A STUDY OF THE EFFECTS OF RIFABUTIN ON ISONIAZID PHARMACOKINETICS AND METABOLISM IN HEALTHY VOLUNTEERS

M. Breda[1], E. Pianezzola[1], M. Strolin Benedetti[1], C. Efthymiopoulos[1], M. Carpentieri[1], D. Sassella[1] and R. Rimoldi[2]

[1]*Farmitalia Carlo Erba, R&D - Erbamont Group, Milan*
[2]*Ospedale di Circolo, Varese*
Italy

SUMMARY

The effect of repeated administration of rifabutin on the pharmacokinetics and metabolism of isoniazid was evaluated in 6 healthy volunteers. The subjects received on day 1 and 9 a single oral dose of 300 mg isoniazid and from day 2 to 8 a single daily oral dose of 300 mg rifabutin. Two out of 6 subjects were shown to be rapid acetylators. No significant modification of the plasma pharmacokinetic profiles of isoniazid and acetylisoniazid was found. Evidence exists in the present study for autoinduction of rifabutin metabolism; this is shown by the lower plasma concentrations obtained 24 h after the seventh dose as compared to the theoretical concentrations.

KEY WORDS

rifabutin, isoniazid, drug interaction

Address for correspondence:
Dr. M. Strolin Benedetti
R&D/Pharmacokinetics and Metabolism
Farmitalia Carlo Erba
Via per Pogliano
20014 Nerviano, Milan
Italy

INTRODUCTION

Rifabutin, 4-deoxo-3,4-[2-spiro-(N-isobutyl-4-piperidyl)]-(1H)-imidazo (2,5-dihydro)-rifamycin S, a semisynthetic derivative of rifamycin S, is a new antimicrobial agent which shows broad-spectrum activity, mainly against gram-positive bacteria and *Mycobacteria* /1/. In addition to being more potent than rifampicin against *Mycobacterium tuberculosis,* rifabutin is effective against *M. leprae* /2/ and against *M. avium* complex and other atypical mycobacteria, which are generally resistant to most antibacterial agents including traditional anti-tuberculosis drugs /3/.

In a comparative study with rifampicin /4/, rifabutin had shown enzyme inducing properties in man, although at the dosages assessed, which are those generally used in the treatment of tuberculosis (300 mg for rifabutin and 600 mg for rifampicin), they were considerably less than those of rifampicin. In fact, previous studies had shown that rifampicin is a potent enzyme inducer in man, as indicated by its capacity to increase microsomal cytochrome P-450 content and oxidative metabolism of steroids in liver biopsies from patients treated with the drug (600 mg/ day for 6-10 days) /5/.

As observed for rifampicin in human volunteers /6/ and in patients with tuberculosis /7/, autoinduction of rifabutin metabolism was observed in healthy volunteers following repeated oral administration of 450 mg once daily for 10 days /8/.

The combination of isoniazid (300 mg) and rifabutin (300 mg) has commonly been employed in clinical trials for the treatment of tuberculosis /9, 10/. Rifampicin is also often given in conjunction with isoniazid. Some reports have suggested that the combination of isoniazid and rifampicin results in a greater incidence of hepatic damage than other combinations /11, 12/, whereas other reports have not supported these findings /13, 14/. The hepatic damage caused by isoniazid is believed to be due to monoacetylhydrazine, a metabolite of isoniazid, which is hepatotoxic in experimental animals, but only after suitable induction of the microsomal enzymes /15/. According to other authors /16, 17/ the hepatotoxic action of metabolites of isoniazid is due to the hydrazine formed directly from isoniazid.

Whichever metabolic pathway(s) is responsible for isoniazid hepatotoxicity, it was considered interesting to study whether or not rifabutin induces isoniazid metabolism. Since rifampicin has been shown to be an inducer in some animal species and not in others /18,

19/ and since rifabutin is extensively metabolized /20/ with important species differences /21/, we decided to study the inducing effects of rifabutin on isoniazid metabolism directly in man.

