Metabolism of Rifabutin and Its 25-Desacetyl Metabolite, LM565, by Human Liver Microsomes and Recombinant Human Cytochrome P-450 3A4: Relevance to Clinical Interaction with Fluconazole

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Rifabutin and fluconazole are often given concomitantly as therapy to prevent opportunistic infections in individuals infected with the human immunodeficiency virus. Recent reports have shown increased levels of rifabutin and its 25-desacetyl metabolite, LM565, in plasma when rifabutin is administered with fluconazole. Since fluconazole is known to inhibit microsomal enzymes, this study was undertaken to determine if this rifabutin-fluconazole interaction was due to an inhibition of human hepatic enzymes. The metabolism of both rifabutin and LM565 was evaluated in human liver microsomes and recombinant human cytochrome P-450 (CYP) 3A4 in the presence of fluconazole and other probe drugs known to inhibit CYP groups 1A2, 2C9, 2D6, 2E1, and 3A. The concentrations of rifabutin (1 μg/ml), LM565 (1 μg/ml), and fluconazole (10 and 100 μg/ml) used were equal to those observed in plasma after the administration of rifabutin and fluconazole at clinically relevant doses. High-performance liquid chromatography was used to assess the metabolism of rifabutin and LM565. Rifabutin was readily metabolized to LM565 by human microsomes, but the reaction was independent of NADPH and was not affected by the P-450 inhibitors. No rifabutin metabolism by recombinant CYP 3A4 was found to occur. LM565 was also metabolized by human microsomes to two products, but metabolism was dependent on NADPH and was affected by certain P-450 inhibitors. In addition, LM565 was readily metabolized by the recombinant CYP 3A4 to the same two products found with its metabolism by human microsomes. Therefore, rifabutin is metabolized by human microsomes but not via cytochrome P-450 enzymes, whereas LM565 is metabolized by CYP 3A4.

Chemoprophylaxis for opportunistic infections associated with the human immunodeficiency virus (HIV) has become the standard of practice in the medical care of patients infected with HIV (7). Two drugs often used concurrently by these patients are rifabutin, for the prevention of Mycobacterium avium complex bacteremia (16), and fluconazole, for the treatment and prevention of fungal infections (14, 18).

Rifabutin, a rifamycin S derivative, has been shown to induce both the activity of the cytochrome P-450 (CYP) enzyme groups (24) and its own metabolism (2, 3, 17, 20, 21). Rifabutin is metabolized into several different products, the major one being LM565, the 25-desacetyl metabolite of the parent compound (11). LM565 is known to be as potent as rifabutin in vitro against M. avium complex; it is further metabolized in the liver prior to its elimination (20). Fluconazole is eliminated essentially unchanged via the kidneys (14). However, fluconazole and a related azole, ketoconazole, are known inhibitors of hepatic microsomal enzymes, especially the CYP 3A group (12). Inhibition of these enzymes has, in turn, been shown to cause clinically significant increases in circulating levels of concomitant drugs that are metabolized via these enzymes (5, 6, 8, 9, 13, 15, 19).

Several groups have reported that concomitant administration of fluconazole and rifabutin has resulted in enhanced rifabutin efficacy (15) and toxicity (5, 6, 8, 19). Trapnell et al. (22) recently reported the results of a pharmacokinetic drug interaction study with HIV-infected subjects which showed a significant increase in the concentrations of both rifabutin and LM565 in serum when rifabutin was administered concurrently with fluconazole (22). These results were hypothesized to be due to fluconazole’s inhibitory effects on the CYP 3A enzyme group, presumed to be responsible for the metabolism of both rifabutin and LM565.

The present study was undertaken to perform an in vitro evaluation of the rifabutin-fluconazole drug interaction by using human liver microsomal enzymes and recombinant human CYP 3A4. Both rifabutin and LM565 were evaluated. Known inhibitors of CYP enzyme groups were used as probe drugs in an effort to determine which CYP group(s), if any, was responsible for the metabolism of these two compounds.

