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INDUCTION AND INHIBITION OF HEPATIC DRUG METABOLIZING ENZYMES BY RIFAMPIN*

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Abstract—The effects of the antibiotic rifampin on microsomal drug metabolizing enzymes were investigated. On acute administration, rifampin (100 mg/kg i.p.) doubled hexobarbital sleeping time and zoxazolamine paralysis time in mice. The *in vitro* metabolism of zoxazolamine (0.25 mM) and 17β-estradiol (10 μM) were inhibited 50% by rifampin in concentrations of 0.4 and 0.3 mM, respectively. The inhibition of ethylmorphine *N*-demethylase was competitive with an apparent K_i of 52 μM. Repeated administration of rifampin to mice (50 mg/kg i.p. daily for 6 days) increased liver weight by 20%, cytochrome P-450 (50%), NADPH cytochrome *c* reductase (43%), ethylmorphine *N*-demethylase (85%), zoxazolamine hydroxylase (77%), benzpyrene hydroxylase (174%), and 17β-estradiol metabolism (89%). Microsomal protein (mg/g), aniline hydroxylase and *p*-nitrophenol glucuronyl transferase activities were unaffected.

Rat microsomal drug metabolizing enzyme activity was also inhibited after acute administration of rifampin as exemplified by an increase in hexobarbital sleeping time of 44% and a competitive inhibition of ethylmorphine *N*-demethylase by rifampin. The K_i (33 μM) was close to that obtained with the mouse enzyme. The similarity in the *in vivo* and *in vitro* inhibition suggests that rifampin binds to microsomes in a similar manner in both species. Chronic administration of rifampin to rats (50 mg/kg i.p.) twice daily for six days did not lead to induction, indicating a species difference with respect to rifampin's inducing ability. Rifampin did not modify microsomal induction in rats by phenobarbital when both drugs were administered concomitantly. The mechanism responsible for the species difference and the clinical relevance of these results are discussed.

Rifampin‡, an antibiotic isolated from *Streptomyces mediterranei* [1] is used mainly in the treatment of tuberculosis in combination with other drugs [2] (isoniazid, ethambutol, streptomycin). At low concentrations (10^{-8}–10^{-7} M) it is a specific inhibitor of bacterial DNA-directed RNA polymerase [3, 4]. At much higher concentrations rifampin or other rifamycins inhibit other polynucleotide polymerases, notably the viral RNA-directed DNA polymerase (reverse transcriptase) [5–7]. Furthermore, the rifamycins have been reported to possess, at high doses, antiviral [8, 9], anticancer [10–13] and immunosuppressive [14–16] activities.

Rifampin has been shown to have a number of effects on the liver. It produces hepatitis [17], especially when given with isoniazid [18], and this is the major drawback to its clinical use. In man the drug has been shown to increase sulfobromophthalein (BSP) half-life and bilirubin levels probably by competing for the hepatic uptake of these drugs [19]. Depending on the dose, in rats, rifampin may increase or decrease bile flow [20]. In humans the antibiotic increased its own metabolism [21], as well as that of warfarin [22] and cortisol [23]. During the administration of rifampin to volunteers Remmer *et al.* found either a small increase or no effect on the metabolism of novaminsulfonum [24]. However this metabolism was increased by 50–100% three to four days after terminating the administration of rifampin, suggesting that in the presence of rifampin the activity of the induced enzyme was inhibited. Thus it appears that rifampin could both inhibit and increase drug metabolizing enzyme activity.

The fragmentary and complex data relative to the effects of rifampin on the liver prompted the present study which examines systematically the actions of this drug on the hepatic drug metabolizing enzymes. The effects of acute and chronic administration of rifampin on both the *in vivo* and the *in vitro* metabolism of drugs by rat and mouse microsomal enzymes are reported.

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* Rifampin, also referred to as rifampicin, belongs to the general class of compounds called rifamycins.

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MATERIALS AND METHODS

Animals. Male ICR–Swiss mice (Flow Res. Lab., Dublin, Va), a strain derived from Swiss–Webster mice, weighing 25–30 g or male Sprague–Dawley rats (Madison, Wisc.) weighing 150–200 g were used throughout this study.