The aim of our study was primarily to assess whether repeated administration of therapeutic doses of rifabutin affects the pharmacokinetics and metabolism of isoniazid in healthy volunteers. In addition, the study was designed to assess whether autoinduction of rifabutin metabolism occurred. The major pathway of isoniazid metabolism in both slow and rapid acetylators being acetylation /22/, isoniazid and acetylisoniazid were measured. Since deacetylation is an important metabolic pathway for rifabutin /8, 20/, the concentrations of its 25-O-deacetyl derivative were also monitored.

MATERIALS AND METHODS

Chemicals

Isoniazid was purchased from Lancaster Synthesis (England), iproniazid (Internal Standard) from Aldrich Chemical Company, Milwaukee (USA), rifabutin and 25-O-deacetylrifabutin were from Farmitalia Carlo Erba, Milan (Italy).

Acetylisoniazid was prepared in our laboratories according to a modification /23/ of the method described by Fox and Gibas /24/.

Analytical procedures

Isoniazid and rifabutin and their respective metabolites were measured separately in plasma using two different HPLC assays. Briefly, after addition of iproniazid (I.S.), isoniazid and acetylisoniazid were extracted from plasma with $CHCl_3$/*n*-butanol (7:3, v/v) according to the procedure described by Hutchings *et al.* /25/. The organic phase was back-extracted with H_3PO_4 and the aqueous phase was injected into the HPLC system (column: Spherisorb-CN, 250 x 4.6 mm I.D., particle size 5 μm; mobile phase: 0.01 M H_3PO_4/acetonitrile, 80:20, v/v; flow rate 1.2 ml/min; UV detection wavelength 266 nm). The linearity of this method was evaluated from eight calibration curves obtained on different days in the range 0.05-10 μg/ml, for both compounds, by linear regression analysis of the peak height ratio (peak height of each analyte/I.S. peak

height) vs the concentration (ng/ml) of each analyte. The mean slope of the calibration curves obtained was 1.72×10^{-3} (CV = 7.40%) for isoniazid and 2.08×10^{-3} (CV = 7.69%) for acetylisoniazid. Correlation coefficients (r) were better than 0.9978 for both compounds. When submitted to Student's t-test, intercept values were not significantly different from zero. The precision of this method expressed as percentage coefficient of variation (CV) of replicate analysis carried out on different days with spiked plasma samples was better than 9% for both compounds (n = 24). The accuracy evaluated on the same samples and expressed as the ratio between the amount found/added was 96.83% for isoniazid and 103.78% for acetylisoniazid. The lower limit of quantification was 50 ng/ml for both compounds. Rifabutin and 25-O-deacetylrifabutin were extracted from plasma samples with CH_2Cl_2/*i*-octane (4:6, v/v) and the organic phase evaporated to dryness. The dried extract was reconstituted with the mobile phase and injected onto the HPLC system (column: Novapak C_{18} 150 x 4.6 mm I.D., particle size 4 μm; mobile phase: 0.05 M KH_2PO_4/acetonitrile 60:40, v/v; flow rate 0.6 ml/min; UV detection wavelength 275 nm) /8, 26/. This method had a lower limit of quantification of 10 and 5 ng/ml for rifabutin and its metabolite, respectively (Breda *et al.*, unpublished results). The linearity of this method was evaluated from three calibration curves obtained on different days in the range 10-908 ng/ml for rifabutin and 5-950 ng/ml for 25-O-deacetylrifabutin by linear regression analysis of the peak height vs the concentration of each analyte. The mean slope was 66.08 (CV = 4.15%) for rifabutin and 171.47 (CV = 4.32%) for 25-O-deacetylrifabutin. Correlation coefficients (r) were better than 0.9948 for both compounds. When submitted to Student's t-test intercept values were not significantly different from zero ($p > 0.05$). The precision evaluated on blank plasma samples spiked with the two compounds and expressed as CV of replicated analyses was better than 8% (n = 9). The accuracy evaluated on the same samples was a mean of 92.7% for rifabutin and 99.2% for 25-O-deacetylrifabutin.

