

PHARMACOKINETICS OF RIFAPENTINE, A NEW LONG  
LASTING RIFAMYCIN, IN THE RAT, THE MOUSE  
AND THE RABBIT

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A study on the pharmacokinetics of rifapentine, a new long-lasting rifamycin, has been carried out in the rat, the mouse and the rabbit. The investigation was made using either radioactive or unlabelled rifapentine and both the total  $^{14}\text{C}$  and the unchanged compound were assayed.

In the rat, the overall evidence obtained was: (a) the oral absorption of rifapentine into central compartment, due to its poor water solubility, appears to be dose-dependent with a satisfactory oral absorption (84%) after a dose of 3 mg/kg, lower (65%) after 10 mg/kg; (b) the antibiotic undergoes rapid liver uptake while it diffuses into the tissue compartment more slowly, with particular affinity for the adrenals, pancreas and kidneys; concentrations higher than in plasma were also measured in the lungs; (c) elimination of rifapentine from the blood and tissue compartments suggests a non linear capacity-limited kinetics where the terminal elimination phase has monoexponential course. Terminal plasma half-life ranged between 14 and 18 hours; (d) the compound is eliminated mainly *via* the bile with the feces (92% of dose).

In mice rifapentine shows a kinetic profile resembling that obtained in rats, whereas in rabbits is metabolized and/or eliminated much more rapidly with a half-life of only 1.8 hours.

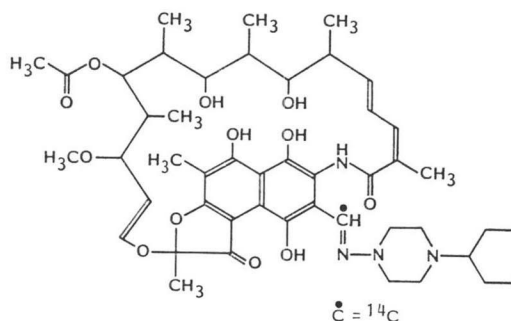
Rifapentine (INN), Fig. 1, is a new semi-synthetic rifamycin belonging to the class of piperazinyl hydrazone derivatives of 3-formyl rifamycin SV<sup>1)</sup>.

The antimicrobial spectrum of this antibiotic substantially resembles that of its homologue rifampicin, with a remarkably greater therapeutic efficacy in the experimental infection against both *Mycobacterium tuberculosis* and *Mycobacterium leprae*<sup>2,3)</sup>.

Preliminary pharmacokinetic studies in the rat<sup>4)</sup> and in volunteers<sup>5)</sup> showed that peak serum levels obtained after iv and/or po administration of rifapentine were comparable to those produced by corresponding doses of rifampicin, but that the two compounds have markedly different half-lives, rifapentine persisting in serum about 4~5 times longer.

The present report deals with the results of a more extensive study of the pharmacokinetics of rifapentine in the rat, with limited observations of the pharmacokinetics of this antibiotic in the mouse and the rabbit.

Fig. 1. Chemical structure of rifapentine.  
MW 877.06



## Materials and Methods

### Products

[38-<sup>14</sup>C]Rifapentine of specific activity 6.15 mCi/mmol was synthesized in the Radioisotopes Laboratory of Lepetit S.p.A. The radiochemical purity of the compound (98%) was determined by means of thin-layer chromatography (solvent system, benzene - Me<sub>2</sub>CO - EtOH, 1: 1: 0.4) autoradiography and liquid scintillation. Unlabeled rifapentine with a chemical purity of 97.6%, as determined by HPLC (stationary phase Lichrosorb RP8-7 $\mu$ , mobile phase 0.05 M NaH<sub>2</sub>PO<sub>4</sub> - CH<sub>3</sub>CN, 55: 45, pH 6.9, UV 254 nm) was supplied by our pilot plant.

### Animal Protocols

Male and female Wistar rats weighing 180~240 g, male (CD-I) mice weighing about 20 g, both supplied by Charles River Italy, and male New Zealand rabbits weighing 1.8~2.5 kg, obtained from the Toxicology Center, Breeding and Animal House, of Lepetit S.p.A. were used in the study. Animals were housed in air conditioned rooms at 22°C and 60% relative humidity with a 14-hour light-10-hour darkness photoperiod for at least one week and were fasted the night prior to treatment.

