aliquot of predose fecal homogenate was spiked with 14C-rifapentine and the sample was prepared as previously described. No peaks other than rifapentine were observed in these spiked radiochromatograms.

Two compounds, rifapentine and 3-formyl-rifapentine, in mouse feces could be confirmed by LC/MS. Injection of 48-hr mouse fecal extracts into the LC/MS resulted in reconstructed ion chromatographic peaks with retention times and mass spectra consistent with authentic standards. No mass spectra (unique from predose fecal extracts) were observed for any of the other radioactive compounds eluting from the LC column, presumably because of low concentrations and/or low ionization yields. The structures for compounds found in mouse, rat, and monkey urine, bile, and fecal extracts based on LC/UV/14C and/or LC/MS data are shown in fig. 1.

Rat Bile. A summary of identified metabolites and their abundance in bile samples is presented in table 2. A representative radiochromatogram from a rat bile extract is shown in fig. 4. Radiochromatograms from rat bile extracts were similar after either iv or oral dosing. Three chromatographic peaks observed in the LC/14C chromatograms were assigned as 25-desacetyl-rifapentine, rifapentine, and 3-formyl-rifapentine, respectively. The assignments were based upon a retention time match of chromatographic peaks from authentic standards to those from rat fecal extracts. The largest individual region consistently corresponded to rifapentine (3%–35% of dose). Generally, no significant new peaks were observed as time progressed postdose. Instead, a decrease in the intensity of the peaks that were already present at earlier times was found. In the feces, one of the significant chromatographic regions of radioactivity (1%–19% of dose) was a very broad region (8- to 10-min rise) in the background (about 7%- to 17-min retention time), with few distinct peaks within it. The presence of rifapentine in the rat fecal extract could be confirmed by LC/MS, based upon a comparison of the retention time and mass spectrum to an authentic standard. The other identified metabolites, based upon LC/UV/14C data, were not observed by LC/MS, probably because of the relatively poor MS response of rifapentine and analogs combined with their low concentration.

### Table 2

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mouse Urine</th>
<th>Mouse Feces</th>
<th>Rat (No Bile Duct Cannulation) Urine</th>
<th>Rat (No Bile Duct Cannulation) Feces</th>
<th>Rat (Bile Duct Cannulation) Urine</th>
<th>Rat (Bile Duct Cannulation) Feces</th>
<th>Monkey Urine</th>
<th>Monkey Feces</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rifapentine</td>
<td>&lt;2</td>
<td>33</td>
<td>ND</td>
<td>30</td>
<td>ND</td>
<td>8</td>
<td>ND</td>
<td>29</td>
</tr>
<tr>
<td>25-Desacetyl-rifapentine</td>
<td>ND</td>
<td>11</td>
<td>ND</td>
<td>12</td>
<td>ND</td>
<td>1</td>
<td>ND</td>
<td>20</td>
</tr>
<tr>
<td>3-Formyl-rifapentine</td>
<td>&lt;3</td>
<td>4</td>
<td>ND</td>
<td>11</td>
<td>ND</td>
<td>2</td>
<td>ND</td>
<td>10</td>
</tr>
<tr>
<td>3-Formyl-25-desacetyl-rifapentine</td>
<td>ND</td>
<td>1</td>
<td>ND</td>
<td>6</td>
<td>ND</td>
<td>1</td>
<td>ND</td>
<td>9</td>
</tr>
<tr>
<td>All other regions of radioactivity</td>
<td>ND</td>
<td>26</td>
<td>ND</td>
<td>41</td>
<td>ND</td>
<td>5</td>
<td>13</td>
<td>&lt;3</td>
</tr>
</tbody>
</table>

*Urine data based upon radiochromatographic analysis of a single sample with the largest percentage of dose.

ND, not detected.
ison of the retention time and mass spectrum to an authentic standard. The other identified metabolites, based upon LC/UV/\(^{14}\)C data, were not observed by LC/MS probably because of the relatively poor MS response of rifapentine and analogs combined with their low concentration. No mass spectra (unique from predose fecal extracts) were observed for any of the other radioactive compounds eluting from the LC column, presumably because of low concentrations and/or low ionization yields.