Specificity tests were carried out to ensure that isoniazid and acetylisoniazid did not interfere with the determination of rifabutin or its metabolite and vice versa.

Clinical procedures

Six healthy male volunteers, aged between 23 and 27 years, with good general health status and normal liver and renal functions, participated in the study, after giving written informed consent.

On days 1 and 9 of the investigation, the volunteers received a single oral dose of 300 mg isoniazid (capsules, Farmitalia Carlo Erba). Blood samples were collected into heparinized tubes at the following times: before and 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 9, 12 and 24 hours after dosing. Plasma was immediately separated by centrifugation (1200 *g* for 10 min) and stored at -80°C, in order to prevent loss of isoniazid and acetylisoniazid /27/.

From day 2 to day 8 each subject received a single daily dose of 300 mg rifabutin orally (two capsules of 150 mg, Farmitalia Carlo Erba) and blood samples were collected 24 hours after the administration of the 1st, 5th, 6th and 7th dose. Both isoniazid and rifabutin were given under fasting conditions.

Safety tests were carried out at the screening visit and on days 1, 2, 9 and 10. Tests included haematology (haemoglobin, haematocrit, RBC, WBC and platelet count), blood chemistry (glucose, creatinine, SGOT, SGPT, LDH, gamma-GT, total and indirect bilirubin, alkaline phosphatase, prothrombine time, sodium, potassium, chloride, calcium, phosphorus, total protein, albumin), urinalysis (specific gravity, albumin, glucose, acetone, pH and microscopic examination) and creatinine clearance. In addition, haematology and liver function tests were performed on days 3, 5, 7 and 16 and at the final visit (2 weeks after the study's end). Vital signs were monitored at the screening visit, on days 1 and 10, and at the final visit.

Sample storage and thawing

Plasma samples were kept strictly frozen at -80°C during the whole storage period (< 5 weeks); in particular, the samples were transported from the clinic to the analytical centre in thermal containers on dry ice within about 1 h. Particular care was adopted in thawing; after removal from storage, plasma samples were thawed in a water bath at 22°C for 6 min under stirring and immediately assayed in order to avoid decomposition of the compounds of interest.

Pharmacokinetic analysis

Data plotting, calculations and analysis were performed using an IBM PS2 PC with SIPHAR software /28/. Non-compartmental methods were used for the pharmacokinetic analysis of the data. In order to evaluate whether autoinduction of rifabutin metabolism occurred after repeated administration of the compound, theoretical concentrations after each dose were calculated according to the principle of superposition. To calculate the theoretical concentrations, the experimental concentrations observed 24 hours after the first dose of rifabutin obtained in the present study and the average value of the elimination rate constant of rifabutin (0.015 h^{-1}) reported in a previous study /8/ were used. Monoexponential decline of rifabutin plasma concentrations beyond 24 h from drug administration was assumed. The theoretical concentrations obtained each time by adding contributions from preceding doses were compared with the experimentally obtained values.

RESULTS

Treatments were well tolerated. No adverse events were reported nor clinically significant changes in vital signs or laboratory tests observed.

The chromatographic profile of a plasma extract from a subject who received 300 mg isoniazid is shown in Figure 1. Figure 2 shows the profile of a plasma extract from a subject 24 h after the administration of 300 mg rifabutin.

Both plasma profiles (Figures 3, 4) and pharmacokinetic parameters (Tables 1, 2) suggested that volunteers #1 and #3 were rapid acetylators, the remaining 4 volunteers being slow acetylators of isoniazid. The subjects were considered rapid or slow acetylators as a function of isoniazid half-life, rapid acetylators being those with elimination half-life less than 1.8 h /13, 29/.

Maximum plasma concentrations of isoniazid in the rapid acetylators were on average 5.12 μg/ml and 5.22 μg/ml after the first and the second administration of isoniazid respectively, and were observed within 2 h from dosing in both cases. The corresponding C_{max} values in the slow acetylators were on average 6.76 μg/ml and 6.42 μg/ml and were observed within 1 h after administration.

