The Effect of RPR 102341 on Theophylline Metabolism and Phenacetin *O*-Deethylase Activity in Human Liver Microsomes

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Purpose. RPR 102341 is structurally similar to the fluoroquinolone class of antibiotics. Because some fluoroquinolones have been shown to inhibit theophylline metabolism, concomitant administration may increase plasma levels of theophylline resulting in serious adverse effects. The purpose of this study was to determine if RPR 102341 affects theophylline metabolism *in vitro* and, thus, predict whether a clinically significant drug interaction is likely to occur. In addition, the effect of RPR 102341 on phenacetin *O*-deethylase activity was determined to address the enzymatic basis of a potential drug interaction.

Methods. The *in vitro* theophylline metabolism assay was conducted according to a modification of a published procedure. The phenacetin *O*-deethylase assay was conducted according to a modification of a published procedure.

Results. The rate of conversion of theophylline to 3-methylxanthine in human liver microsomes in the presence of 100 μM and 500 μM RPR 102341 was 93.6 and 106 percent of the control reactions, respectively. The formation of 1-methylxanthine was 97.6 and 100 percent of the control, and 1,3-dimethyluric acid formation was 88.9 and 95.2 percent of control at 100 μM and 500 μM RPR 102341, respectively. In agreement, RPR 102341 caused no inhibition of human liver CYP1A2—catalyzed phenacetin *O*-deethylase activity. Finally, no inhibition was observed when RPR 102341 was incubated with human liver microsomes and an NADPH regenerating system prior to the addition of theophylline.

Conclusions. Based on these studies, RPR 102341 is not expected to cause significant drug interactions with theophylline.

KEY WORDS: drug metabolism; human liver; antibiotics; cytochrome P450; theophylline; phenacetin; cytochrome P4501A2.

INTRODUCTION

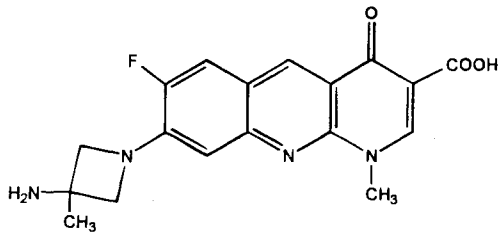
The benzonapthyridones are a novel class of compounds that were designed to be structurally similar to fluoroquinolones in order to reproduce their antibacterial effects by inhibiting type II bacterial topoisomerases. However, a possible problem with the quinolone structure is that it may inhibit the metabolism of the common bronchodilator theophylline when concomitantly administered (1). This drug interaction leads to adverse effects such as nausea and vomiting. Previous studies have indicated that the quinolone antibiotics are competitive inhibitors of human liver cytochrome P450 1A2 (CYP1A2) (2). RPR 102341 is a benzonapthyridone that has been shown to be active against both gram positive and gram negative bacteria. The purpose of this study was to determine if RPR 102341 inhibits theophylline metabolism or CYP1A2—catalyzed phenacetin *O*-deethylation *in vitro* and, thus, predict whether a clinically significant drug interaction is likely to occur.

There are three major metabolites of theophylline. The predominant metabolite, 1,3-dimethyluric acid (1,3-DMU) is formed by 8-hydroxylation (3). The other two metabolites, 1-methylxanthine (1-MX), and 3-methylxanthine (3-MX) are formed by *N*-demethylation reactions. Cytochrome P450 1A2 has been shown to catalyze 50% of the 8-hydroxylation and 80–90% of the *N*-demethylation reactions *in vitro* (4–6). Enoxacin has been reported to inhibit all three metabolic routes, whereas norfloxacin only inhibits the *N*-demethylation pathways (1). These two inhibitors were included as positive controls in our evaluation of the possible inhibition of theophylline metabolism by RPR 102341. Structures of all three compounds are presented in Figure 1.

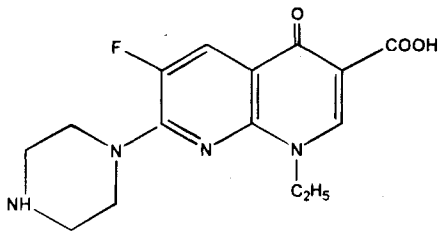
MATERIALS AND METHODS

Analytes and Chemical Reagents

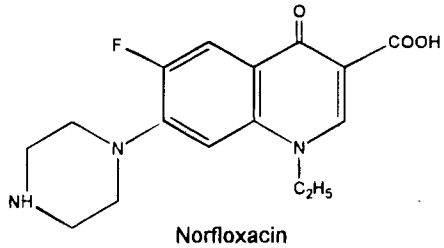
RPR 102341 was obtained through the Collegeville Chemical Processing Center. HPLC grade acetonitrile, methanol,



RPR 102341



Enoxacin



Norfloxacin

Fig. 1. Structure of RPR 102341, enoxacin, and norfloxacin.