Monkeys. A representative LC/\(^{14}\)C chromatogram for the 10 mg/kg iv dose in monkeys that was obtained 72 hr postdose is presented in fig. 5. Most radiochromatograms obtained after the iv and oral doses were similar, with seven regions of significant radioactivity observed. As with the other species, one of the abundant regions of radioactivity (12%–19% of the dose) eluted from the column as a peak with retention time from 6 to 15 min. Four peaks in each radiochromatogram were assigned as 25-desacetyl-rifapentine, rifapentine, 3-formyl-25-desacetyl-rifapentine, and 3-formyl-rifapentine. In general, rifapentine represented 17–29% of the dose in a sample, while 25-desacetyl-rifapentine represented 14–20% of the dose. These were the largest amounts of radioactivity eluting from the column.

Identification or characterization by LC/MS was successful for four of the radioactive compounds in monkey feces eluting from the column. Fig. 6 illustrates a typical reconstructed ion chromatogram obtained after the injection of 200 μl of reconstituted fecal extract (72 hr postdose) from a monkey dosed with rifapentine 10 mg/kg iv. Four peaks were observed and their mass spectra were consistent with the assignment of 25-desacetyl-rifapentine, rifapentine, 3-formyl-25-desacetyl-rifapentine, and 3-formyl-rifapentine, respectively. As each compound eluted from the column, the (M+H)\(^+\) ions were prominent in the respective mass spectra at m/z 684.6, 726.6, 835.7, and 877.7 for 3-formyl-25-desacetyl-rifapentine, 3-formyl-rifapentine, 25-desacetyl-rifapentine, and rifapentine.

Discussion

The results of the rifapentine mass balance studies for mouse, rat, and monkey were strikingly similar. During the 120-hr collection period, less than 5% of radioactive drug was excreted in the mouse, rat, and monkey urine, indicating that renal excretion is a minor route of elimination of rifapentine in these species. The major route of elimination of radioactivity was into the feces, where more than 75% of the radioactive dose was recovered. In contrast to rifapentine, mass balance studies of \(^{14}\)C-rifabutin in rats and monkeys revealed that the relative amounts of radioactivity excreted in urine and feces after oral and iv doses were similar (about 44% of the dose each) (Battaglia et al., 1990, 1991).

Fig. 4. Representative \(^{14}\)C radiochromatogram of bile duct–cannulated rat bile extract after iv administration of \(^{14}\)C-rifapentine.

Fig. 5. Radiochromatogram resulting from injection of a monkey sample fecal extract after the animal received a 10 mg/kg iv dose of \(^{14}\)C-rifapentine.

Fig. 6. LC/MS (m/z labeled) and LC/UV (user trace) chromatograms resulting from injection of a monkey sample fecal extract after the animal received a 10 mg/kg iv dose of \(^{14}\)C-rifapentine.

The m/z value corresponds to the (M+H)\(^+\) ion for the labeled analyte. User trace is UV wavelength 480 nm.

Virtually no differences were found between the percentages of dose excreted in urine and feces after oral and iv administration of rifapentine in mouse, uncannulated rats, and monkeys. The relative amounts of radioactivity excreted into bile, feces, and urine after oral administration of rifapentine in bile duct–cannulated rats were also similar to that after iv administration. The ratio of radioactivity excreted in rat bile to feces was approximately 3:2 after both oral and iv administration.
iv-dosing. These data support other reports that absorption of rifampicin is nearly complete (Weber et al., 1983).

Biliary excretion was a major route of elimination of radioactivity (approximately 51% of excreted radioactivity) after iv administration of rifampicin in bile duct–cannulated rats. Approximately 40% of the excreted radioactivity (about 23% of the dose) was recovered in feces after iv administration in bile duct–cannulated rats. This finding suggests that rifampicin may be cleared by another route of elimination, such as direct excretion through the intestinal wall. The role of gastrointestinal secretion in the clearance of rifampicin in rats is currently under investigation. The ratio of rifampicin to 25-desacetyl-rifampicin was approximately 10:1 in fecal samples of the cannulated rats, but for bile, the ratio was approximately 1:3. Since previous studies found no detectable amount of 25-desacetyl-rifampicin in circulating plasma (Weber et al., 1983), it appears that the 25-desacetyl metabolite is rapidly excreted into bile after being formed in the liver.

