BIBLID: ISSN 0253-9756 中国药理学报 *Acta Pharmacologica Sinica* 1992 Nov; **13** (6) : 494-496

# Effects of rifampicin on pharmacokinetics of isoniazid and its metabolite acetylhydrazine in rats[1]

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**ABSTRACT** After iv and ip injections of isoniazid (Iso) 40 mg · kg$^{-1}$ to ♂ Wistar rats, the plasma levels of Iso, acetylisoniazid (AcIso), and acetylhydrazine (AcHz) were determined by spectrophotometric method and gas chromatography. The results suggested that the pharmacokinetic behavior of Iso in rats belonged to a 2-compartment model. The plasma levels of AcHz in rifampicin (Rif 30 mg · kg$^{-1}$)-pretreated rats were lowered *vs* the control ($P < 0.05$ or $< 0.01$). The $T_{\frac{1}{2}}$ of AcHz was shortened by Rif (control group 3.3 h, Rif-pretreated group 1.4 h) after iv injection of AcHz 10 mg · kg$^{-1}$ to rats and the results showed that AcHz was converted to its active metabolites quickly by increasing the oxidative elimination rate of AcHz, which is related to the higher incidence of liver necrosis caused by Iso and Rif in combination.

**KEY WORDS** isoniazid; rifampin; toxic hepatitis; pharmacokinetics; drug interactions

Isoniazid (Iso) is the most effective and commonly used drug available in the treatment of tuberculosis. Hepatic necrosis is a rare adverse reaction during the treatment[1]. Clinical trials have shown that the incidence of toxic hepatitis was obviously higher in patients who received Rif in addition to Iso than in those receiving Iso alone[2,3].

The high incidence of toxic hepatitis caused by Iso and Rif was due to some active metabolite derived from AcHz, a metabolite of Iso[4]. In order to probe the mechanism of this hepatotoxicity, we studied the effect of Rif on the plasma concentrations of Iso and its metabolites, AcIso and AcHz, in rats.

## MATERIALS AND METHODS

Isoniazid (Iso, 860528) and rifampicin (Rif, 870717) were obtained from Shanghai Fifth Pharmaceutical Factory; acetylisoniazid (AcIso) and acetylhydrazine (AcHz) were synthesized in our laboratory. Iso and AcHz were recrystallized from MeOH.

Wistar rats, ♂, weighing 210 ± *s* 30 g, were divided into 2 groups, 22 in each. One group was injected ip Rif (30 mg · kg$^{-1}$ · d$^{-1}$) for 6 d, the other group, a vehicle only.

**1** The plasma levels of Iso and AcIso were examined using samples obtained from the tail vein after a single iv injection of Iso 40 mg · kg$^{-1}$.

**2** The plasma concentrations of AcHz were determined at 1, 2, 4, 6, 8 and 12 h after a single ip injection of Iso 40 mg · kg$^{-1}$.

**3** The elimination rate of AcHz was determined by using plasma of rats at 0.2, 0.5, 1, 2, 3, 4, and 5 h after iv injection of AcHz 10 mg · kg$^{-1}$.

A Type-721 spectrophotometer was used to detect Iso and AcIso[5]. AcHz was detected on a Shimadzu GC-R1A instrument fitted with a hydrogen flame ionization detector[6]. Results were expressed as $\bar{x} \pm s$ and analyzed using a 2-tailed *t* test.

## RESULTS

**1** After iv injection of an aqueous solution of Iso (40 mg · kg$^{-1}$) in rats, the pharmacokinetic behavior of Iso in rats (Tab 1) belonged to a 2-compartment model and the acetylation rate of Iso was not influenced by Rif ($P > 0.05$), which was in agreement with the results obtained in human.

**2** After ip injection of Iso (40 mg · kg$^{-1}$)

Received 1990-06-22 Accepted 1992-07-21

[1] A preliminary account of this work was presented at the 11th International Congress on Pharmacology, 1990 Jul 1-6, Amsterdam, The Netherlands.