The doses administered were 3 and 10 mg/kg. The compound was given orally (po) as a suspension in 0.5% methylcellulose solution (2 ml/kg) containing 10<sup>-3</sup> M ascorbic acid as antioxidant, while in the case of intravenous administration (iv) rifapentine was dissolved in 0.1 M NaHCO<sub>3</sub> solution containing 10% of *N,N*-dimethylformamide and ascorbic acid 10<sup>-3</sup> M adjusted to pH 8.5~8.6 with 1 N NaOH. The volume administered was 1 ml/kg. In the rat, the compound was injected into the femoral vein, in the mouse into the caudal vein, and in the rabbit into the marginal vein of the ear. In the rat, heparinized blood was withdrawn from the abdominal aorta (HPLC assay) or retro-orbital sinus (<sup>14</sup>C assay), in the mouse from the carotid (by decapitation), and in the rabbit from the marginal vein of the ear. The number of animals used in the different experiments is reported in Tables and Figures. Bile samples were collected for periods of 4 hours from anesthetized rats, (1.25 g/kg ip of ethyl urethane) cannulated at the common bile duct. For tissue distribution studies rats were sacrificed under ether anesthesia by bleeding from the abdominal aorta. Feces and urine were collected by means of glass metabolic cages. For <sup>14</sup>CO<sub>2</sub> collection the cages were hermetically sealed, while aeration inside these was achieved by means of a suitable pump, passing the outgoing air through a trap containing ethanolamine - MeOH in the proportions of 4: 1.

### Radiochemical Determinations

Total radioactivity measurements were made with an Intertechnique SL 40/4000 scintillation counter. Samples were prepared as follows: plasma and urine were added directly to the scintillation cocktail Instagel (Packard Inst., Downers Grove, Ill.). Samples of lyophilized feces were solubilized in Soluene 350 (Packard) for 2 hours at 50°C and decolorized by the addition of 30% H<sub>2</sub>O<sub>2</sub> and isopropanol for another 2 hours at 50°C. Water and Dimilume T 30 (Packard), were then added for counting.

The various organs were weighed immediately after removal, homogenized, freeze-dried, and 30 mg samples were moistened with H<sub>2</sub>O, added with a solution of Soluene 350 - 2-propanol, 10: 2, plus H<sub>2</sub>O<sub>2</sub>, and digested at 50°C for 4 hours; Dimilume T 30 was used as scintillation cocktail. The remainder carcasses, deprived of skin, tail and paws, were ground and homogenized by a Polytron model PT 20 ST (Kinematica GMBH, Luzern, Switzerland) before lyophilization and <sup>14</sup>C assay.

The radioactivity values (dpm) were calculated from efficiency curves obtained with the external standard method.

### High Performance Liquid Chromatography (HPLC)

The plasma and biliary levels of rifapentine and of one of its metabolites, 25-deacetyl rifapentine were determined by means of HPLC. A Waters Associates liquid chromatograph equipped with a 660-gradient former, a 440-UV detector at 254 nm (Hg) of fixed wavelength, and an automatic sampling device Wisp 710A, was used to carry out the analyses. The substances to be determined were extracted from the biological fluids according to the method already reported<sup>9)</sup>, while the conditions of chromatographic separation were suitably modified in order to elute the products under isocratic conditions as follows: stationary phase, Supelcosil LC-18, 5  $\mu$ m, 0.46  $\times$  15 cm (Supelco Inc., Bellefonte, PA); mobile

phase, (A) 0.05 M  $\text{Na}_2\text{HPO}_4$  at pH 7.6 with 0.1 M  $\text{H}_3\text{PO}_4$ , (B)  $\text{CH}_3\text{CN}$  containing 10% (v/v) of tetrahydrofuran; isocratic elution, 40% of B in A; flow rate 2 ml/minute; room temperature. Quantitation was effected with a Hewlett Packard 3380A integrator, using the internal standard method. The sensitivity of the analytical method was 0.2  $\mu\text{g/ml}$  for both rifapentine and 25-deacetyl rifapentine.

#### Protein Binding Assay

Aliquots (150  $\mu\text{l}$ ) of plasma samples collected up to 48 hours from rats (N=4) given iv 10 mg/kg of [ $^{14}\text{C}$ ]rifapentine were pooled, diluted 1:1 with Krebs-phosphate buffer, pH 7.4, and total radioactivity bound to proteins was determined at 37°C by the batch gel filtration procedure according to ASSANDRI and SEMENZA<sup>7</sup>.