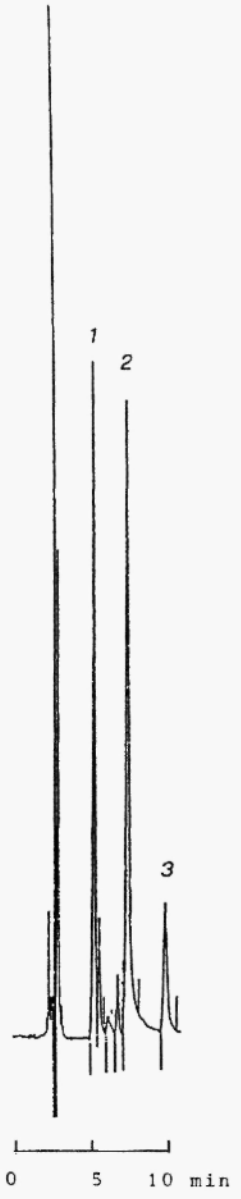

Fig. 1: Chromatographic analysis of a plasma sample from a volunteer after administration of 300 mg isoniazid. Peaks are identified as: 1 = acetylisoniazid (R_t = 5.29); 2 = isoniazid (R_t = 7.49); 3 = iproniazid (I.S., R_t = 10.01).

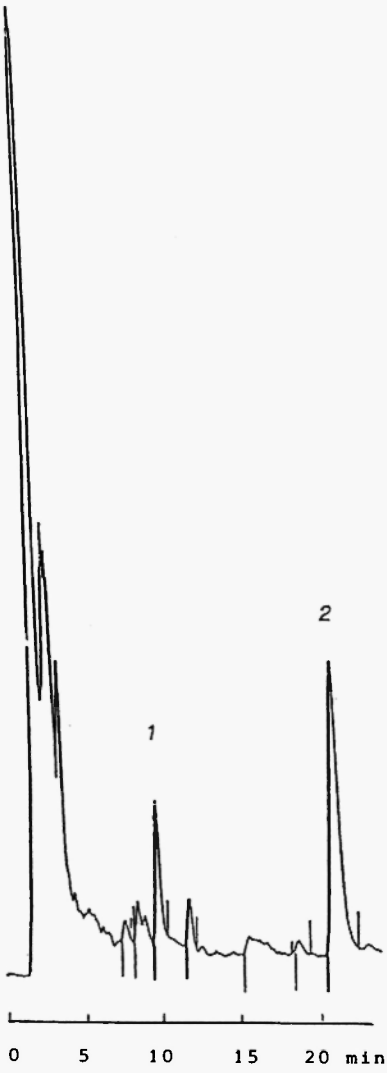

Fig. 2: Chromatographic analysis of a plasma sample from a volunteer after administration of 300 mg rifabutin. Peaks are identified as: 1 = 25-O-deacetylrifabutin (R_t = 10.13); 2 = rifabutin (R_t = 21.81).