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**Tab 1. Effect of ip Rif 30 mg · kg$^{-1}$ · d$^{-1}$ × 6 d pretreatment and after iv or ip Iso 40 mg · kg$^{-1}$ on plasma Iso, AcIso, and AcHz in rats.** $n=8$, $\bar{x}\pm s$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$.

| Time / min | Drug concentrations in plasma / μg · ml$^{-1}$ | | | | | |
|---|---|---|---|---|---|---|
| | Iso | | AcIso | | AcHz (ip Iso) | |
| | Control | Rif | Control | Rif | Control | Rif |
| 5 | 46 ± 8 | 50 ± 9$^{*}$ | 0.9 ± 0.4 | 1.0 ± 0.5$^{*}$ | | |
| 15 | 28 ± 5 | 28 ± 4$^{*}$ | 3.3 ± 0.6 | 3.3 ± 0.9$^{*}$ | | |
| 30 | 15.6 ± 2.6 | 15 ± 3$^{*}$ | 5.3 ± 1.0 | 5.0 ± 1.1$^{*}$ | | |
| 60 | 5.8 ± 1.8 | 6.6 ± 1.9$^{*}$ | 7.2 ± 1.6 | 6.2 ± 1.1$^{*}$ | 1.4 ± 0.8 | 0.30 ± 0.14$^{**}$ |
| 120 | 3.4 ± 1.1 | 3.0 ± 1.1$^{*}$ | 7.6 ± 1.8 | 7.0 ± 1.1$^{*}$ | 2.3 ± 1.1 | 0.54 ± 0.14$^{***}$ |
| 180 | 2.2 ± 0.6 | 1.8 ± 0.6$^{*}$ | 7.0 ± 1.8 | 5.8 ± 1.2$^{*}$ | | |
| 240 | 1.32 ± 0.26 | 1.19 ± 0.22$^{*}$ | 5.6 ± 1.5 | 4.8 ± 0.9$^{**}$ | 3.1 ± 1.0 | 1.1 ± 0.3$^{***}$ |
| 300 | 0.80 ± 0.28 | 0.67 ± 0.29$^{*}$ | 4.3 ± 1.4 | 3.0 ± 0.9$^{*}$ | | |
| 360 | | | 2.0 ± 1.0 | 1.3 ± 0.7$^{*}$ | 2.5 ± 1.0 | 0.81 ± 0.21$^{***}$ |
| 480 | | | | | 1.8 ± 0.4 | 0.73 ± 0.22$^{***}$ |
| 720 | | | | | 0.73 ± 0.13 | 0.35 ± 0.15$^{***}$ |

in rats, the plasma levels of AcHz in the Rif-pretreated groups were lower than those in the control groups (Tab 1).

3 After iv injection of AcHz (10 mg · kg$^{-1}$) in rats, the plasma concentrations of AcHz decreased significantly in Rif-pretreated groups *vs* the control group (Tab 2). The elimination $T_{\frac{1}{2}}$ was calculated as 3.3 h for the control group, 1.4 h for the Rif-pretreated groups ($P<0.05$), and the area under the curve was prominently decreased in Rif-pretreated groups (Tab 3).

**Tab 2. Effect of ip Rif 30 mg · kg$^{-1}$ × 6 d pretreatment on plasma concentrations of AcHz in rats after iv AcHz 10 mg · kg$^{-1}$.** $n=6$, $\bar{x}\pm s$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$.

| Time / min | AcHz in plasma / μg · ml$^{-1}$ | |
|---|---|---|
| | Control | Rif-pretreated |
| 10 | 7.6 ± 1.7 | 7.1 ± 1.4$^{*}$ |
| 30 | 5.5 ± 1.1 | 5.0 ± 1.4$^{*}$ |
| 60 | 3.3 ± 0.6 | 2.5 ± 0.5$^{**}$ |
| 120 | 1.5 ± 0.4 | 1.12 ± 0.21$^{*}$ |
| 180 | 1.15 ± 0.27 | 0.72 ± 0.26$^{**}$ |
| 240 | 0.87 ± 0.27 | 0.36 ± 0.23$^{***}$ |
| 300 | 0.61 ± 0.22 | 0.22 ± 0.12$^{***}$ |

