

# The Effect of Rifampicin and Pyrazinamide on Isoniazid Pharmacokinetics in Rats

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**ABSTRACT:** Tuberculosis chemotherapy involves combination of the drugs isoniazid (INH), rifampicin (RMP) and pyrazinamide (PYR) for a 6-month period. The present work investigated the influence of RMP and PYR on the pharmacokinetic parameters of INH when groups of rats were pre-treated for 21 days with INH alone or in combination with RMP and/or PYR, in the following amounts per kg body weight: INH 100 mg; INH 100 mg + RMP 100 mg; INH 100 mg + PYR 350 mg; INH 100 mg + PYR 350 mg + RMP 100 mg. It was found that the co-administration of PYR caused an increase in the INH distribution volume ( $V_d/F$ ), half-life of elimination ( $t_{1/2\beta}$ ) and clearance ( $Cl_T/F$ ), and a decrease in the area under curve 0 to 24 h ( $AUC$ ). Co-administration of RMP caused an increase in the  $Cl_T/F$  and a decrease in the  $AUC$ . The combination INH + PYR + RMP caused an increase in the  $Cl_T/F$  and a decrease in the  $AUC$ . These significant pharmacokinetic interactions between the tuberculostatic drugs might be related to differences in the therapeutic and toxic effects. Copyright © 2007 John Wiley & Sons, Ltd.

**Key words:** pharmacokinetics; isoniazid; pyrazinamide; rifampicin

## Introduction

Tuberculosis (TB) chemotherapy involves the combination of the drugs isoniazid (INH), rifampicin (RMP) and pyrazinamide (PYR) for a period as long as 6 months [1]. These combinations result in an increased therapeutic effectiveness; however, serious side-effects are also common. Indeed, the side-effects during tuberculosis chemotherapy play an important role in the patient's adherence to the treatment. In general, gastrointestinal intolerance symptoms

such as inappetency, nausea, heartburn, epigastric and abdominal pain are the most frequent effects [2]. These symptoms have also been associated with the development of hepatitis as a result of the INH toxic effect [2,3].

A number of mechanisms have been proposed to elucidate the pathogenesis of the INH-induced hepatitis in humans [4,5]. Among these, the most widely accepted one is that in which INH metabolism leads to the formation of hepatotoxic compounds [6,7]. The metabolism of INH involves an initial acetylation phase in order to form acetylisoniazid (AcINH) [4,8,9]. Hydrolysis is the subsequent stage, leading to isonicotinic acid and acetylhydrazine (AcHz). The AcHz may undergo further acetylation to diacetylhydrazine

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(DAcHz) or microsomal oxidation, leading to reactive metabolites which are well known hepatotoxic substances. At the initial stage of its metabolism, INH may also undergo direct hydrolysis to originate hydrazine (Hz), for which further oxidation also results in the formation of hepatotoxic metabolites [4,10]. There is also evidence of a considerable first-pass effect after oral isoniazid administration; however, oral bioavailability values have not been established [11].

The successful treatment of TB is highly dependent on the co-administration of INH, RMP and PYR; and it is evident that drug interactions could exacerbate the hepatotoxicity. For instance, RMP is well known as an inducer of several CYP isoenzymes, especially CYP3A. Parthasarathy *et al.* [12] found no evidence that PYR would contribute to this hepatotoxic effect; however, other studies found a significant contribution of PYR in the development of hepatotoxicity during treatment of humans with INH and PYR [13,14]. There are also studies suggesting that RMP, being a powerful CYP2E1 inducer, would increase the INH toxicity as a function of the increase in the production of reactive metabolites [3,14–16].

Since hepatotoxicity is strongly related to drug bioavailability, this study aimed to evaluate the influence of RMP and PYR on the pharmacokinetic parameters of INH administered to rats.

## Materials and Methods

### Chemicals

INH, RMP and PYR standards were granted by the Popular Drug Foundation; potassium phosphate and trichloroacetic acid (TCA) were purchased from Labsynth (São Paulo, SP, Brazil) and cinnamaldehyde (CA) from Sigma-Aldrich (St Louis, MO, USA). Acetonitrile and isopropanol were obtained from J.T. Baker (Phillipsburg, NJ, USA).