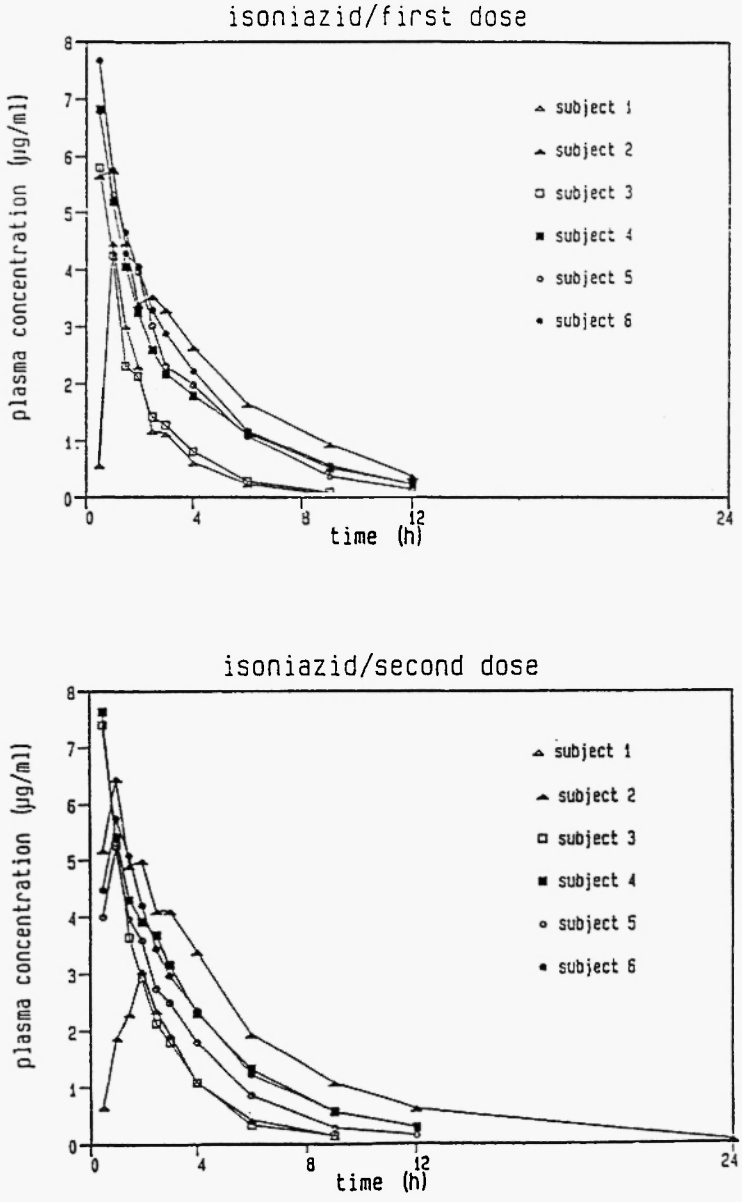

Fig. 3: Plasma levels of isoniazid in six healthy male volunteers (#1 to #6) after administration of 300 mg isoniazid before (upper graph) and after (lower graph) treatment with rifabutin.