MATERIALS AND METHODS

Materials. Rifabutin and LM565 were obtained from Pharmacia-Upjohn, Inc., Kalamazoo, Mich. Human liver samples, medically unsuitable for transplantation, were obtained from the Washington Regional Area Transplant Consortium (Washington, D.C.) and immediately sectioned and stored at −70°C until they were used as described previously (10). Microsomal preparations containing recombinant human CYP 3A4 and CYP reductase were obtained from Gentest Corp., Woburn, Mass.

Metabolism of rifabutin and LM565 by human liver microsomes. Metabolic time studies were performed for both rifabutin at 1 μg/ml (1.2 μM) and LM565 at 1 μg/ml (1.2 μM) to determine the optimal incubation times. Each 1 ml of incubation mixture contained 2.25% bovine serum albumin in 5 mM MgCl₂, (pH 7.4), an NADPH-generating system (10 mM glucose-6-phosphate, 1 mM NADP⁺, and 1 Sigma unit of glucose-6-dehydrogenase), 1 mg of protein from a mixture of liver microsomes from three human donors, the inhibitors, and either rifabutin or LM565. The inhibitors and the respective CYP that each inhibits (indicated in parentheses) included ciprofloxacin HCl at 200 μM (CYP 1A2), sulfaphenazole at 100 μM (CYP 2C9), quinidine sulfate at 0.5 μM (CYP 2D6), sodium diethyldithiocarbamate (DDTC) at 100 μM (CYP 2E1), ketoconazole at 1 μM (CYP 3A4), and fluconazole at 10 μg/ml (33 μM) and 100 μg/ml (330 μM) (CYP 2C9 and 3A4). Rifabutin, LM565, fluconazole, and ketoconazole were
FIG. 1. LM565 metabolism in human liver microsomes. Data represent LM565’s percentage (mean ± standard deviation) of the total area of the LM565 peak at 278 nm obtained by HPLC with no NADPH in the mixture after 60 min of incubation and in the presence of selected inhibitors. Comparisons were made to the percentage of LM565 remaining in incubations at 60 min (T = 60 min). CIPRO, ciprofloxacin; SULFA, sulfaphenazole; QUIN, quinidine sulfate; KETO, ketoconazole; FLU, fluconazole; *, statistically significant P value (P < 0.0056) by analysis of variance (the P value was adjusted for multiple comparisons by using Bonferroni’s inequality).

DISCUSSION

Rifabutin’s metabolism was unaffected when the NADPH-generating system was excluded from the reactions. This indicates that rifabutin’s metabolism is independent of hepatic CYP enzymes. None of the inhibitors, including fluconazole, exerted any effect on rifabutin’s metabolism, which is consistent with the first finding. These findings were supported by the fact that rifabutin also was not metabolized by recombinant human CYP 3A4.

Trapnell et al. (22) reported an 82% increase in rifabutin’s area under the concentration-time curve in the presence of fluconazole and hypothesized that these findings were due to inhibition of CYP enzyme 3A by fluconazole. However, our in vitro data refute that hypothesis. This observed increase in the rifabutin area under the concentration-time curve could have been due to other factors such as enhanced rifabutin absorption. It is also possible that rifabutin was inducing its own metabolism. A 40% decrease in rifabutin’s area under the concentration-time curve after 10 to 28 days of rifabutin administration has been reported (21). The rifabutin concentrations obtained after 28 days of rifabutin therapy in the clinical study were lower than the rifabutin concentrations obtained after 14 days of rifabutin therapy. The in vivo findings were observed because the concentrations measured were always at least 10% greater than the background signal.

RESULTS

Metabolism of rifabutin and LM565 by human liver microsomes. Rifabutin and LM565 were both metabolized by human liver microsomes. The metabolism of each compound was linear for up to 90 min. The metabolism of rifabutin was independent of NADPH, and its metabolism was unchanged by the presence of any of the inhibitors. Under these conditions, LM565 was the only product observed (data not shown). For LM565, two metabolic products were observed, but their quantitation was problematic. Therefore, LM565’s metabolism was monitored by measuring substrate disappearance, as indicated in Fig. 1. LM565’s metabolism was dependent on NADPH. The addition of ciprofloxacin, sulfaphenazole, and quinidine did not affect its metabolism. However, DDTC at 100 μM inhibited 50% of LM565’s metabolism, while ketoconazole at 1 μM inhibited 75% of its metabolism. Finally, fluconazole at 100 μg/ml (330 μM) appeared to completely block the metabolism of LM565.