Chemicals. Cytochrome *c*, 17β-estradiol, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, NADP, NADPH, neotetrazolium, *p*-nitrophenol, reduced glutathione, rifampin and uridine 5′-diphospho-glucuronic acid (UDPGA) were purchased from Sigma Chemical Co., St. Louis, Mo. Benzpyrene was

purchased from Calbiochem., Los Angeles, Ca.; ethylmorphine from Merck and Co., West Point, Pa.; and hexobarbital from Winthrop Lab., N.Y., N.Y. 3-Hydroxybenzpyrene was kindly supplied by Dr. Harry Gelboin of the National Cancer Institute, and zoxazolamine by McNeil Lab., Fort Washington, Pa.

[4-^{14}C]17β-Estradiol (sp. act., 58.2 mCi/m-mol) was purchased from New England Nuclear, Boston, Ma. Its purity was checked by t.l.c. on Baker-flex Silica gel IBF plates developed with ethylacetate–cyclohexane–ethanol (45:45:10).

All other chemicals were of the highest grade commercially available.

Treatment. Solutions of rifampin were prepared immediately before use. For the *in vivo* studies the drug was dissolved in HCl in a concentration of 15 mg rifampin/ml (pH 3.0) and injected i.p. in doses of 50 mg/kg or 100 mg/kg. Controls received HCl (pH 3.0) only. For the *in vitro* experiments, rifampin was dissolved in water.

In vivo *metabolism.* Hexobarbital sleeping time and zoxazolamine paralysis time were measured as the time between loss and recovery of the righting reflex after injection i.p. of 100 mg/kg of the appropriate drug. Liver and whole blood 'rifamycin' levels were measured by the spectrophotometric assay of Maggi *et al.* [25]. This technique measures both rifampin and metabolite(s). Hepatic zoxazolamine levels were determined by the method of Trevor [26].

Tissue preparation and in vitro *metabolism.* The hepatic 10.000 *g* supernatant and microsomal fractions were prepared as previously described [27]. Incubations were carried out aerobically at 37° using a Warner–Chilcott shaker-incubator. Ethylmorphine *N*-demethylase, aniline hydroxylase and neotetrazolium reductase were assayed as previously described [27]. The cofactor mixture was modified in that no nicotinamide was added to the incubates. Ethylmorphine *N*-demethylase activity was determined for 20 min with the mouse liver fraction and for 10 min only when the rat preparation was used. The ether extraction method was used for the assay of aniline hydroxylase. Benzpyrene hydroxylase activity was determined by the method of Kuntzman *et al.* [28], using microsomes from 5 mg wet wt liver. Conversion of [^{14}C]17β-estradiol into water-soluble–ether-insoluble metabolites was measured by the method of Jellinck and Perry [29], using microsomes from 50 mg wet wt liver. Zoxazolamine hydroxylase activity was determined by the method of Trevor [26] using 10.000 *g* supernatant equivalent to 250 mg of liver. *p*-Nitrophenol glucuronyl transferase activity was assayed by a modification of the method of Lueders and Kuff [30]. In the non-detergent-activated assay, microsomes from 125 mg of liver were incubated at 37° for 20 min with 0.5 μmole of *p*-nitrophenol, 5 μmole UDPGA, 25 μmole $MgCl_2$, in a total vol of 3.5 ml of 0.05 M Tris buffer pH 7.4. The detergent-activated assay was the same except that 0.05% Triton X-100 was added, microsomes from 50 mg of liver were used and the incubation time was 10 min.

When studying the *in vitro* effects of rifampin, the antibiotic was added at the concentrations shown in the figures in both the blank and the test flasks. Rifampin is a highly colored compound [1]. Thus, when necessary, special precautions were taken to be certain that rifampin did not affect the quantitative aspects of the various procedures. In the ethylmorphine demethylase assay, where a formaldehyde complex was being measured at 415 nm, the absorbance of an incubated blank containing rifampin but no ethylmorphine was subtracted from the absorbance of the test. It was ascertained by adding various amounts of formaldehyde to non-incubated flasks that formaldehyde was indeed accurately being measured under these conditions. The zoxazolamine and estradiol assays were unaffected by rifampin.

Microsomal NADPH cytochrome *c* reductase activity, cytochromes P-450 and b_5 levels, and microsomal protein were assayed as previously described [27]. Total microsomal heme was measured by the method of Omura and Sato [31].