### Animals

Male Wistar rats (weighing around 250 g) were housed at a constant temperature ( $23 \pm 1^\circ\text{C}$ ), humidity ( $55 \pm 5\%$ ) and light cycle (12/12) with

food and water *ad libitum*. The experiments were conducted during the light phase.

### Administration of drugs in rats

The animals received the following drug dosage for a period of 21 days: Group I: INH (100 mg/kg/day)  $n = 50$ ; Group II: INH (100 mg/kg/day) + PYR (350 mg/kg/day)  $n = 50$ ; Group III: INH (100 mg/kg/day) + RMP (100 mg/kg/day)  $n = 50$ ; Group IV: INH (100 mg/kg/day) + RMP (100 mg/kg/day) + PYR (350 mg/kg/day)  $n = 50$ . These doses were based on hepatotoxicity studies in rats [17]. The sample size was based on the method published by Chow and Liu [18]. After 21 days, the animals were killed by decapitation before (time zero) and 0.25, 0.5, 0.75, 1, 1.5, 3, 6, 12, 24 h after administration. Five animals were used for each point. The blood samples were centrifuged and submitted to HPLC analysis.

### HPLC analysis of INH

The analysis of INH in serum samples was performed by HPLC based on the method developed by Seifart *et al.* [19]. The mixture was separated on a RP  $\text{C}_{18}$  column (5  $\mu\text{m}$ ,  $3.9 \times 300$  mm, Symmetry<sup>®</sup>, Waters<sup>®</sup>) through a linear gradient consisting of isopropanol 8–14%, acetonitrile 32–56% and 0.05 mol/l phosphate buffer 60–30% in a 15 min run time, delivered at a flow of 1 ml/min and detected at 340 nm. Briefly, to 300  $\mu\text{l}$  serum, was added 300  $\mu\text{l}$  10% trichloroacetic acid, vortexed and centrifuged at 2500 rpm, for 10 min. To the deproteinized supernatant 30  $\mu\text{l}$   $\text{H}_2\text{O}$  and 60  $\mu\text{l}$  1% CA was added. After vortexing and incubation for 10 min, at room temperature, the samples (50  $\mu\text{l}$ ) were injected into the HPLC system (Alliance separation model and 2487 UV-Vis detector, Waters).

Samples of serum, spiked with stock methanol solution of INH, were prepared and submitted to the analytical protocol. The calibration curve was linear ( $r = 0.9999$ ,  $n = 8$ ) in the range 0.78–50.0  $\mu\text{g/ml}$ , and the limit of quantitation was 70 ng/ml. Precision, expressed as the inter- ( $n = 5$ ) and intraday ( $n = 10$ ) coefficient of variation, was  $\leq 2.5\%$  on the same day and  $\leq 7.2\%$  between days for each quality control sample of 1, 10 and 40  $\mu\text{g/ml}$ , respectively. Accuracy expressed as the inter- and intraday %bias was

−7.6 to 1.2 on the same day and −7.3 to 1.1 between days at each quality control sample.

### Pharmacokinetic analysis

The INH kinetic disposition was evaluated after administration of the drug combinations within a period of 21 days. The pharmacokinetic parameters were calculated based on the plasma concentration  $\times$  time curves. The half-life of absorption ( $t_{1/2a}$ ), half-life of distribution ( $t_{1/2\alpha}$ ), half-life of elimination ( $t_{1/2\beta}$ ), constant of absorption ( $K_a$ ), constants of distribution ( $K_z$ ) and constant of elimination ( $K_{el}$ ) were determined through the bi-compartmental model. The constants were calculated by the equation  $0.693/t_{1/2}$ . The area under curve 0 to 24 h (AUC) was calculated by the trapezoidal method. The AUC was used for the calculation of the apparent total clearance ( $Cl_T/F = \text{dose}/AUC$ ) and for the calculation of the distribution apparent volume ( $V_d/F = Cl_T/F/\beta$ ).