#### Mathematical Processing

An open one-compartment model<sup>8</sup>) has been used in this pharmacokinetic study:

$$C_p^i = C_p^o \cdot e^{-\beta \cdot t} \quad (\text{iv administration})$$

$$C_p^i = (B \cdot e^{-\beta \cdot t}) - (A \cdot e^{-K_a \cdot t}) \quad (\text{po administration})$$

where:

$C_p$  = plasma concentration of the drug ( $\mu\text{g/ml}$ ),  $\beta$  = elimination rate constant ( $\text{hour}^{-1}$ );  $K_a$  = absorption rate constant ( $\text{hour}^{-1}$ );  $t$  = time;  $A$  and  $B$  = arbitrary constants.

The pharmacokinetic parameters calculated were: peak-level,  $C_{\text{max}}$  ( $\mu\text{g equiv/ml}$ ); terminal plasma half-life,  $t_{1/2\beta}$  (hours); area under the curve of the plasma levels  $\text{AUC}_{0 \rightarrow t_n}$ , or  $\text{AUC}_{0 \rightarrow \infty}$  ( $\mu\text{g equiv} \cdot \text{hour} \cdot \text{ml}^{-1}$ ); volume of distribution,

$\frac{V}{F} = \frac{\text{Dose}}{\text{AUC}_{0 \rightarrow \infty} \times \beta}$  ( $\text{liter} \cdot \text{kg}^{-1}$ ), where in iv administration  $F=1$  while in po treatments  $0 < F < 1$ ; extent of bioavailability,  $F_p$ , and total clearance,  $C_t$ ;

$$F_p = \frac{\text{AUC}_{0 \rightarrow \infty} \text{ po administration}}{\text{AUC}_{0 \rightarrow \infty} \text{ iv administration}} \times 100\%, \quad C_t = \frac{\text{Dose}}{\text{AUC}_{0 \rightarrow \infty} \times W} \quad (\text{ml} \cdot \text{kg} \cdot \text{hour}^{-1}).$$

## Results

### Plasma Levels in the Rat

Table 1 reports the concentration of total plasma radioactivity in male rats after iv and po treatment with 3 and 10 mg/kg of [ $^{14}\text{C}$ ]rifapentine. At both doses, the concentration of plasma radioactivity after iv administration fell rapidly during the distribution phase and then remained constant to about the 8th hour. Starting 24 hours after treatment, an elimination phase was observed characterized by a first-order kinetics, with mean half-lives of 14.4 (3 mg/kg) and 17.7 hours (10 mg/kg). The shape of these curves suggests parallel capacity-limited and first-order eliminations<sup>9</sup>), where the "steady-state" phase, besides an entero-hepatic recirculation of the drug, probably reflects the drug distribution and an incomplete fit to a one-compartment model.

As can be deduced from the high plasma levels measured 1 hour after administration, absorption of the radioactive dose from the gastro-intestinal tract into the bloodstream appears to be rapid, however peak concentrations ( $C_{\text{max}}$ ) were reached only 8 hours after administration or even later. In addition a comparison of the values of the areas under the curves (AUC) after po and iv administration (Table 2) revealed a reduced oral absorption at the higher dose ( $F_p=84\%$  or  $65\%$  after 3 or 10 mg/kg).

HPLC determination of the levels of unchanged drug after both parenteral and po administration (Table 1) gave plasma profiles substantially identical to those obtained by radiochemical assay. In particular, the values of  $C_{\text{max}}$ ,  $t_{1/2\beta}$  and AUC were very similar (Table 2). No significant pharmacokinetic differences linked to sex could be found after po administration of rifapentine to female rats (Table 2).

Table 1. Plasma levels of total radioactivity ( $^{14}\text{C}$ ) and rifapentine (R) in rats, mice and rabbits given iv or po [ $^{38}\text{-}^{14}\text{C}$ ]rifapentine or unlabelled rifapentine.

The values reported, expressed as  $\mu\text{g}$  or  $\mu\text{g}$  equiv of rifapentine per ml, are the means of 4 rats and 7 rabbits  $\pm$ S.E.M. or of groups of 4 mice each.