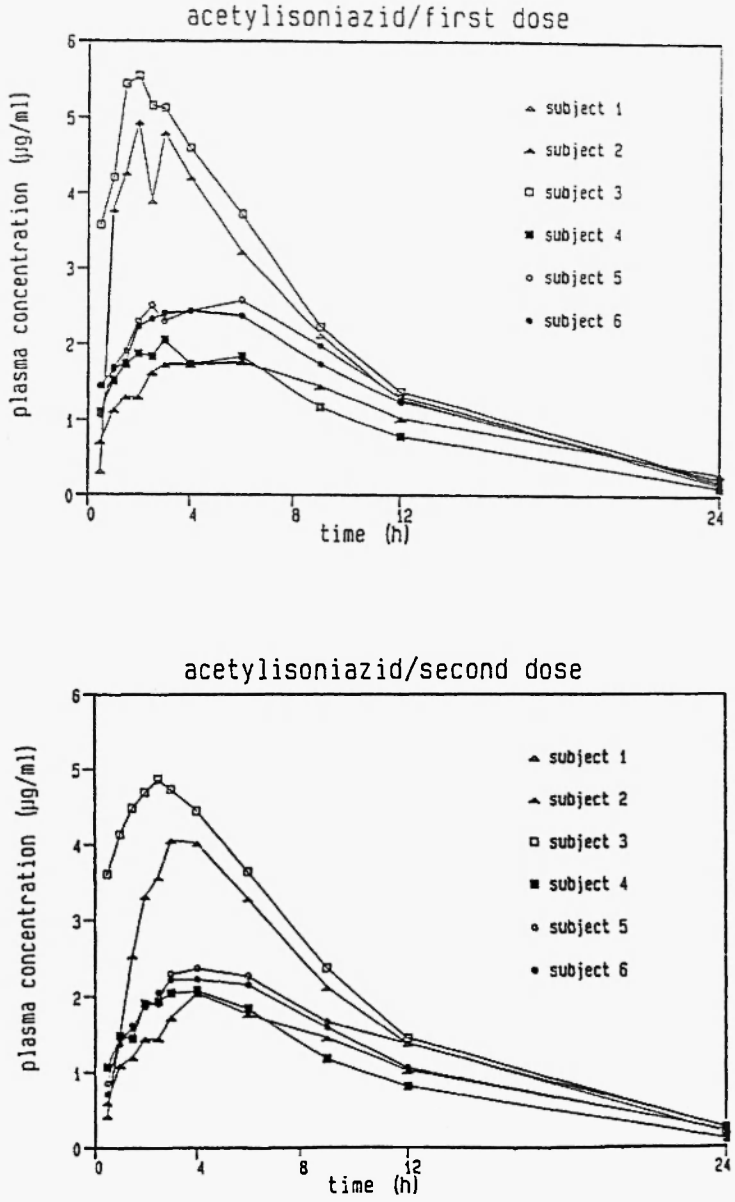

Fig. 4: Plasma levels of acetylisoniazid in six healthy male volunteers (#1 to #6) after administration of 300 mg isoniazid before (upper graph) and after (lower graph) treatment with rifabutin.

TABLE 1
Pharmacokinetic parameters of isoniazid in six male healthy volunteers after a single oral dose of 300 mg isoniazid

		Subjects (acetylator phenotype)							
		Fast Acetylators			Slow Acetylators				
	Administration	1	3	Mean value (n=2)	2	4	5	6	Mean value ± SD (n=4)
Cmax ($\mu g \cdot ml^{-1}$)	First	4.47	5.78	5.12	5.74	6.84	6.77	7.69	6.76 ± 0.80
	Second	3.06	7.37	5.22	6.44	7.64	5.22	6.39	6.42 ± 0.99
Tmax (h)	First	1.0	0.5	0.8	1.0	0.5	0.5	0.5	0.6 ± 0.2
	Second	2.0	0.5	2.2	1.0	0.5	1.0	1.0	0.9 ± 0.2
AUC ($0-\infty$) ($\mu g \cdot ml^{-1} \cdot h$)	First	8.26	11.02	9.64	26.96	20.92	21.00	23.74	23.16 ± 2.86
	Second	9.74	14.89	12.32	34.37	24.61	17.87	23.69	25.14 ± 6.84
MRT (h)	First	2.41	2.15	2.28	4.57	3.75	3.24	3.56	3.78 ± 0.57
	Second	3.18	2.20	2.69	5.28	3.74	3.31	3.86	4.05 ± 0.85
$t_{\frac{1}{2}}$ el (h)	First	1.30	1.41	1.36	3.05	2.51	2.11	2.36	2.51 ± 0.40
	Second	1.52	1.43	1.48	3.55	2.48	2.08	2.46	2.64 ± 0.63

TABLE 2

Pharmacokinetic parameters of acetylisoniazid in six male healthy volunteers after a single oral dose of 300 mg isoniazid

	Administration	Subjects (acetylator phenotype)							
		Fast Acetylators			Slow Acetylators				
		1	3	Mean value (n=2)	2	4	5	6	Mean value ± SD (n=4)
Cmax ($\mu g \cdot ml^{-1}$)	First	4.95	5.53	5.24	1.76	2.06	2.58	2.44	2.21 ± 0.37
	Second	4.06	4.86	4.46	2.03	2.07	2.36	2.23	2.17 ± 0.15
Tmax (h)	First	2.0	2.0	2.0	6.0	3.0	6.0	4.0	4.6 ± 1.5
	Second	3.0	2.5	2.8	4.0	4.0	4.0	4.0	4.0 ± 0
AUC ($0-\infty$) ($\mu g \cdot ml^{-1} \cdot h$)	First	44.04	51.44	47.74	27.87	23.06	34.26	33.13	29.58 ± 5.15
	Second	41.98	51.07	46.52	26.43	24.08	33.34	29.85	28.42 ± 4.04
MRT (h)	First	7.11	7.14	7.12	11.35	7.72	8.52	8.87	9.12 ± 1.57
	Second	8.05	7.53	7.79	9.68	7.87	9.68	9.29	9.13 ± 0.86
$t_{\frac{1}{2}}$ el (h)	First	4.02	4.37	4.20	6.71	4.09	4.20	4.95	4.99 ± 1.21
	Second	4.35	4.58	4.46	5.20	4.24	5.46	5.28	5.04 ± 0.55

