NON-COMPETITIVE INHIBITION OF HEPATIC AND INTESTINAL ARYL HYDROCARBON HYDROXYLASE ACTIVITIES FROM RATS BY RIFAMPIN

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Abstract.—Rifampin is a semisynthetic antibiotic which is known to alter hepatic cytochrome P-450 mediated drug metabolizing enzymes. Using benzo(a)pyrene as the substrate we have shown that rifampin acts as a non-competitive inhibitor of hepatic microsomal aryl hydrocarbon hydroxylase *in vitro* at or below 0.10 mM and that it is also a non-competitive inhibitor of intestinal aryl hydrocarbon hydroxylase at or below 0.075 mM. These results suggest that the administration of rifampin with other drugs may result in altered drug biotransformation.

Rifampin is a semisynthetic antibiotic derived from the natural product rifamycin B which is produced by a strain of *Streptomyces mediterranea* together with other minor components. The rifamycins belong to the chemical class of naphthalenic ansamycins (1) and possess high bactericidal activity against a broad spectrum of organisms (2). The metabolism and pharmacokinetics of rifampin have recently been reviewed (3). Various drug interactions and metabolic alterations have been attributed to the use of rifampin, including (4): increased estradiol metabolism; precipitation of withdrawal during methadone treatment; pregnancy while taking oral contraceptives; and altered cortisol metabolism. Pessayre and Mazel (5) have shown that the administration of rifampin to mice results in an 85% increase in hepatic ethylmorphine *N*-demethylase activity and a 174% increase in aryl hydrocarbon hydroxylase. However, when added to hepatic microsomes *in vitro*, rifampin acted as a competitive inhibitor of ethylmorphine-*N*-demethylase with a Ki of approximately 0.05 mM and also inhibited estradiol and zoxazolamine metabolism (5). Thus, it appears that rifampin acts as an enzyme inducer when used *in vivo* but may act as an *in vitro* inhibitor of the metabolism of some xenobiotics. The intestine has been shown to be a site of active drug metabolism (6,7); and following the oral administration of drugs it may play an important role in biotransformations. We have, therefore, examined the effect of rifampin *in vitro* on hepatic and intestinal microsomal aryl hydrocarbon hydroxylase activities using benzo(a)pyrene as the substrate.

RESULTS AND DISCUSSION

The effect of four concentrations of rifampin on aryl hydrocarbon hydroxylase (AHH) activity from hepatic and intestinal microsomes of male rats is presented in table 1. Rifampin was shown to be a potent inhibitor of microsomal AHH activity from both tissues, producing complete inhibition at a 1.0 mM concentration.

In order to determine the type of inhibition of AHH activity produced by rifampin, microsomes were incubated in the presence and absence of various fixed concentrations of rifampin using substrate (benzo(a)pyrene) concentrations ranging from 0.375 to 6.0 mM. Lineweaver-Burk plots were then prepared and are presented in figures 1 and 2 for hepatic and intestinal microsomes, respectively. The R values for correlation of the data points for each line were greater than 0.91 in all cases, indicating excellent agreement.

For hepatic AHH activity (figure 1), concentrations below 0.10 mM rifampin resulted in a change in the slope but not the intercept of the plots, indicating that rifampin was producing non-competitive inhibition of benzo(1)pyrene metabolism. The good correlation between the data points and the linear plots supports this interpretation. The Km value for benzo(1)pyrene was 3.8 mM and the Ki for

TABLE 1. Effects of rifampin on aryl hydrocarbon hydroxylase (AHH) activity of intestinal and hepatic microsomes from male rats.