RESULTS

Effect of acute rifampin treatment

Hexobarbital sleeping time and zoxazolamine paralysis. Administration of rifampin (100 mg/kg) i.p. to mice 30 min prior to the test doubled the duration of action of hexobarbital and zoxazolamine above controls (Table 1). Since both hexobarbital and zoxazolamine are metabolized by the microsomal mixed function oxidase system [32], these results imply that rifampin may be inhibiting microsomal activity. When the hexobarbital experiment was repeated in rats, rifampin also significantly ($P < 0.05$) increased the sleeping times from 27 ± 2 to 39 ± 4 min (mean of 9 rats ± S.E.M.).

Correlation of hepatic 'rifamycin' levels and hexobarbital sleeping times. Based on the above observation an experiment was performed to determine the duration of rifampin's effect. Hexobarbital sleeping times were therefore determined 30 min, 3, 6, and 18 hr after the administration of rifampin (100 mg/kg) i.p. to mice. The data in Fig. 1 indicate that hexobarbital sleeping time, which was increased by 108% 30 min after rifampin treatment, was only 33% above controls 3 hr later and 20% at the end of 6 hr. On

Table 1. Effect of acute rifampin treatment of mice on hexobarbital sleeping time and zoxazolamine paralysis

	Control	Rifampin	Increase %
Hexobarbital sleeping time (min)	35 ± 3	73* ± 6	108
Zoxazolamine paralysis time (min)	37 ± 4	87* ± 10	135

Rifampin (100 mg/kg i.p.) was injected 30 min before either hexobarbital (100 mg/kg i.p.) or zoxazolamine (100 mg/kg i.p.). Values are the mean of 12 mice ± S.E.M.
* $P < 0.001$ with respect to control.

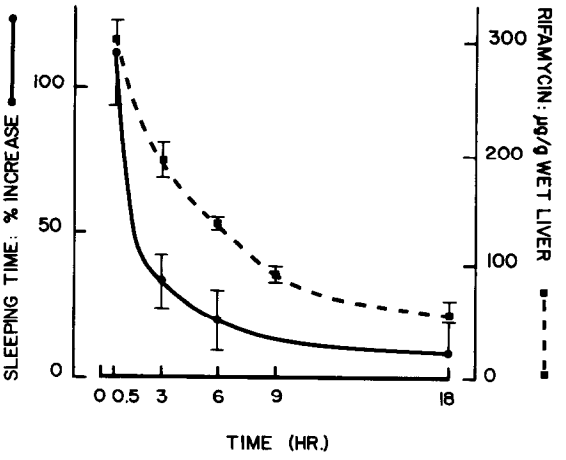

Fig. 1. Hexobarbital sleeping times and hepatic 'rifamycin' levels at various time periods after rifampin administration. Rifampin (100 mg/kg) was injected i.p. at zero time. At 4 different time periods thereafter, hexobarbital (100 mg/kg) was administered i.p. to 4 different groups of 12 rifampin-treated and 12 control mice. The sleeping times (solid line) are expressed as the per cent increase (mean ±S.E.) above the mean of the simultaneously run controls. Hepatic 'rifamycin' levels were measured as described in Methods in 5 separate other groups of 4 mice per group. They are shown (mean ± S.E.M.) on the dotted line.

the other hand, the hepatic 'rifamycin' levels had only decreased by 35% at the end of 3 hr and by 54% at the end of 6 hr. Thus, although the effect of rifampin on sleeping time rapidly diminished, the hepatic 'rifamycin' levels slowly decreased. The half-life of 'rifamycin' in the liver and in the whole blood was found to be 6 hr.

In vitro *effects of rifampin on drug metabolism.* Three different substrates were selected to study these effects: zoxazolamine as an example of drug hydroxylation and a compound already studied *in vivo*, ethylmorphine as an example of *N*-demethylation, and 17β-estradiol as an example of steroid metabolism. The inhibition by rifampin of zoxazolamine hydroxylation by mouse 10,000 *g* supernatant is shown in Fig. 2. The substrate concentration of zoxazolamine was 0.25 mM, which was equivalent to the zoxazolamine liver concentration (0.25 μmole/g) in mice just recovering from zoxazolamine paralysis. *In vitro* zoxazolamine metabolism was decreased by 50% with a rifampin concentration of 0.4 mM. This concentration is close to the liver 'rifamycin' levels (0.35 μmole/g) which *in vivo* resulted in a doubling of the paralysis time. The conversion of 17β-estradiol into water-soluble-ether-insoluble metabolites by mouse microsomes was decreased by 50% with a rifampin concentration of 0.3 mM (Fig. 3). Both mouse and rat microsomal ethylmorphine *N*-demethylase activities were competitively inhibited by rifampin (Figs. 4 and 5). When the 10,000 *g* supernatant fraction of mouse liver was used as a source of enzyme the apparent kinetic constants (mean ± S.E.M.) were determined graphically to be: $K_m = 0.50 \pm 0.11$ mM and $K_i = 52 \pm 13$ μM. The kinetic constants were similar when the rat fraction was used. The K_m was 0.80 ± 0.09 mM and the K_i was 33 ± 4 μM.