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ABBREVIATIONS: CYP1A2, cytochrome P450 1A2; 1,3-DMU, 1,3-dimethyluric acid; 1-MX, 1-methylxanthine; 3-MX, 3-methylxanthine.

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isopropanol, and dichloromethane were obtained from EM Science. Zinc sulfate heptahydrate was purchased from Aldrich Chemical Company. HPLC grade ammonium acetate was purchased from Fisher Scientific. Enoxacin, norfloxacin, 1-methylxanthine, 3-methylxanthine, 1,3-dimethyluric acid, theophylline, theobromine, phenacetin, bucetin, and paracetamol were purchased from Sigma Chemical Company.

Biological Reagents

Fresh human tissue was obtained from organ donors following ethical guidelines for informed consent and supplied by procurement agencies (National Disease Research Interchange, International Institute for the Advancement of Medicine, and The Anatomic Gift Foundation). Human liver microsome samples HL05, HL06, and HL08 were mixed in equal protein proportions to create a pool. Sample HL05 was from a 41 year-old Caucasian female, HL06 was from a 10 year-old African American male, and HL08 was from a 43 year-old Caucasian female.

Incubation Conditions and Sample Analysis

Stock solutions of RPR 102341, norfloxacin, and enoxacin (10 mM) were prepared by dissolving each compound in 100 mM potassium phosphate adjusted with sodium hydroxide as indicated (RPR 102341, 0.25 N; enoxacin, 0.025 N; and norfloxacin, 0.042 N).

For the theophylline metabolism studies, incubations were conducted according to the procedure of Rasmussen *et al.* (7). Human liver microsomes were incubated with 5 or 25 μl enoxacin, norfloxacin, or RPR 102341 (100 or 500 μM). Control samples contained equal volumes of the vehicle used to prepare the test compound or distilled water. Theophylline metabolites were identified by comparing HPLC profiles and retention times with the primary standards. Approximate retention times were: 9.3 min., 3-MX; 11.2 min., 1-MX; 16.7 min., 1,3-DMU; 20 min., theobromine; and 25 min., theophylline. Results were processed using the Beckman Gold software. Neither RPR 102341, enoxacin or norfloxacin interfered with the quantitation of theophylline metabolites.

In one study, the above procedure was modified in order to incubate the NADPH-generating system with RPR 102341 for 15 minutes prior to the addition of theophylline and continuing with the 45 minute incubation. The purpose of this study was to verify that neither RPR 102341 nor metabolites of RPR 102341 affected theophylline metabolism either by irreversibly binding to the enzyme or by competitive inhibition.

For the phenacetin *O*-deethylase assays, incubations of phenacetin with human liver microsomes were conducted according to a modification of the procedure of Sattler *et al.* (8). Briefly, incubations were carried out in a total volume of 0.5 ml at the final concentrations given in parentheses. Incubations contained potassium phosphate buffer (100 mM, pH 7.4), microsomal protein (0.5 mg/ml), $NADP^+$ (0.5 mM), glucose-6-phosphate dehydrogenase (2 units/ml), phenacetin (40, 100, or 200 μM), and RPR 102341 (125, 250, or 500 μM), enoxacin (125, 250, or 500 μM), or norfloxacin (125, 250, or 500 μM). Samples were incubated in a shaking water bath for 3 minutes at 37°C. Glucose-6-phosphate (10 mM final concentration) was added to the appropriate tubes, and samples were incubated for 30 minutes at 37°C. Control reactions contained distilled water in lieu of glucose-6-phosphate. Reactions were stopped with 100 μl of a solution of 2% sodium azide containing 10 μM bucetin as the internal standard. Reaction products were extracted with 4 ml of ethyl acetate; 3 ml of the organic phase was transferred to a clean tube and evaporated under a stream of nitrogen. The samples were dissolved in 300 μl of mobile phase and 50 μl was analyzed by HPLC using an Alltech Nucleosil C_8 column (5 micron, 250 mm × 4.6 mm) with an Alltech All-Guard C_8 cartridge (7.5 mm × 4.6 mm) and an Upchurch pre-column filter. The column was eluted with a mobile phase of 75% 10 mM $NaH_2PO_4 \cdot H_2O$, pH 2.6, 25% acetonitrile, at a flow rate of 1 ml/min followed by peak detection at 246 nm. Bucetin and phenacetin were identified by co-chromatography with the primary standards. Approximate retention times were: 5.0 min., paracetamol; 11.5 min., bucetin; and 14.4 min., phenacetin. Results were processed using the Nelson Analytical Chromatographic software (version 5.1). The data was analyzed using Graph Pad™ (San Diego, CA) software.