Collection time (hours)	Rat (N=4)						Mouse (composite sample)		Rabbit (N=7)	
	3 mg/kg		10 mg/kg		10 mg/kg		10 mg/kg		10 mg/kg	
	$^{14}\text{C}$ (iv)	$^{14}\text{C}$ (po)	$^{14}\text{C}$ (iv)	$^{14}\text{C}$ (po)	R (iv)	R (po)	$^{14}\text{C}$ (iv)	R (iv)	$^{14}\text{C}$ (iv)	R (iv)
5 <sup>a</sup>	7.71 $\pm$ 0.71	—	26.42 $\pm$ 2.88	—	23.05 $\pm$ 1.07	—	11.46	12.04	29.27 $\pm$ 2.19	18.24 $\pm$ 1.31
0.5	—	—	—	—	13.03 $\pm$ 0.82	—	9.12	9.92	22.20 $\pm$ 2.04	16.98 $\pm$ 1.87
1	4.43 $\pm$ 0.34	3.49 $\pm$ 0.24	14.86 $\pm$ 1.01	4.92 $\pm$ 0.63	15.95 $\pm$ 0.70	5.53 $\pm$ 0.67	9.86	10.92	15.62 $\pm$ 0.94	11.92 $\pm$ 1.07
2	3.94 $\pm$ 0.33	3.43 $\pm$ 0.23	12.52 $\pm$ 0.83	5.34 $\pm$ 0.65	10.98 $\pm$ 0.45	7.58 $\pm$ 0.68	8.73	10.01	10.05 $\pm$ 0.68	8.21 $\pm$ 0.93
4	3.33 $\pm$ 0.26	3.78 $\pm$ 0.19	11.35 $\pm$ 0.93	6.32 $\pm$ 0.45	14.22 $\pm$ 0.93	8.47 $\pm$ 1.19	7.93	9.01	6.33 $\pm$ 0.45	4.75 $\pm$ 0.84
6									2.97 $\pm$ 0.15 <sup>b</sup>	1.73 $\pm$ 0.12
8	3.70 $\pm$ 0.25	4.50 $\pm$ 0.25	11.64 $\pm$ 0.66	7.29 $\pm$ 0.58	12.84 $\pm$ 0.65	8.44 $\pm$ 0.70	5.65	6.09	1.72 $\pm$ 0.08	0.73 $\pm$ 0.07
16							3.26	3.57		
24	1.85 $\pm$ 0.16	2.06 $\pm$ 0.24	8.08 $\pm$ 0.94	4.38 $\pm$ 0.38	5.71 $\pm$ 0.69	5.09 $\pm$ 0.31	2.43	2.00	0.34 $\pm$ 0.05	
48	0.51 $\pm$ 0.07	0.81 $\pm$ 0.08	3.51 $\pm$ 1.08	2.70 $\pm$ 0.11	2.27 $\pm$ 0.11	1.86 $\pm$ 0.31	1.24	0.92		
72	0.18 $\pm$ 0.02	0.28 $\pm$ 0.01	1.24 $\pm$ 0.24	1.48 $\pm$ 0.12	0.88 $\pm$ 0.11					
96			0.50 $\pm$ 0.07	0.55 $\pm$ 0.11						

<sup>a</sup> Minutes. <sup>b</sup> Mean of 4 animals.

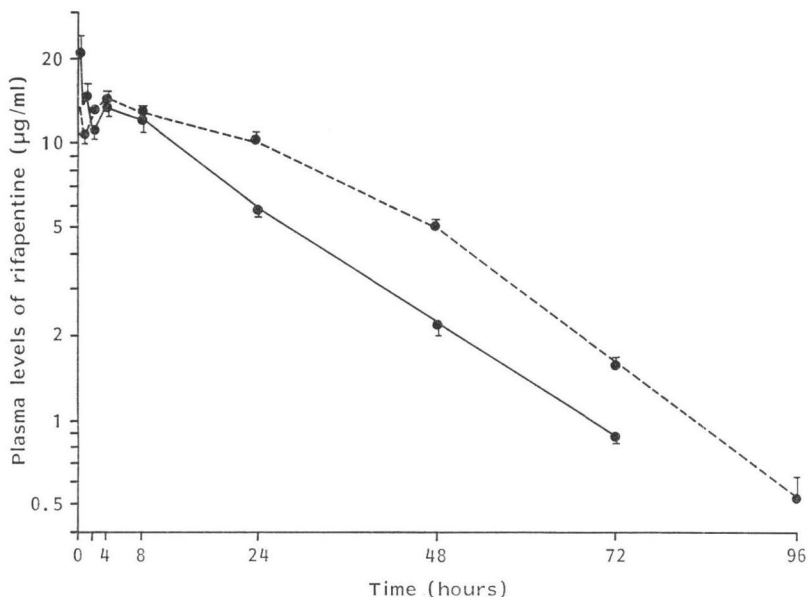
Table 2. Kinetic parameters of rifapentine (R) and total radioactivity ( $^{14}\text{C}$ ) in rats, mice and rabbits given po or iv [ $^{38-14}\text{C}$ ]rifapentine or unlabeled rifapentine.

Data (mean  $\pm$  S.E.M.) calculated on the basis of the plasma concentrations.