**Tab 3. Effect of ip Rif 30 mg · kg$^{-1}$ · d$^{-1}$ × 6 d pretreatment and after iv AcHz 10 mg · kg$^{-1}$ on pharmacokinetics of AcHz in rats.** $n=6$, $\bar{x}\pm s$. $^{*}P>0.05$, $^{**}P<0.05$.

| Parameter | Control | Rif-pretreated |
|---|---|---|
| $K_{12}$ / h$^{-1}$ | 0.50 ± 0.17 | 0.51 ± 0.29$^{*}$ |
| $K_{21}$ / h$^{-1}$ | 0.80 ± 0.6 | 1.0 ± 0.4$^{*}$ |
| $K_{10}$ / h$^{-1}$ | 0.68 ± 0.15 | 1.03 ± 0.19$^{**}$ |
| $T_{\frac{1}{2}}$ / h$^{-1}$ | 3.3 ± 2.0 | 1.4 ± 0.8$^{**}$ |
| $Cl$ / l · h$^{-1}$ | 0.15 ± 0.05 | 0.23 ± 0.04$^{**}$ |
| $V_1$ / l | 0.23 ± 0.07 | 0.23 ± 0.05$^{*}$ |
| $V_d$ / l | 0.62 ± 0.16 | 0.50 ± 0.22$^{*}$ |
| AUC / μg · h$^{-1}$ · ml$^{-1}$ | 14 ± 5 | 9.0 ± 1.5$^{*}$ |

## DISCUSSION

The major pathway of Iso in both the rat and human[7,8] is by acetylation to AcIso which is then hydrolyzed to AcHz and isonicotinic acid. AcHz is further acetylated to di-AcHz and oxidized to other metabolites by oxidases (Fig 1).

The data from our investigation demonstrated that the elimination rate of AcHz in Rif-pretreated rats increased significantly.

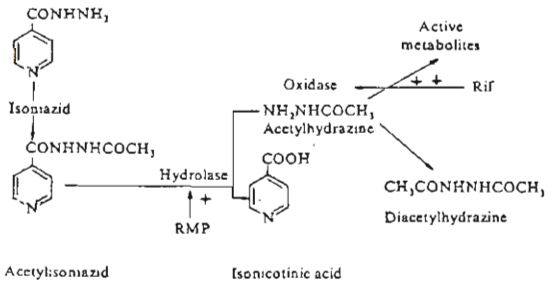


**Fig 1. Metabolism of isoniazid and inductive effect of rifampicin on hydrolase and oxidase.**

This can be explained by the potent increase of a kind of hepatic cytochrome P-450 which is responsible for the metabolic activation of AcHz through oxidative pathway since the acetylation of AcHz was not influenced by Rif. Moreover, it could be assumed that the AcHz oxidase was more inducible than the hydrolase of AcIso by Rif (Fig 1).

According to our results and the relevant documents, we postulated that AcHz was converted by Rif into active metabolites, which were responsible for the higher incidence of toxic hepatitis on receiving Iso and Rif.

Further research will be carried on to ascertain the chemical nature of the metabolites of AcHz.

**ACKNOWLEDGMENT** We are grateful to technician MO Guo-Qiang, Department of Instrumental Analysis Center, for his keen help at all stages of the investigation.

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## 利福平对大鼠异烟肼及其代谢物乙酰肼药物动力学的影响

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**提要** 给 Wistar ♂大鼠 iv 和 ip 异烟肼(Iso) 40 mg · kg$^{-1}$ 后，分别用分光光度法和气相谱法测定利福平(Rif)对血浆中 Iso，AcIso，AcHz 浓度的影响. Rif 30 mg · kg$^{-1}$ 诱导组的 AcHz 血浓度明显低于对照组. AcHz iv 10 mg · kg$^{-1}$ 后，Rif 明显缩短 AcHz 的 $T_{\frac{1}{2}}$ (对照组为3.3 h, Rif 诱导组为1.4 h)，提示: AcHz 可因 Rif 诱导加速氧化代谢生成反应性中间产物的消除而导致肝坏死发生率的提高.

**关键词** 异烟肼；利福平；中毒性肝炎；药物动力学；药物相互作用