RESULTS

Theophylline Metabolism

Enoxacin inhibited all three pathways of theophylline metabolism. Rates of 3-MX and 1,3-DMU formation were 40.2 and 78.8 percent of the control values, respectively, in the presence of 100 μM enoxacin. At 500 μM enoxacin the formation rates for 3-MX and 1,3 DMU were 22.1 and 68.4 percent of the control values. The conversion of theophylline to 1-MX was inhibited to below the level of quantitation at both enoxacin concentrations.

Similarly, 100 μM norfloxacin decreased the formation rates of the N-demethylations to 60.9 and 72.7 percent of the control (3-MX, 1-MX), however little effect on theophylline 8-hydroxylation (96.4% of control) was observed. The concentration dependency of the inhibition was evident as 500 μM norfloxacin decreased formation rates to 44.6 and 40.8 percent of the control for 3-MX and 1-MX, respectively. The formation rate for 1,3 DMU was 88.2 percent of the control.

In contrast, RPR 102341 did not inhibit either pathway of theophylline metabolism. As shown in Figure 2, the formation rates of 3-MX in the presence of 100 μM and 500 μM RPR 102341 were 93.6 and 106 percent of the control reactions, respectively. The formation of 1-MX was 97.6 and 100 percent of the control, and 1,3-DMU formation was 88.9 and 95.2 percent of the control tubes at 100 μM and 500 μM RPR 102341, respectively. Pre-incubation of either 100 μM or 500 μM RPR 102341 and human liver microsomes in the presence of an NADPH-generating system followed by a determination of remaining enzyme activity showed no enzyme inactivation. Specifically, the formation rates of 3-MX, 1-MX and 1,3-DMU were 95.6, 97.0, and 94.2 percent of the control tubes at 100 μM RPR 102341 and 112.5,112.4 and 101.0 percent of the control tubes at 500 μM RPR 102341.

Phenacetin *O*-Deethylase Assay

The phenacetin *O*-deethylase assay is a well established probe for CYP1A2 activity. This assay was included in order to confirm the specificity of inhibition of the three test compounds. The concentrations of phenacetin were chosen based on the reported K_m for phenacetin *O*-deethylation (40 μM) (9).

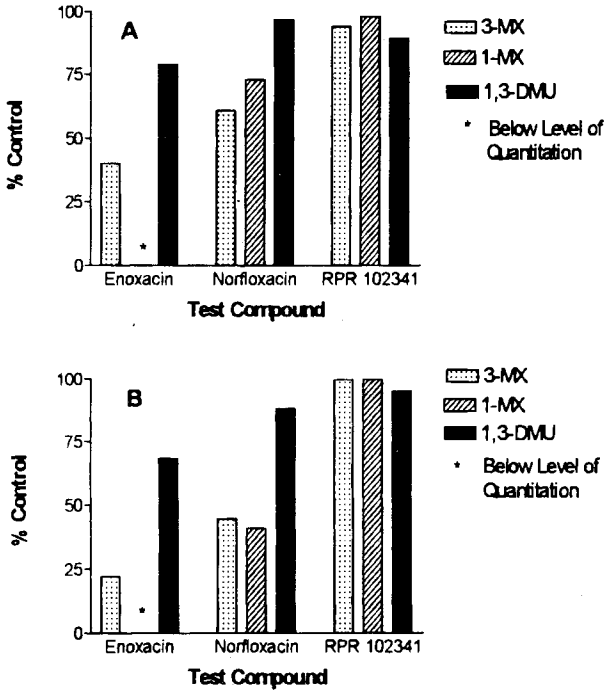

Fig. 2. Inhibition of human liver microsomal theophylline metabolism by 100μM (A) or 500 μM (B) test compound. Each bar represents an average of duplicate values.