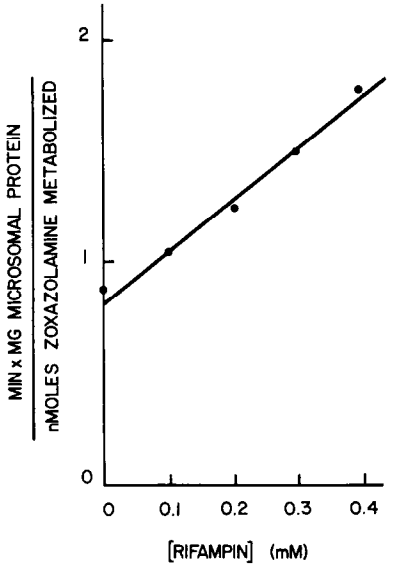

Fig. 2. Rifampin inhibition of zoxazolamine metabolism by mouse 10,000 *g* supernatant. Zoxazolamine (0.25 mM) disappearance was measured as described in Methods. The reciprocal of the rate is plotted against the rifampin concentration. Each point is the mean of 3 distinct experiments.

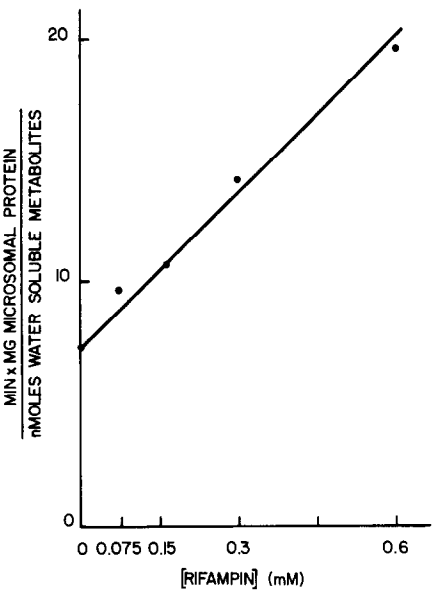

Fig. 3. Rifampin inhibition of estradiol metabolism by mouse microsomes. Conversion of [^{14}C]17β-estradiol (10 μM) to water-soluble–ether-insoluble metabolites was measured as described in Methods. The reciprocal of the rate is plotted against the rifampin concentration. Each point is the mean of 3 distinct experiments.

Effect of chronic rifampin treatment

In mice rifampin pretreatment (50 mg/kg i.p. for 6 days) affected the components of the mixed function oxidase system as well as enzymatic activity. As shown in Table 2, liver weight, cytochrome P-450, total heme and NADPH-cytochrome *c* reductase were all significantly increased. The maximum absorbance of the carbon monoxide binding pigment after rifampin was at 450 nm. In contrast to the phenobarbital type of induction, the microsomal protein (mg/g)

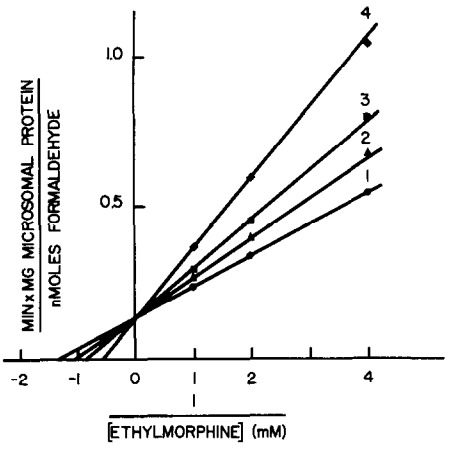

Fig. 4. Competitive inhibition by rifampin of ethylmorphine *N*-demethylation by mouse 10,000 *g* supernatant. The formation of formaldehyde was measured as described in Methods. The rifampin concentrations were: (1) 0 μM, (2) 25 μM, (3) 50 μM, (4) 100 μM. A double reciprocal plot of a single experiment is shown. The kinetic constants (mean of 3 experiments $\pm$ S.E.M.) were respectively: $K_m = 0.50 \pm 0.11$ mM and $K_i = 52 \pm 13$ μM.