Concentration of rifampin (mM)	Aryl Hydrocarbon Hydroxylase (AHH) Activity			
	Intestine		Liver	
	Units	% of control	Units	% of control
0	10.9±0.5	100	2124±52	100
0.05	7.2±0.3*	66	1752±43*	82
0.10	6.3±0.4*	58	1012±30*	48
0.50	0.1±0.0*	1	85± 6*	4
1.00	0.0±0.0*	0	0± 0*	0

*$P<0.05$ with respect to the control group. Values are the means of four determinations± SD. Rifampin was added in 10 μl of DMSO to give the final desired concentrations. One unit is defined as the amount of enzyme which catalyzes the formation of 1.0 nmole of product/min/mg of microsomal protein.

rifampin was 0.10 mM. When the concentration of rifampin was increased to 0.50 mM, the apparent Km value increased to 33.3 mM, suggesting that other less specific binding sites may exist on the enzyme complex. Other investigators have similarly observed the inhibition of hepatic mixed function oxidase at high inhibitor concentrations by mixed inhibition kinetics (8).

For intestinal microsomal AHH activity (figure 2), non-competitive inhibition by rifampin was also observed at concentrations below 0.075 mM. The Km for

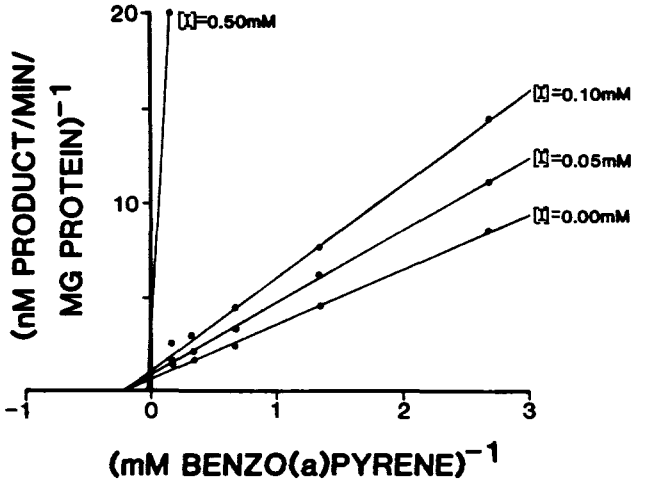

FIGURE 1. Lineweaver-Burke plots of benzo(a)pyrene metabolism by hepatic microsomal AHH in the presence and absence of fixed concentrations of rifampin (I). Each point is the mean of four determinations. The R values for correlation of the lines for each set of points exceeded 0.98.

benzo(a)pyrene was 0.10 mM, while the Ki for rifampin was 0.14 mM. Raising the concentration of rifampin to 0.150 mM caused the apparent Km value to increase to about 0.14 mM. Again, these results are in agreement with the assumption that multiple binding sites may exist on the enzyme complex and non-specific binding occurs at the higher concentrations.

Passayre and Mazel (5) demonstrated that rifampin given 30 min prior to hexobarbital or zoxazolamine doubled the duration of sleeping times and paralysis, respectively, in rats. Rifampin also competitively inhibited the metabolism

by mouse hepatic 10,000 x G supernatant fractions of ethylmorphine, zoxazolamine and estradiol, and ethylmorphine-*N*-demethylation by rat hepatic microsomes (5). The effect of rifampin on hepatic AHH activity and on intestinal metabolism in general had not previously been examined.

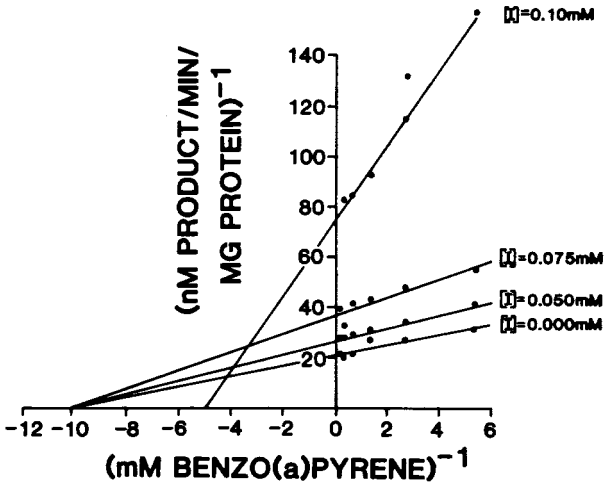

FIGURE 2. Lineweaver-Burke plots of benzo(a)pyrene metabolism by intestinal microsomal AHH in the presence and absence of various fixed concentrations of rifampin (I). Each point is the mean of four determinations. R values for each line exceeded 0.91.