The total radioactivity recovered in urine and feces over 96 hr postdose in orally dosed uncannulated rats (about 104.6%) was much greater than the recovery in urine, bile, and feces found in orally dosed bile duct–cannulated rats (about 41.1%). The balance of the radioactivity was primarily found in the carcass (table 1). This difference was most likely due to changes in the physiology of the rats, stemming from the stress of cannulation and/or surgical trauma. Further investigation of dose recovery after bile duct cannulation in rats is planned.

In general, examination of urine samples using LC/UV/14 C was not done in any of the species because the total and individual sample recoveries of radioactivity in urine was less than 5% and 0.7% of dose, respectively. Only the urine sample with the highest level of radioactivity in each species was examined using LC/14 C. The radiocromatograms contained small peaks corresponding to rifampicin and its hydrolysis product, 3-formyl-rifampicin.

The radiocromatograms from rat fecal and bile samples after oral and iv administration of 14 C-rifampicin were similar. By comparing the HPLC retention times of existing standards and/or mass spectral analysis of chromatographic peaks, components identified in the fecal and bile samples included rifampicin, 25-desacetyl-rifampicin, 3-formyl-rifampicin, and 3-formyl-25-desacetyl-rifampicin. The primary peak observed in fecal samples was rifampicin in all three species. Rifampicin is known to be unstable in solution and one major degradation product is 3-formyl-rifampicin. Similar to rifampicin, the 3-formyl derivatives found in fecal samples are probably formed by nonenzymatic hydrolysis (Battaglia et al., 1990). Since these metabolites have not been observed in plasma, formation most likely occurs as the excreta residues in the gut.

In all three species, one region of radioactivity occurred as a broad rise in the background with few distinct peaks. The approximate retention time of this area was 9–17 min in mice (accounting for 8%–18% of dose), 7–17 min in rats (accounting for 1%–19% of dose), and 6–15 min in monkeys (accounting for approximately 12%–19% of dose). Attempts to resolve the broad peaks into individual components using other columns and mobile phase conditions were unsuccessful; therefore, structures could not be assigned. No unique mass spectra (from predose fecal extracts) were observed for any of the radioactive compounds eluting from the LC column other than rifampicin, 25-desacetyl-rifampicin, 3-formyl-rifampicin, or 3-formyl-25-desacetyl-rifampicin, presumably because of low concentrations and/or because the ionization efficiency was insufficient to yield discernible mass spectra.

A search of the published literature failed to identify any studies that evaluated the metabolism and excretion of rifampicin in animals. The mass balance and metabolic profile of another rifamycin analog, rifabutin, has been reported in animals and humans (Battaglia et al., 1990, 1991; Utkin et al., 1997; Koudriakova et al., 1996). Parent drug accounted for only 8.5% of the radioactive rifabutin dose in urine of rats, and less than 0.5% of the dose in rabbits and monkeys (Battaglia et al., 1990). Most of the urinary radioactivity (more than 93%) after a single oral dose of 14 C-rifabutin in rats, rabbits, and monkeys was constituted by unidentified peaks of polar compounds (Battaglia et al., 1990). The most abundant polar metabolite identified was N-isobutyl-4-hydroxy-piperidine. Lipophilic metabolites, which accounted for less than 20% of urinary radioactivity in rats and humans, included 25-O-desacetyl-rifabutin, 25-O-demethyl-rifabutin, and 31-hydroxy-rifabutin (Battaglia et al., 1990; Utkin et al., 1997; Koudriakova et al., 1996). Only small amounts of the 25-desacetyl derivative were found in the urine of rats (2.1% of the dose) (Battaglia et al., 1990). In bile duct–cannulated rats, approximately 24% of the rifabutin dose was excreted in bile, almost exclusively (98%) as metabolites (Koudriakova et al., 1996). These data suggest rifabutin is more extensively metabolized in animals than rifampicin is, with metabolic pathways that include oxidation.

In summary, the disposition and biotransformation of rifampicin in mouse, rat, and monkey are quite similar. Rifampicin is excreted primarily as intact drug in feces; less than 5% of rifampicin and its metabolites are excreted in the urine. The primary metabolite in bile and feces is 25-desacetyl-rifampicin, with smaller amounts of the degradation byproducts, 3-formyl-rifampicin and 3-formyl-25-desacetyl-rifampicin, formed in the gut.

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References


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