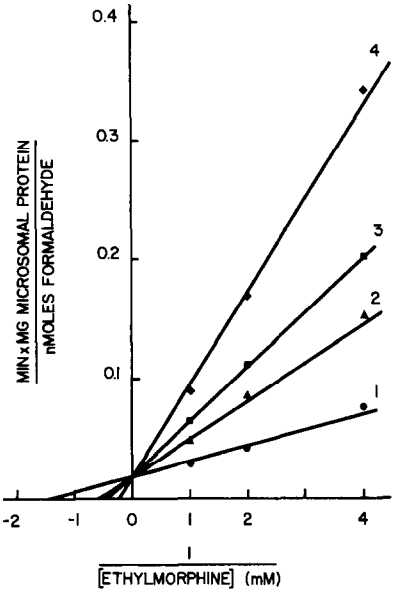

Fig. 5. Competitive inhibition by rifampin of ethylmorphine *N*-demethylation by rat 10,000 *g* supernatant. The formation of formaldehyde was measured as described in Methods. The rifampin concentrations were: (1) 0 μM, (2) 25 μM, (3) 50 μM, and (4) 100 μM. A double reciprocal plot of a single experiment is shown. The kinetic constants (mean of 3 experiments $\pm$ S.E.M.) were respectively: $K_m = 0.80 \pm 0.09$ mM and $K_i = 33 \pm 4$ μM.

was not increased. The effect of 6 days of rifampin treatment on the *in vitro* microsomal drug metabolism is shown in Table 3. These data indicate that the metabolism of ethylmorphine, zoxazolamine, benzpyrene and 17β-estradiol were all significantly increased. In contrast to the above substrates aniline hydroxylation and the glucuronoconjugation of *p*-nitrophenol were essentially unaffected.

When rat microsomal activity was tested against 6 different substrates (Table 4) no significant difference between the rifampin treated and the control rats was observed. Table 4 also shows that microsomal protein, cytochrome P-450 and liver weight were unaffected. Clearly this demonstrated a sharp species difference with respect to induction by rifampin of microsomal mixed function oxidase activity.

Coadministration of rifampin (50 mg/kg) twice daily with phenobarbital (80 mg/kg) once daily for 3 days to rats did not affect the induction observed with phenobarbital alone. The percent increase above controls were essentially equal in both the phenobarbital and the phenobarbital plus rifampin-treated rats. The liver weights were increased respectively 29 and 27%, the concentrations of microsomal protein 20 and 23%, and of cytochrome P-450 146 and 153%.

DISCUSSION

In the experiments presented here it has been shown that the antibiotic rifampin can affect the activity of the hepatic drug metabolizing enzymes in two ways: (1) by inhibiting microsomal activity on acute administration and (2) by inducing microsomal activity upon chronic treatment.

Table 2. Effect of rifampin pretreatment of mice on liver weight and some microsomal components

	Control	Rifampin	Increase %
Liver wt (g)	1.92 $\pm$ 0.10	2.30‡ $\pm$ 0.05	20
Microsomal protein (mg/g liver)	27.3 $\pm$ 0.6	28.4 $\pm$ 0.5	4
Cytochrome P-450*	0.60 $\pm$ 0.04	0.90‡ $\pm$ 0.02	50
Cytochrome b_5*	0.39 $\pm$ 0.02	0.45 $\pm$ 0.02	15
Total heme*	1.09 $\pm$ 0.05	1.34‡ $\pm$ 0.03	23
NADPH-cytochrome *c* reductase†	49 $\pm$ 3	70‡ $\pm$ 3	43

Rifampin = 50 mg/kg i.p. daily for 6 days. Animals were sacrificed 24 hours after the last injection of rifampin. Data are the mean $\pm$ S.E.M. of 10 values; each individual value was obtained from 2 pooled livers.

* nmole/mg microsomal protein.

† nmole of cytochrome *c* reduced/min/mg microsomal protein.

‡ $P < 0.005$ with respect to control.