The average $AUC_{0-\infty}$ values of isoniazid in the rapid acetylators were 9.64 $\mu g.ml^{-1}.h$ and 12.32 $\mu g.ml^{-1}.h$ after the first and the second treatment respectively, whereas the corresponding average values in the slow acetylators were 23.16 and 25.14 $\mu g.ml^{-1}.h$. The extrapolated part of the AUC was less than 6% in all cases.

Average MRT values of isoniazid of 2.28 h and 2.69 h (after the first and the second treatment) and of 3.78 h and 4.05 h were obtained in the rapid and slow acetylators respectively.

A similar trend was observed for the elimination half-life. Average values of $t_{1/2}$ were 1.36 h and 1.48 h in the rapid and 2.51 h and 2.64 h (first and second treatment) in the slow acetylators.

For acetylisoniazid, average maximum plasma concentrations of 5.24 $\mu g/ml$ and 4.46 $\mu g/ml$ were observed in the rapid acetylators after the first and the second treatment with isoniazid, respectively. In the slow acetylators, average C_{max} values were 2.21 $\mu g/ml$ and 2.17 $\mu g/ml$ for the first and the second treatment, respectively. Maximum plasma concentrations of acetylisoniazid were achieved within 3 h in the rapid and within 6 hours in the slow acetylators. In the rapid acetylators, average $AUC_{0-\infty}$ values of the metabolite were 47.74 $\mu g.ml^{-1}.h$ and 46.52 $\mu g.ml^{-1}.h$ (first and second treatment, respectively), whereas in the slow acetylators the average $AUCO_{0-\infty}$ values determined were 29.58 and 28.42 $\mu g.ml^{-1}.h$ (first and second treatment, respectively). Extrapolated AUC never exceeded 10% in all cases. MRT values of acetylisoniazid were on average 7.12 h and 7.79 h in the rapid and 9.12 h and 9.13 h in the slow acetylators, after the first and the second treatment with isoniazid, respectively. The corresponding average elimination half-life values were 4.20 h and 4.46 h (fast acetylators) and 4.99 h and 5.04 h (slow acetylators).

The plasma concentrations of rifabutin and 25-O-deacetyl-rifabutin observed 24 h after the first, fifth, sixth and seventh dose of rifabutin are shown in Table 3.

Figure 5 shows the theoretical and experimental concentrations of rifabutin 24 hours after the seventh dose of rifabutin in the six subjects.

TABLE 3

Plasma levels (ng/ml) of rifabutin (RIF) and 25-O-deacetyl rifabutin (DRIF) measured in six healthy male volunteers 24 hours after the administration of the 1st, 5th, 6th and 7th dose of rifabutin (300 mg, p.o.)

SUBJECT	1st DOSE		5th DOSE		6th DOSE		7th DOSE	
	RIF	DRIF	RIF	DRIF	RIF	DRIF	RIF	DRIF
1	57.13	5.07	41.32	n.d.	47.40	n.d.	48.36	n.d.
2	67.75	10.86	86.89	7.23	76.42	5.44	79.93	n.d.
3	80.33	n.d.	84.91	n.d.	88.47	n.d.	92.07	n.d.
4	56.54	6.31	96.72	5.19	90.19	5.63	88.43	5.65
5	51.48	10.27	137.89	16.22	95.60	9.37	59.73	n.d.
6	54.96	9.80	42.40	n.d.	46.76	n.d.	38.16	n.d.
MEAN	61.37	7.05	81.69	4.77	74.14	3.41	67.78	0.94
±S.D.	10.77	4.16	36.32	6.41	21.88	3.99	22.29	2.31

n.d. = not detectable (<5 ng/ml)