### Statistical analysis

The data were expressed as mean and SEM, and analysed by one-way analysis of variance (ANOVA) followed by the Tukey test for multiple comparison among groups (Sigma-Stat software). The calibration and variation coefficient (CV%) curve calculations were performed through the Origin<sup>®</sup> program.

### Results

Figure 1 shows the time-dependent plots of the serum concentration of INH for the animals treated with isoniazid and associations. The pharmacokinetic parameters of INH are displayed in Table 1. The combination of INH with PYR (Group II), with RMP (Group III) and with RMP + PYR (Group IV) did not result in statistically significant differences in parameters  $K_a$  and  $t_{1/2a}$  of INH; it is important to point out that the drugs were administered at different times, eliminating the possibility of physical–chemical interactions.

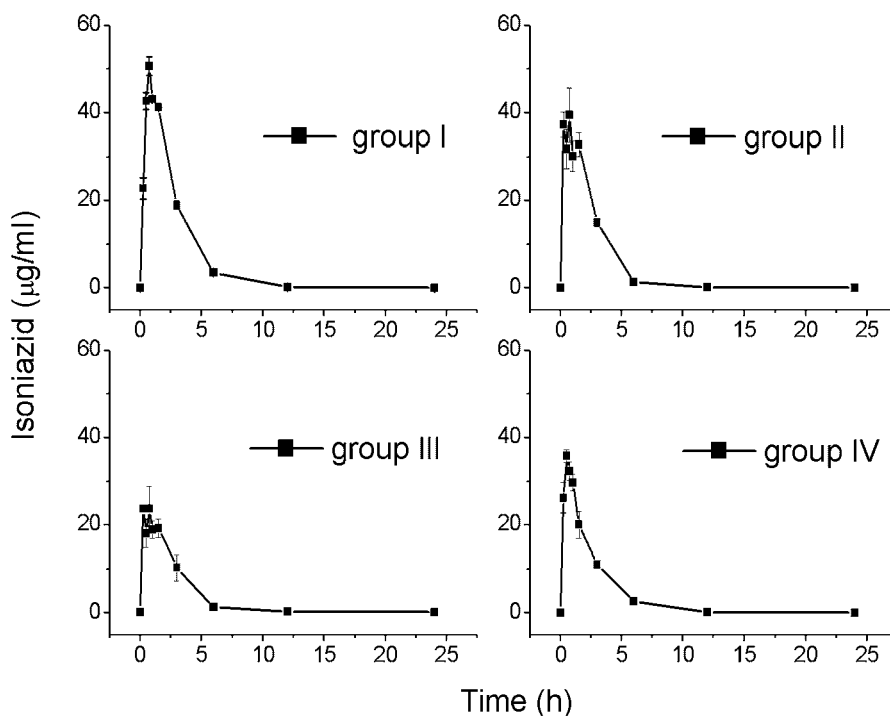


Figure 1. Time-dependent plasma concentration of isoniazid. The results are expressed as mean and SEM ( $n=5$ )

