

Original Article

The Role of Cytochrome P450 3A5 Enzyme on the Metabolism of Tacrolimus in Rats

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Abstract

Purpose: The present study was designed to determine the effect ratio of CYP3A5 on the metabolism of tacrolimus that is used as an immunosuppressant for tissue transplantation.

Methods: To determine the role of CYP3A4 and CYP3A5 on tacrolimus metabolism, rats were divided into five groups as: group 1 (control group, tacrolimus only 1 mg/kg i.v.), group 2 (pretreated with ritonavir 5 mg/kg, i.v. 1 h before administration of tacrolimus), group 3 (pretreated with indinavir 10 mg/kg, i.v. 1 hour before administration of tacrolimus), groups 4 and 5, in addition to the protocol of groups 2 and groups 3. Dexamethasone (100 mg/kg, p.o.) was administered for 2 days before the experimental study to each group. To estimate the area under curve (AUC) of tacrolimus, the blood samples were collected after 15, 30, 60, 75, and 90 min and after 2, 3, 4, 8, and 24 h, and MEIA method was used to determine whole blood levels of tacrolimus.

Results: Although the AUCs of tacrolimus in group 2 (533.5±139.85 ng.h/mL) and group 3 (3428±683 ng.h/mL) were higher than the control group (394±127 ng.h/mL), the only significant difference was found in ritonavir pretreated group (group 3). In dexamethasone pretreated groups, the AUC values were similar to control group.

Conclusion: These results suggest that the role of CYP3A5 enzyme has to be taken into account for probable drug interactions and sufficient immunosuppression in patients who are treated with tacrolimus.

Keywords: AUC, CYP3A5, drug metabolism, rat, tacrolimus

INTRODUCTION

Tacrolimus (FK 506) is a calcineurin inhibitor, a macrolide lactone with potent immunosuppressive properties (1). FK 506 was isolated from *Streptomyces tsukubaensis* as a white crystalline powder. It was reported that FK 506 is 100-fold more potent than cyclosporine (2). Tacrolimus was applied in 1994 for the prevention of graft rejection in liver transplant patients. It is now used for the prevention of rejection in kidney, lung, heart, small bowel, pancreas, and bone marrow transplant patients (3). Tacrolimus is metabolized primarily by cytochrome P450 (CYP) 3A4 and 3A5. CYP3A members are the most abundant CYPs in rat and human liver and small intestine (4, 5). Furthermore, tacrolimus is also a substrate of P-glycoprotein (1). Most of CYP 3A substrates are also recognized as substrates or inhibitors of P-glycoprotein (P-gp) because of the strong overlapping substrate specificities and inhibitors between CYP 3A and P-gp. For this reason, many drug interactions may involve both CYP 3A and P-gp (4, 6). In addition to the pharmacokinetic drug-drug interactions between P-gp substrates and CYP3A-related compounds, a concomitant induction of P-gp and CYP 3A by dexamethasone is reported in vivo and in cultured cells (7). In the in vitro study, it has been shown that dexamethasone pretreatment increased P-gp level in the intestine 1.9-fold but not in the liver and different ratios of CYP 3A in the intestine and liver of rats (8). Indinavir and ritonavir are anti-HIV agents that selectively inhibit the HIV type I protease (9). It has been shown that the protease inhibitors indinavir and ritonavir are metabolized primarily by isozymes of the CYP 3A subfamily (10, 11) and substrates of P-gp (12). Koudriakova et al. (13) showed that ritonavir is metabolized by CYP 3A4 and CYP 3A5 at the same ratio, but indinavir is metabolized by CYP 3A4 than CYP 3A5 in human intestinal microsomes and expressed CYP 3A4/3A5.

Although the role of CYP3A4 and CYP3A5 enzyme at the tacrolimus metabolism is reported, the fraction of CYP 3A5 involvement remains unclear. The aim of our study is to show the percentage of tacrolimus metabolized by CYP 3A5 enzyme and also to show whether drug-drug interaction takes place when tacrolimus is used with the drugs which are substrates of this enzyme.