Metabolism of rifabutin and LM565 by recombinant human CYP 3A4. Rifabutin was not metabolized by recombinant human CYP 3A4. LM565 incubations showed substrate disappearance with the formation of at least two species, comparable to what was seen with the liver microsomal incubations (data not shown). Similarly, the metabolism of LM565 was inhibited by the addition of ketoconazole to the incubation mixture (Fig. 2). These results were nearly identical to what was observed with the metabolism of LM565 by the human liver microsome incubations.

FIG. 2. LM565 metabolism in human liver microsomes and recombinant human CYP 3A4. LM565’s percentage (mean ± standard deviation) of the total area of the LM565 peak at 278 nm obtained by HPLC after 0 and 60 min of incubation (T = 0 and 60 min, respectively) and in the presence of ketoconazole (KETO). White bars, human liver microsomes; black bars, recombinant CYP 3A4.
otherwise. Finally, the increasing LM565 concentrations might, in turn, have caused inhibition of the biotransformation of rifabutin. This phenomenon has been reported with diltiazem (23). However, our in vitro data showed a linear relationship between both rifabutin disappearance and LM565 formation over 90 min, suggesting that the increasing LM565 concentrations had no impact on rifabutin’s metabolism. The metabolism of rifabutin’s LM565, 25-desacetyl metabolite, appears to be catalyzed by CYP 3A4. Ketoconazole at 1 μM, a specific inhibitor of CYP 3A4, almost completely inhibited the metabolism of LM565 in both the human and recombinant microsomal systems. Furthermore, in the human microsomes, complete inhibition of the metabolism of LM565 was observed with fluconazole at 100 μg/ml (330 μM) and partial inhibition of the metabolism of LM565 was seen with fluconazole at 10 μg/ml (33 μM) and DDTC at 100 μM. DDTC at lower concentrations is an inhibitor of CYP 2E1; however, at this higher concentration, it has overlapping inhibition with CYP 3A4 (4). This explains the partial inhibition of LM565’s metabolism by the addition of DDTC at 100 μM.

The change in LM565 exposure reported from the in vivo drug-drug interaction study was significantly greater than the change observed in the rifabutin area under the concentration-time curve (22). This could be due to increased formation of LM565 secondary to the observed increase in rifabutin concentrations and/or decreased LM565 metabolism from enzyme inhibition by fluconazole. The in vitro data demonstrate that one mechanism for the observed in vivo changes in the LM565 area under the concentration-time curve is due, at least in part, to the inhibition of CYP 3A4 enzymes by fluconazole.

We chose in vitro concentrations of rifabutin, LM565, and fluconazole in microsomes equal to the concentrations in serum observed in the clinical study when rifabutin was administered at a dosage of 300 mg daily and fluconazole was administered at a dosage of 200 mg daily (22). It is, however, not known if the drug concentrations in microsomes are higher than, lower than, or similar to drug concentrations in serum. Fluconazole is currently administered at doses of up to 2,000 mg daily, with corresponding average steady-state peak concentrations in plasma of 90 μg/ml (1), making it possible that, in some clinical settings, in vivo fluconazole concentrations in serum and/or microsomes may equal or exceed the higher concentration of fluconazole used in our in vitro study. The results of this in vitro CYP study together with the in vivo data from the clinical pharmacokinetic study (22) suggest that the changes seen in rifabutin exposure with fluconazole therapy are not due to an inhibition of CYP enzymes. In contrast, the observed changes with LM565 are due, at least in part, to an inhibition of its metabolism at the level of CYP 3A4. It is probable that the observations of enhanced efficacy and the increase in the observed toxicity of rifabutin therapy may be primarily due to the presence of significantly higher concentrations of rifabutin and/or LM565 when rifabutin and fluconazole are coadministered. The relative contributions of rifabutin and LM565 to these findings are not known. A complete understanding of the contribution of these two compounds to the efficacy and toxicity profile observed with rifabutin therapy as well as the mechanism behind the observed changes in rifabutin’s in vivo concentrations in plasma would be useful for providing a more complete understanding of the clinical findings for patients receiving these therapies.

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