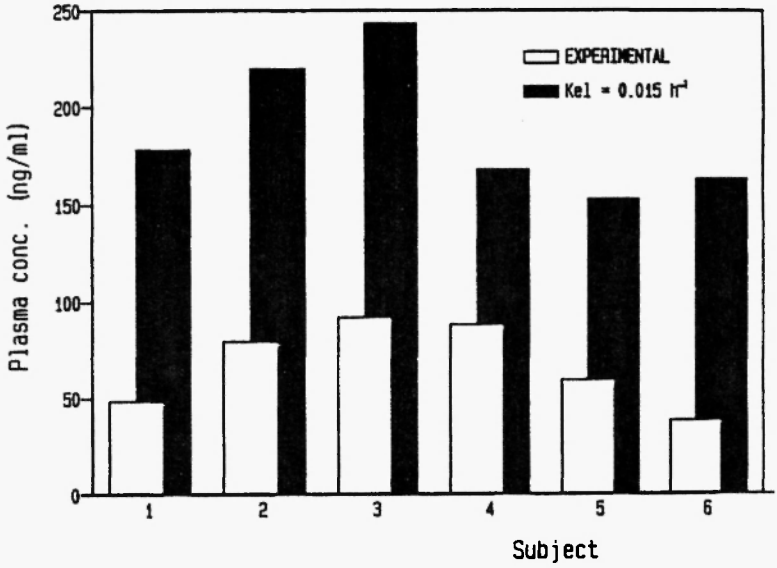

Fig. 5: Experimentally observed and theoretical (calculated using $K_{el} = 0.015$ h^{-1}) plasma concentrations (ng/ml) of rifabutin in six healthy male volunteers 24 h after the seventh dose of rifabutin (300 mg daily).

DISCUSSION

This study shows that no significant modification of the plasma pharmacokinetic parameters of both isoniazid and acetylisoniazid occurs, following treatment with 300 mg/day oral rifabutin for 7 days. To our knowledge there is no evidence in the literature of induction of acetyltransferases. A decrease of plasma levels of acetylisoniazid might have occurred in this study as a consequence of induction by rifabutin of further metabolism of acetylisoniazid and/or mono-acetylhydrazine.

The distribution of acetylator phenotype observed in this study is consistent with data in the literature indicating that about 60% of caucasians are slow acetylators /29/. The individual values of the molar AUC ratio (i.e. AUC acetylisoniazid/AUC isoniazid) obtained (about 4 for rapid and about 1.5 for slow acetylators) are similar to those previously reported in the literature /30/.

A study of the effect of rifampicin on isoniazid metabolism in human volunteers, showed no significant modifications of the urinary excretion of isoniazid, acetylisoniazid, mono and diacetylhydrazine

after treatment with therapeutic doses of isoniazid (300 mg daily) and rifampicin (450 mg daily) for 7 days, although there was a rise in the urinary excretion of 6-β-hydroxycortisol, indicating that induction of the microsomal enzymes by rifampicin had occurred /30/.

A minor pathway of isoniazid metabolism is the direct hydrolysis /16, 17/ by isoniazid hydrolase. Our results showed no modification of the plasma kinetics of unchanged isoniazid following rifabutin treatment, indicating that rifabutin does not induce this pathway, although further work is needed to draw a final conclusion.

Finally, the plasma concentrations of rifabutin observed 24 hours after the seventh dose were compared with the theoretical concentrations evaluated for each subject by the principle of superposition. In all subjects the actual plasma concentrations were lower than the theoretical concentrations, showing that autoinduction of rifabutin metabolism occurred after repeated 300 mg doses. This is in agreement with the findings of a previous study, in which, however, the daily dose of rifabutin and the duration of treatment were different from those of this study /8/.

In conclusion, the results obtained in this study showed no relevant modification in the plasma pharmacokinetics of isoniazid and acetylisoniazid either in the two rapid or in the four slow acetylators, following repeated treatment with rifabutin, although autoinduction of rifabutin metabolism occurred.

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