Table 3. Effect of rifampin pretreatment of mice on *in vitro* drug metabolism

Enzyme activity	Control	Rifampin	Increase %
Ethylmorphine *N*-demethylase	2.0 ± 0.2	3.7* ± 0.2	85
Zoxazolamine hydroxylase	0.61 ± 0.02	1.08* ± 0.05	77
Benzpyrene hydroxylase	0.073 ± 0.006	0.200* ± 0.012	174
17β-Estradiol metabolism	0.09 ± 0.01	0.17* ± 0.02	89
Aniline hydroxylase	0.17 ± 0.01	0.17 ± 0.01	0
p-Nitrophenol glucuronyl transferase			
without Triton X-100	1.8 ± 0.1	1.7 ± 0.1	−6
with Triton X-100 (0.05%)	11.2 ± 0.4	12.2 ± 0.3	9

Rifampin = 50 mg/kg daily for 6 days. Animals were sacrificed 24 hr after the last injection of rifampin. All activities, measured as described in Methods, are expressed in nmole/min/mg of microsomal protein. Data are mean ± S.E.M. of 10 values; each individual value was obtained from two pooled livers.

* $P < 0.05$ with respect to control.

Microsomal inhibition in mice was strongly suggested by the finding that hexobarbital sleeping time and zoxazolamine paralysis were doubled by treatment of the animals 30 min earlier with rifampin (100 mg/kg i.p.) (Table 1). The effect of rifampin on the hexobarbital sleeping time rapidly diminished with time (Fig. 1). An attempt was made to correlate the observed inhibition of hexobarbital metabolism *in vivo* with the hepatic 'rifamycin' levels. Although there was a gross correlation, there were some differences (Fig. 1). While 'rifamycin' slowly disappeared from the liver, its effect on hexobarbital sleeping time rapidly diminished. Obviously a number of factors may have contributed to this lack of absolute correlation, one of which being that the analytical procedure measures both rifampin and its metabolite(s). Rifampin metabolite(s) may have no effect on hexobarbital sleeping time. Further evidence supporting the inhibitory effect of rifampin was obtained from the *in vitro* experiments which demonstrated that rifampin inhibited zoxazolamine, 17β-estradiol, and ethylmorphine metabolisms in mice (Figs. 2, 3, and 4). The rifampin concentration (0.4 mM), which *in vitro* inhibited the metabolism of zoxazolamine by 50%, was close to the liver 'rifamycin' levels (0.35 μmole/g) which *in vivo* resulted in a doubling of the paralysis time. The inhibition of ethylmorphine *N*-demethylase was competitive; the apparent K_i (52 μM) was one order of magnitude higher than that obtained for SKF-525A (6 μM) [33], a very potent inhibitor of microsomal drug metabolizing enzymes.

The acute inhibitory effect of rifampin was also observed in rats, both *in vivo*, as measured by the hexobarbital sleeping time and *in vitro* on ethylmorphine *N*-demethylation (Fig. 5). The apparent K_i for ethylmorphine *N*-demethylase obtained with the rat enzyme was very close to that obtained with the mouse enzyme. This suggests that rifampin binds to the microsomes in a similar manner in both species. A number of attempts were made to determine the microsomal binding spectrum of rifampin. No binding spectrum could be obtained. Rifampin absorbance at these wavelengths is very high and this may have concealed a binding spectrum.

The clinical significance of the acute inhibitory effect of rifampin on drug biotransformations is not known. If one were to extrapolate our animal data it would imply that initial therapy with rifampin could inhibit the biotransformation of concomitantly administered drugs in humans.

In the chronic studies all tests were done 24 hr after the last dose of rifampin. At this time period there is very little rifampin remaining in the liver of both rats and mice. Thus, it is unlikely that inhibition by any residual rifampin could significantly mask an induction process.

On chronic administration, in mice, rifampin shared a number of effects with the classical enzyme inducer, phenobarbital. Similar to phenobarbital [34], rifampin (50 mg/kg i.p. daily for 6 days) increased liver weight, cytochrome P-450 hemoprotein, NADPH cytochrome *c* reductase, ethylmorphine

Table 4. Absence of induction after rifampin pretreatment of rats

	Control	Rifampin
Hexobarbital sleeping time (min)	23 ± 5	26 ± 4
Ethylmorphine *N*-demethylase*	5.7 ± 0.4	5.2 ± 0.3
Aniline hydroxylase*	0.32 ± 0.02	0.31 ± 0.01
Neotetrazolium reductase*	55 ± 4	53 ± 1
NADPH-cytochrome *c* reductase*	60 ± 5	58 ± 8
p-Nitrophenol glucuronyl transferase*	2.2 ± 0.4	1.9 ± 0.2
Cytochrome P-450 (nmol/mg protein)	0.59 ± 0.01	0.54 ± 0.02
Microsomal protein (mg/g liver)	36 ± 1	35 ± 1
Liver weight (g)	5.6 ± 0.2	5.4 ± 0.1

Rifampin = 50 mg/kg i.p. twice daily for 6 days. Animals were tested 24 hr after the last injection of rifampin. All values are mean ± S.E.M. of 7 rats.