Previous studies have shown that prostaglandin E_1 (PGE_1) is also a non-competitive inhibitor of hepatic microsomal AHH activity, while PGE_1 produces a concentration-dependent stimulation of intestinal AHH activity (9). Disulfiram inhibits benzo(a)pyrene metabolism by intestinal and hepatic microsomes, with the liver system being more sensitive (10,11). α-Naphthoflavone stimulates *in vitro* AHH activity of control intestinal microsomes from male rats while inhibiting the AHH activity of intestinal microsomes from 3-methylcholanthrene-induced rats (7). Similar results have been reported for the effects of α-naphthoflavone on hepatic microsomal AHH activity from control and 3-methylcholanthrene-induced animals (12). These results suggest that different forms of AHH may exist in the intestine and liver.

The above results demonstrate that rifampin can inhibit AHH activity associated with hepatic and intestinal microsomes. In both cases a non-competitive mechanism is involved, suggesting that rifampin binds to the enzyme at a site other than the binding site of the varied substrates. Furthermore, the concomitant administration of rifampin with other drugs may result in the altered metabolism of co-administered drugs.

EXPERIMENTAL

ANIMALS.—Male Sprague-Dawley rats (Sasco Inc., Omaha, NE), weighing 175–200 g, were used. The animals were kept in stainless steel cages at 22° with 50% relative humidity and lighting from 6 am to 6 pm daily, and were given Purina Lab Chow and tap water *ad libitum*. All animals were allowed to acclimate to the environment for 3–5 days prior to use. Four rats were used for each experiment.

MICROSOMAL PREPARATION.—Liver microsomes were prepared in 1.15% KCl–0.15 M Tris buffer, pH 7.4, by differential centrifugation as previously described (13). Preparation of intestinal microsomes was accomplished by the method of Stohs *et al.* (7) in a Tris-KCl buffer (0.05 M Tris chloride in 1.15% KCl, pH 7.8) containing soybean trypsin inhibitor (5 mg wet weight of small intestine), glycerol (20%, v/v, final concentration), and heparin (3 units/ml). The washed microsomal pellet was resuspended to a concentration of approximately 0.5 mg of microsomal protein per ml in the Tris-KCl buffer, pH 7.8. The final microsomal suspensions obtained from four rats were pooled.

ASSAYS.—AHH activity was determined by the assay procedure of Dehnen *et al.* (14) in an Amino-Bowman spectrophotofluorometer. Protein was determined by the method of Lowry *et al.* (15) with bovine serum albumin as the reference standard. One AHH unit is defined as the amount of enzyme which catalyzes the formation of 1.0 nmole of product/min/mg of microsomal protein. Rifampin was dissolved in DMSO. The concentration of rifampin were prepared such that 10 μl of solution added per ml of the incubation mixture yielded the final desired concentrations. Control incubation flasks contained 10 μl of the solvent per ml of reaction mixture.

CHEMICALS.—Benzo(a)pyrene, $NADP^+$, and trypsin inhibitor were purchased from Sigma Chemical Co. (St. Louis, MO). Rifampin were generously donated by Dow Pharmaceuticals. Indianapolis, Indiana. The 3-hydroxybenzo(a)pyrene which was used as reference standard for the AHH assay was kindly supplied by Dr. Eleanor Rogan. All other chemicals were of analytical grade, obtained from local commercial sources and used without further purification.

DATA ANALYSIS.—The data was fit by linear regression analysis with a Hewlett Packard calculator (see figures 1 and 2), and Pearson's R for correlation was calculated.

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