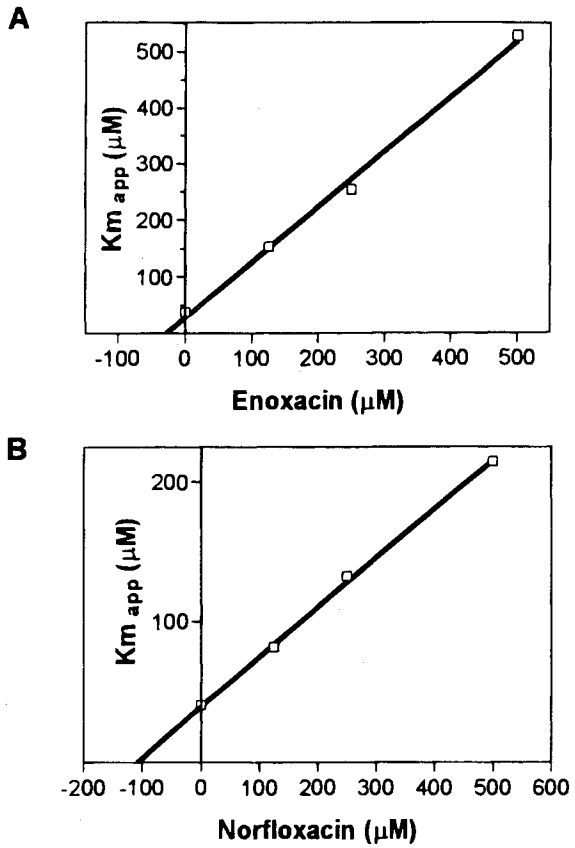

Fig. 3. Replot of data taken from Lineweaver-Burk plot of the effect of enoxacin (A) or norfloxacin (B) on human liver microsomal phenacetin-*O*-deethylation. Each point represents an average of duplicate values.

RPR102341 did not effect phenacetin *O*-deethylation at the concentrations tested (Table I). However, enoxacin and norfloxacin did inhibit phenacetin *O*-deethylase activity. K_i values of 30 μM for enoxacin and 101 μM for norfloxacin were obtained by transformation of Lineweaver—Burk plots. In agreement with previous studies (2), the type of inhibition was competitive. K_m vs. [I] replots of the data from the double reciprocal plots are shown in Figure 3A for enoxacin and Figure 3B for norfloxacin. Reciprocal plots are not presented for RPR 102341 since this compound had no effect on phenacetin *O*-deethylation.

DISCUSSION

Due to recent advances in technology for studying drug metabolism/ drug interactions *in vitro,* regulatory agencies have begun to encourage *in vitro* studies to determine when clinical studies are most beneficial (10). In general, when *in vitro* studies are performed using appropriate concentrations of the compounds in question, the studies may be acceptable in lieu of a clinical study (11). A previous study that investigated the effect of 44 quinolone antibacterial agents found that nearly all of them had an inhibitory effect on CYP1A2 activity (2). Because of the structural similarity of RPR 102341 to quinolone antibacterial agents, the question arose as to whether RPR 102341 would inhibit theophylline metabolism or CYP1A2—catalyzed phenacetin O-deethylation.

For the theophylline metabolism assay, the microsome mixture was incubated with 1 mM theophylline and either 100 or 500 μM RPR 102341, enoxacin, or norfloxacin. Although high concentrations of both theophylline and the quinolones were used in our *in vitro* study, they were necessary to allow quantitation of the theophylline metabolites. However, the ratios of the theophylline and quinolone concentrations are similiar to those observed in a clinical situation (1). The results of the inhibition of theophylline metabolism by enoxacin and norfloxacin correspond well with a previous study by Sarker, *et al.* (1) in that both compounds inhibited the N-demethylation pathways, but only enoxacin had an effect on the 8-hydroxylation pathway. RPR 102341 did not affect either pathway. For the measurement of phenacetin *O*-deethylase activity, a range of substrate concentrations were tested against a range of test compound concentrations. Consistent with the theophylline metabolism results, enoxacin and norfloxacin inhibited phenacetin *O*-deethylation whereas RPR 102341 did not, thus indicating that RPR 102341 does not inhibit CYP1A2. In summary,

Table I. Effect of RPR102341 on Phenacetin *O*-Deethylase Activity in Human Liver Microsomes[a]

		RPR102341 Concentration		
Phenacetin	Control	125 μM	250 μM	500 μM
40 μM	0.638	0.618 (96.9)[b]	0.624 (97.8)	0.592 (92.8)
100 μM	0.967	0.809 (83.7)	0.775 (80.1)	0.786 (81.3)
200 μM	1.034	1.021 (98.7)	0.939 (90.8)	0.918 (88.8)

[a] Activities shown are nmoles paracetamol formed per min per mg protein and represent an average of duplicate values.
[b] Percent of control activity.

these results suggest that coadministration of RPR 102341 and theophylline or any other drug metabolized by CYP1A2 would not be expected to produce the drug interaction problems seen with other quinolone antibiotics.

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