Table 1. Pharmacokinetic parameters of isoniazid

Parameter	INH alone Group I	INH + PYR Group II	INH + RMP Group III	INH + PMP + PYR Group IV
$t_{1/2\alpha}$ (h)	0.4 (0.030)	0.5 (0.060)	0.394 (0.08)	0.4 (0.020)
$K_a$ ( $\text{h}^{-1}$ )	1.61 (0.100)	1.63 (0.210)	2.138 (0.500)	1.71 (0.080)
$t_{1/2\alpha}$ (h)	1.4 (0.070)	1.0 (0.100) <sup>a</sup>	0.98 (0.08) <sup>a</sup>	1.2 (0.060) <sup>b</sup>
$K_z$ ( $\text{h}^{-1}$ )	0.51 (0.024)	0.77 (0.100) <sup>a</sup>	0.7246 (0.0676) <sup>a</sup>	0.56 (0.025)
$V_d/F$ (l/kg)	3.32 (0.19)	9.52 (1.52) <sup>a</sup>	5.18 (0.20)	6.62 (0.99)
$Cl_T/F$ (l/h <sup>-1</sup> kg <sup>-1</sup> )	0.68 (0.016)	0.90 (0.047) <sup>a</sup>	1.3494 (0.085) <sup>a,b</sup>	1.09 (0.061) <sup>a,b,c</sup>
$t_{1/2\beta}$ (h)	3.4 (0.026)	7.2 (0.910) <sup>a</sup>	2.7 (0.135) <sup>b</sup>	4.8 (0.400) <sup>c</sup>
$K_{el}$ ( $\text{h}^{-1}$ )	0.21 (0.015)	0.10 (0.018) <sup>a</sup>	0.2606 (0.012) <sup>b</sup>	0.14 (0.027) <sup>a,c</sup>
$AUC$ ( $\mu\text{g h ml}^{-1}$ )	146.36 (3.25)	111.96 (5.60) <sup>a</sup>	75.42 (5.23) <sup>a,b</sup>	92.71 (5.00) <sup>a,b,c</sup>

The results are expressed as mean and SEM ( $n = 5$ ).

$t_{1/2\alpha}$ , half-life of absorption;  $t_{1/2\alpha}$ , half-life of distribution;  $t_{1/2\beta}$ , half-life of elimination;  $K_a$ , constant of absorption;  $K_z$ , constants of distribution;  $K_{el}$ , constant of elimination;  $AUC$ , the area under curve 0 to 24 h;  $Cl_T/F$ , apparent total clearance;  $V_d/F$ , distribution apparent volume.

<sup>a</sup>  $p < 0.05$  relative to the Group I.

<sup>b</sup>  $p < 0.05$  relative to the Group II.

<sup>c</sup>  $p < 0.05$  relative to the Group III.

The co-administration of PYR (Group II) caused a significant increase in the  $V_d/F$ ,  $K_z$ ,  $t_{1/2\beta}$  and  $Cl_T/F$ . However, the parameters  $t_{1/2\alpha}$  and  $AUC$  were lower compared with the control.

The animals that had received INH + RMP (Group III) presented the biggest decrease in the  $AUC$  and increase in the  $Cl_T/F$  when compared with all the other groups. Animals that received INH + RMP + PYR (Group IV) presented a decrease in the  $AUC$  and an increase of the  $Cl_T/F$  for INH when compared with the control.

## Discussion

The most relevant finding was the statistically significant increase in the  $Cl_T/F$  for all drug combinations studied here. Moreover, it is important to observe that Group I presented the lowest  $Cl_T/F$  value followed by Groups II and IV in increasing order, and finally, Group III presented the highest  $Cl_T/F$ . These data suggest that the RMP presented a higher effect on the  $Cl_T/F$  of INH in relation to PYR, leading to a more significant decrease in the  $AUC$  of INH. Corroborating this finding, it is well known that RMP is a potent inducer of CYP isozymes [3] and this could explain the increase of the  $Cl_T/F$  and reduction of the  $AUC$  of INH. The finding that the increase in the  $Cl_T/F$  was higher in Group II than Group IV could be explained by an interference in the metabolism of RMP when

co-administered with PYR. Indeed, Jain and colleagues [20] observed differences in the pharmacokinetic parameters of RMP among patients treated with INH and RMP. In that case, the concomitant administration of PYR in patients treated with RMP produced a decrease in  $AUC$  and an increase in  $Cl_T/F$  of RMP. These observations could explain the intermediate increase of the  $Cl/F$  of INH when the administration involves INH + PYR + RMP.

In conclusion, the results show that pharmacokinetic interactions among the drugs used in the treatment of TB are evident. These interactions can lead to differences in the therapeutic and toxic effects when the drugs are administered simultaneously. The reduction in  $AUC$  of INH, observed in the groups that received the combinations could lead to a decrease in their therapeutic effect. Since all studies highlight that the hepatotoxicity of INH is related to its metabolism, the significant increase in the  $Cl_T/F$  is a clear indication that association of these drugs can lead to an augmented production of potentially hepatotoxic metabolites.

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