Experiment		$C_{\max}$ ( $\mu\text{g/ml}$ )	$T_{\max}$ (hours)	$t_{1/2\beta}$ (hours)	$\text{AUC}_{0\rightarrow\infty}$ ( $\mu\text{g}\cdot\text{hour/ml}$ )	V/F (liter/kg)	$C_r$ (ml/kg $\cdot$ hour)
Rat, 3 mg/kg	$^{14}\text{C}$ (iv)	$7.7\pm 0.7$	5 minutes	$14.4\pm 1.3$	$137\pm 3$	$0.54\pm 0.04$	26.0
	$^{14}\text{C}$ (po)	$4.5\pm 0.3$	8	$17.8\pm 0.7$	$115\pm 4$	$0.56\pm 0.02$	21.9
10 mg/kg	$^{14}\text{C}$ (iv)	$26.4\pm 2.9$	5 minutes	$17.7\pm 0.8$	$491\pm 18$	$0.52\pm 0.07$	20.4
	R (iv)	$23.1\pm 1.1$	5 minutes	$16.9\pm 1.1$	$421\pm 21$	$0.59\pm 0.06$	23.8
10 mg/kg	$^{14}\text{C}$ (po)	$7.3\pm 0.6$	8	$20.9\pm 2.4$	$317\pm 13$	$0.95\pm 0.10$	31.6
	R (po)	$8.4\pm 0.7$	4~8	$16.9\pm 1.2$	$297\pm 11$	$0.82\pm 0.04$	33.6
10 mg/kg (females)	R (po)	$8.6\pm 0.5$	4~8	$15.1\pm 0.5$	$317\pm 5$	$0.69\pm 0.03$	31.5
Mouse, 10 mg/kg	$^{14}\text{C}$ (iv)	11.5	5 minutes	22.8	207	1.59	48.3
	R (iv)	12.0	5 minutes	17.0	190	1.29	52.7
Rabbit, 10 mg/kg	$^{14}\text{C}$ (iv)	$29.3\pm 2.2$	5 minutes	$2.13\pm 0.18$	$70\pm 4$	$0.44\pm 0.08$	143.5
	R (iv)	$18.2\pm 1.3$	5 minutes	$1.82\pm 0.18$	$51\pm 5$	$0.57\pm 0.10$	197.9

Fig. 2. Plasma levels of rifapentine in rats given iv 10 mg/kg of the antibiotic as single dose (1st dose, solid line) or 24 hours after the 7th daily po 10 mg/kg dose (8th dose, dashed line).

The values reported are the means obtained from 4 animals  $\pm$  S.E.M.



In order to assess whether repeated administrations could influence the antibiotic's kinetics, male rats were treated orally with 10 mg/kg for 7 consecutive days, and then with the same dose by iv injection on the 8th day. The plasma rifapentine levels measured following the 8th iv dose are shown in Fig. 2 in comparison to those obtained after the single dosing.

The minimum plasma rifapentine levels increased about twice (from  $3.7\pm 0.3$  to  $6.3\pm 0.7$   $\mu\text{g/ml}$ ) between the 1st and 7th po dose, while the kinetic profile obtained after the 8th iv dose did not appear substantially different from that obtained after a single dose, and the terminal half-life was almost iden-

tical ( $15.38 \pm 1.70$  hours). However, probably because of the saturation of the elimination processes, the non-linear kinetics already recognized for the single dose was even more apparent. The plasma values of 25-deacetyl rifapentine determined in all the tests were always less than the sensitivity of the analytical method (HPLC) used.

#### Plasma Levels in the Mouse and the Rabbit

A comparative study was carried out in the mouse and the rabbit to explore possible pharmacokinetic and/or metabolic interspecies differences. The plasma levels of the radioactivity, unaltered drug, and its 25-deacetylated metabolite were determined after iv administration of 10 mg/kg of [ $^{14}\text{C}$ ]rifapentine (Table 1). Table 2 summarizes the relative kinetic parameters calculated from the plasma concentration curves. As already observed for the rat, the course and value of the plasma radioactivity levels in the mouse do not differ substantially from those obtained for rifapentine only. In comparison with the rat, it can be seen that, in spite of the fact that the terminal plasma half-life values are practically equal, the concentrations in the plasma are lower to the extent that the areas under the curve are about 54.9% less than those calculated for the rat. These results, in agreement with the distribution volume values,  $V/F$  rat=0.59, mouse=1.29 (liters/kg), suggest for the mouse a higher distribution of the drug in the extravascular tissue space.