* All activities are expressed in nmole/min/mg microsomal protein.

N-demethylase, zoxazolamine hydroxylase, benzpyrene hydroxylase, and 17β-estradiol metabolism (Tables 2 and 3). However rifampin differed from phenobarbital [34, 35] in that microsomal protein (mg/g), aniline hydroxylase, and *p*-nitrophenol glucuronyl transferase activities were unaffected. Rifampin differed from 3MC [32] in that rifampin induced P-450 formation and not P-448. Rifampin induced NADPH-cytochrome *c* reductase whereas 3MC does not. They were similar in that both 3MC and rifampin did not increase microsomal protein/g liver.

Although the acute inhibitory effects of rifampin were similar in rats and mice, there was a sharp species difference with respect to induction of drug metabolizing enzymes after repeated doses of rifampin. Chronic rifampin treatment did not induce these enzymes in rats (Table 4). It is unlikely that the faster rate of rifampin metabolism in rats was responsible for this lack of effect. Rifampin half-life in rats is half of that in mice [1]. Therefore, rifampin (50 mg/kg) was injected i.p. for 6 days twice daily in rats as opposed to once daily in mice. Qualitatively the metabolism of rifampin by deacetylation and *N*-demethylation was reported to be similar in rats and mice [36]. Although rifampin did not induce in rats, neither did it affect microsomal induction by phenobarbital. Thus rifampin does not inhibit the transcriptional and translational steps necessary for enzyme induction in rats. Rather it seems probable that rifampin failed in rats to trigger the unknown initial event which starts the transcriptional step.

Our results agree with those of Barone *et al.* [37]. Rifampin treatment of mice increased pentobarbital and hexobarbital metabolisms, as well as the cytochrome P-450 levels. These workers found no induction in rats or guinea pigs, although in this last species an increase in the smooth endoplasmic reticulum was reported [38].

In humans, there is little doubt that rifampin leads to microsomal enzyme induction. Chronic administration of rifampin to humans decreased the plasma half-life of rifampin itself [21], and of warfarin [22]. In man, the daily production rate of cortisol [23], D-glucaric acid [23], and the amount of smooth endoplasmic reticulum in liver cells [38, 39] were increased.

Induction of drug metabolizing enzymes by rifampin may have harmful consequences in man. In a patient with Addison's disease, maintained on steroid therapy, an addisonian crisis developed when rifampin was added to the treatment regimen [23]. The patient was reequilibrated with higher doses of steroid which led to cushinoid manifestations upon rifampin withdrawal. Patients on long-term therapy with oral anticoagulants required an increase in their daily doses, in order to maintain adequate prothrombin levels, whenever rifampin was concomitantly administered [40, 41]. Furthermore, a high incidence of pregnancies (5 out of 88) was reported in women taking oral contraceptives in conjunction with rifampin [42]. Bolt *et al.* found a fourfold increase in the *in vitro* metabolism of ethynylestradiol by liver microsomes from patients receiving rifampin [43]. These findings and our mice studies could explain the reduced effectiveness of oral contraceptives in patients on chronic rifampin therapy. Finally, it has been suggested that the seemingly increased incidence of hepatitis when rifampin and isoniazid are given together could result from an induction by rifampin leading to an increased production of a hepatotoxic reactive metabolite of isoniazid [44].

In summary the results presented here suggest that: (1) In mice rifampin has a biphasic effect. It inhibits microsomal drug metabolizing enzymes on acute administration and induces on chronic treatment. (2) Acutely, the inhibitory effect of rifampin is similar in rats and mice. Thus rifampin probably binds to microsomes similarly in both species. (3) The induction by rifampin was species specific in that no induction was observed in rats. (4) These results and those of others suggest that, in man, rifampin may modify the metabolism and the effectiveness or toxicity of concomitantly administered drugs.

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