Among the three animal species studied, the rabbit appears to be the one differing most greatly: the plasma half-life of the antibiotic is short ( $t_{1/2}=1.82 \pm 0.18$  hours), the calculated distribution volume is the lowest of all the species considered ( $V/F=0.44$  liter/kg), the AUC values for the unaltered drug are not only much lower than those calculated for the rat and the mouse ( $\text{AUC}=50.54 \pm 4.90 \mu\text{g} \times \text{hour} \times \text{ml}^{-1}$ ), but are also significantly less ( $P < 0.01$ ) than those measured for the total radioactivity ( $\text{AUC}=69.7 \mu\text{g} \times \text{hour} \times \text{ml}^{-1}$ ). Both in the mouse and the rabbit, the plasma levels of 25-deacetyl rifapentine were always less than 0.2  $\mu\text{g}/\text{ml}$ .

#### Binding to Plasma Proteins (Rat)

Plasma samples, collected 5 minutes, 1, 2, 4, 8, 24 and 48 hours following iv administration of the labelled antibiotic, were processed for measuring the fraction of radioactivity bound to proteins. In these samples, the fraction of  $^{14}\text{C}$  bound did not vary in time ranging between 92.7 and 96.4% of total, with standard errors ( $N=4$ ) of  $\pm 8.1$  to  $\pm 3.5\%$ . These figures are in good agreement with the values of the affinity constants of rifapentine calculated for bovine ( $3.6 \times 10^4$  liters/mol) and human ( $1.7 \times 10^4$  liters/mol) serum albumin<sup>(10)</sup>.

#### Distribution in the Tissues (Rat)

Four groups of rats were orally administered 10 mg/kg of [ $^{14}\text{C}$ ]rifapentine and autopsied 1, 8, 24 and 72 hours after administration to determine the  $^{14}\text{C}$ -levels in a series of tissue and organs (Table 3).

The highest concentration of radioactivity was measured in the following decreasing order in the adrenals, liver, pancreas, kidneys and submaxillary glands. The ratios between the tissue and plasma concentrations were shown to vary in time; they all increased up to the 8th hour, remained constant until the 24th hour and thereafter decreased. The main exception to this general trend was that the tissue plasma ratios of the liver were constantly high.

#### Biliary Excretion (Rat)

Biliary excretion of rifapentine and its metabolite 25-deacetyl rifapentine, was measured in rats cannulated at the common bile duct (enterohepatic circulation interrupted) after iv administration

Table 3. Tissue distribution of the total radioactivity in the rat after po administration of [ $^{14}\text{C}$ ]rifapentine at the dose of 10 mg/kg.

The values are expressed as  $\mu\text{g}$  equivalents of rifapentine per gram of fresh tissue or ml of plasma and are the means obtained from two animals.

Tissue or organ	$\mu\text{g}$ equiv/g of fresh tissue				$\frac{\mu\text{g equiv/g of tissue}}{\mu\text{g equiv/ml of plasma}}$			
	Time from administration (hours)				Time from administration (hours)			
	1	8	24	72	1	8	24	72
Adrenals	22.6	53.83	20.40	4.52	3.97	7.35	6.31	3.80
Brain	0.41	1.25	0.60	0.21	0.07	0.17	0.19	0.18
Fat	4.77	13.98	9.33	1.63	0.84	1.91	2.88	1.37
Femur	2.51	5.65	3.00	0.76	0.44	0.77	0.93	0.64
Heart	6.47	13.70	6.35	1.87	1.14	1.87	1.96	1.57
Kidneys	9.04	18.55	9.03	2.72	1.59	2.53	2.79	2.29
Liver	27.51	39.71	18.08	7.81	4.83	5.43	5.58	6.53
Lung	5.53	9.49	5.32	1.42	0.97	1.30	1.64	1.19
Muscle	3.08	8.20	3.76	1.14	0.54	1.12	1.16	0.96
Pancreas	7.89	27.49	10.09	3.78	1.38	3.76	3.11	3.18
Spleen	4.61	10.35	5.13	1.44	0.81	1.41	1.58	1.21
Submaxillary glands	7.08	18.61	8.22	2.09	1.24	2.54	2.54	1.76
Testes	0.45	3.55	2.81	1.04	0.08	0.49	0.87	0.87
Thymus	2.00	11.53	5.21	1.57	0.35	1.58	1.61	1.32
Plasma	5.70	7.32	3.24	1.19	1.00	1.00	1.00	1.00

Table 4. Biliary concentrations of rifapentine (R) and 25-deacetyl-rifapentine (DR) in rats given iv 10 mg/kg of rifapentine.

The values reported are the means obtained from 4 animals  $\pm$  S.E.M.

Collection time interval (hours)	Biliary concentration ( $\mu\text{g/ml}$ )		Ratio R/DR	Collection time interval (hours)	Biliary concentration ( $\mu\text{g/ml}$ )		Ratio R/DR
	R	DR			R	DR	
0~1	36.5 $\pm$ 8.2	1.1 $\pm$ 0.3	33.2	22~23	17.4 $\pm$ 2.3	3.0 $\pm$ 0.2	5.8
1~2	42.3 $\pm$ 7.2	2.0 $\pm$ 0.2	21.2	23~24	16.0 $\pm$ 2.2	4.5 $\pm$ 0.7	3.6
2~3	30.0 $\pm$ 3.0	2.6 $\pm$ 0.3	11.5	44~45	8.7 $\pm$ 1.6	1.9 $\pm$ 0.2	4.6
3~4	26.0 $\pm$ 4.0	3.7 $\pm$ 0.2	7.0	45~46	8.3 $\pm$ 1.5	2.2 $\pm$ 0.1	3.8
20~21	22.3 $\pm$ 2.5	3.7 $\pm$ 0.8	6.0	46~47	7.1 $\pm$ 1.3	1.9 $\pm$ 0.1	3.7
21~22	23.9 $\pm$ 2.2	4.5 $\pm$ 0.1	5.3	47~48	5.7 $\pm$ 0.9	1.8 $\pm$ 0.1	3.2

(10 mg/kg). Fractions of bile were collected between 0~4, 20~24 and 44~48 hours after administration. As Table 4 shows, the concentration of rifapentine in the bile, which was very high immediately after administration (25.97 $\pm$ 4.03~42.27 $\pm$ 7.20  $\mu\text{g/ml}$ ) successively fell and from the 44th to the 48th hour oscillated between 5.75 $\pm$ 0.89 and 8.66 $\pm$ 1.55  $\mu\text{g/ml}$ . The concentration of 25-deacetyl-rifapentine, which was very low at first, increased until it reached levels of about 3 to 5 times lower than those of the unaltered antibiotic.

#### Urinary and Fecal Elimination (Rat)

Urinary and fecal elimination of the radioactivity in rats given iv and po 10 mg/kg of [ $^{14}\text{C}$ ]-rifapentine were followed for a 12-day period; the cumulative values, expressed as percentage of the dose administered, are given in Table 5.

Table 5. Fecal and urinary elimination of radioactivity in the rat after iv and po administration of  $[38\text{-}^{14}\text{C}]$ -rifapentine at the dose of 10 mg/kg; mean cumulative values obtained from 5 animals  $\pm$ S.E.M.

Time (days)	% of the dose administered ( $\pm$ standard error)			
	Urine		Feces	
	iv	po	iv	po
0.33	2.91 $\pm$ 0.20	1.77 $\pm$ 0.10	ns	ns
1	5.12 $\pm$ 0.25	3.30 $\pm$ 0.27	17.20 $\pm$ 2.60	18.37 $\pm$ 2.77
2	6.83 $\pm$ 0.30	4.76 $\pm$ 0.32	50.11 $\pm$ 2.88	54.63 $\pm$ 3.38
3	7.40 $\pm$ 0.28	5.48 $\pm$ 0.34	62.80 $\pm$ 2.82	73.20 $\pm$ 2.34
4	7.80 $\pm$ 0.27	5.75 $\pm$ 0.35	76.42 $\pm$ 2.91	81.72 $\pm$ 2.35
5	8.10 $\pm$ 0.32	5.91 $\pm$ 0.37	82.12 $\pm$ 2.81	86.61 $\pm$ 1.81
6	8.42 $\pm$ 0.35	6.08 $\pm$ 0.37	85.44 $\pm$ 2.90	89.86 $\pm$ 2.05
7	8.72 $\pm$ 0.36	6.19 $\pm$ 0.37	86.55 $\pm$ 2.70	91.11 $\pm$ 2.24
8	8.90 $\pm$ 0.34	6.23 $\pm$ 0.38	87.71 $\pm$ 2.51	91.62 $\pm$ 2.24
9	9.10 $\pm$ 0.34	6.27 $\pm$ 0.38	88.19 $\pm$ 2.35	92.00 $\pm$ 2.28
10	9.40 $\pm$ 0.33	6.30 $\pm$ 0.38	88.70 $\pm$ 2.24	92.19 $\pm$ 2.28
11	9.76 $\pm$ 0.33	6.31 $\pm$ 0.38	88.94 $\pm$ 2.10	92.31 $\pm$ 2.27
12	9.91 $\pm$ 0.33	6.33 $\pm$ 0.38	89.10 $\pm$ 2.11	92.38 $\pm$ 2.28

ns, No samples.

A mean of  $89.10\pm 2.11$  and  $92.38\pm 2.28\%$  of the dose, was recovered in the feces, while  $9.91\pm 0.33$  and  $6.33\pm 0.38\%$  was eliminated in the urine following the iv and po dosing, respectively. Measurable concentrations of  $^{14}\text{C}$  were not found in the exhaled air, while the residual radioactivity in the carcasses of animals sacrificed at the end of the experiments amounted to 0.62 to 0.96% of the dose.

### Discussion

The synthesis and development of rifapentine was aimed at obtaining a new semisynthetic rifamycin with a pharmacokinetic profile with a prolonged duration of action. In fact, preliminary studies on healthy volunteers, administered single doses of 4~8 mg/kg<sup>5)</sup>, demonstrated that rifapentine had a high plasma half-life of at least 13~15 hours which is 4 to 5 times longer than that of rifampicin<sup>11,12)</sup>. In the rat, the plasma concentration profile of the antibiotic obtained after the po treatment substantially reflects that previously reported in volunteers. Both, the rates of absorption (peak time 8 hours) and elimination ( $t_{1/2}\beta \approx 17$  hours) as well as the values of peak level ( $C_{\text{max}}=9.7$   $\mu\text{g/ml}$  after 10 mg/kg dose) and AUC (279  $\mu\text{g}\cdot\text{hour/ml}$  after 10 mg/kg dose) were found to be close enough to those measured in man (ref 5 and BUNIVA *et al.* 13th International Congress of Chemotherapy, Wien, 1983, proceedings part III, PS 4.6/6-9).

The preliminary data obtained in mice following the iv administration indicate, also in this species, a similar kinetic behavior of rifapentine. In the rabbit instead, rifapentine is eliminated ten times more rapidly than in the rat, mouse and man.

A series of data, completing the pharmacokinetic profile of the antibiotic were obtained in the rat. After a single dose, it was not possible to demonstrate any kinetic difference related to sex. The po adsorption of rifapentine was found to be high at the 3 mg/kg dose ( $F_p=84.4\%$ ), lower after 10 mg/kg ( $F_p=64.5\%$ ), moreover in spite of the high plasma levels of rifapentine measured 1 hour post-administration, the peak levels were reached in 4~8 hours. This suggests that the absorption rate of the dissolved antibiotic is rapid but is related to its dissolution time.

Repeated treatment with 7 po doses at daily intervals followed by 1 iv dose, each of 10 mg/kg, made it possible to reach the stationary-state. Afterwards the terminal half-life was unchanged, so that it can be presumed that a metabolic self-induction phenomenon may not arise at these dose levels.



As regards the metabolism of rifapentine it should be noted that in both the rat and the mouse and to a lesser extent in the rabbit, levels and AUC values of the drug were almost identical to those calculated for the total radioactivity, indicating a negligible or low concentration of plasma metabolites.

As reported in literature, rifapentine and rifamycins<sup>13)</sup> are mainly eliminated in the bile, either unchanged or as metabolites. In the rat, rifapentine is almost completely eliminated in the feces, *via* the bile (92% of the dose), with a rate of excretion of about 4~5 times less than that of rifampicin, (8% of the dose eliminated in 4 against 38%), which is consistent with the half-life ratio of the two rifamycins. Among the metabolites formed, only the 25-deacetyl derivative has been identified and measured although in this species it does appear to be one of the major biotransformation products<sup>4)</sup>.

Besides the hepato-biliary excretion system, rifapentine displays great affinity for the adrenals, pancreas, kidneys and salivary glands. Concentrations slightly higher than in plasma have been measured in the lungs, while the drug does not appear to cross the blood-brain barrier to any quantitatively important extent. Consistent with the findings of IRVESEN and co-workers obtained in dogs<sup>14)</sup> the levels of radioactivity found in the bone were much higher than those reported for rifampicin. At this point it appears important to observe that the concentrations of rifapentine in the various organs 24 hours after administration are about 40-fold those measured for rifampicin<sup>4)</sup> and that the plasma and/or tissue levels of the antibiotic 72 hours after treatment are still decisively higher than the MIC values measured *in vitro* for several strains of *M. tuberculosis* var. *hominis*<sup>1)</sup>.

This overall evidence suggests that with rifapentine, more advantageous treatment schedules may be expected than with rifampicin.

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