METHODS

Animals

Male Wistar rats weighing 200–250 g were used throughout the study. All animals were kept in standardized conditions of temperature (21°C–22°C) and illumination (12-h light/12-h darkness) and cages with mesh bottoms and free access to tap water and pelleted food. The animals were fasted for 12 h before the experiment but had free access to water right up to the beginning of the experiment. This study was approved by Dokuz Eylul University Animal Care Committee.

Experimental Procedure and Groups

Rats were anesthetized with diethyl ether. During the study, body temperature was maintained with appropriate heating lamps. The jugular vein was cannulated with polyethylene tube for intravenous bolus injection of tacrolimus and other drugs (indinavir and ritonavir). In our study, there were five groups. Group 1 (control group, n=7) was administered only tacrolimus i.v. bolus injection at a dose of 1 mg/kg. Rats in group 2 (n=6) and group 3 (n=5) were, respectively, given 10 mg/kg i.v. indinavir and 5 mg/kg ritonavir 1 h before 1 mg/kg tacrolimus i.v. bolus injection. Rats in group 4 (n=5) and group 5 (n=5) were first given dexamethasone at a dose of 100 mg/kg daily for 2 consecutive days by gavage and these rats were subjected to the protocol of groups 2 and 3 after the last dose of dexamethasone. All the blood samples (0.2 mL) were drawn from tail vein at 15, 30, 60, 75, 90 min and 2, 3, 4, 8, and 24 h after administration of tacrolimus bolus injection. The last blood sample (24 h) was collected by cardiac puncture.

Analysis of Blood Samples

All the blood samples were analyzed by a microparticle enzyme immunoassay (MEIA) method that can be performed on the Abbott IMx analyzer at the end of the studying day. The lower and upper limits of quantitation of the assay were 1.5 and 30 ng/mL, respectively. Therefore, all the samples were studied after dilution procedure.

Drugs

To adjust the solution for intravenous bolus injection to a dose of 1 mg/kg, 200 µL/kg tacrolimus injection solution (Prograf) was diluted with saline. Indinavir was administered in dimethyl sulfoxide (DMSO) solution (0.5 mL/kg) at a concentration of 10 mg/kg. Ritonavir was freshly formulated in 5% ethanol/95% PEG for i.v. dosing at a dose of 5 mg/kg. Dexamethasone was dissolved in distilled water at a concentration of 50 mg/mL.

Pharmacokinetic Analysis

Pharmacokinetic analysis was performed by using a two-compartment method. The area under the concentration-time curve (AUC) was estimated by the linear trapezoidal rule. The elimination rate constant k was calculated by linear regression of the log concentration versus time curve during the log-linear decline phase. Elimination half-life and clearance were estimated as follows:

$$t_{1/2} = \ln 2 / k$$

$$CL = \text{Dose} / \text{AUC}$$

Figure 1. Time courses of blood concentration of tacrolimus after i.v. administration (1 mg/kg) to all the groups. Each point with bar represents the mean±SD (n=5–7)

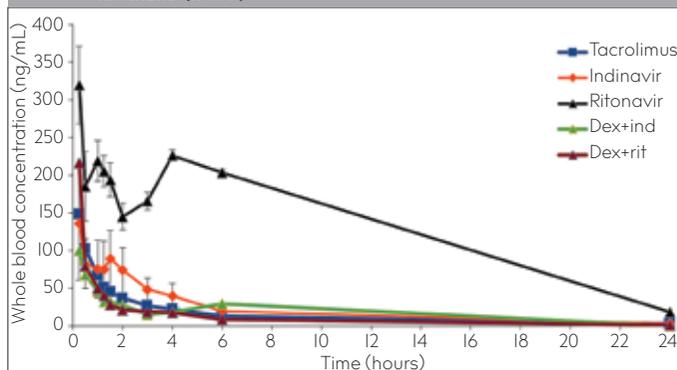
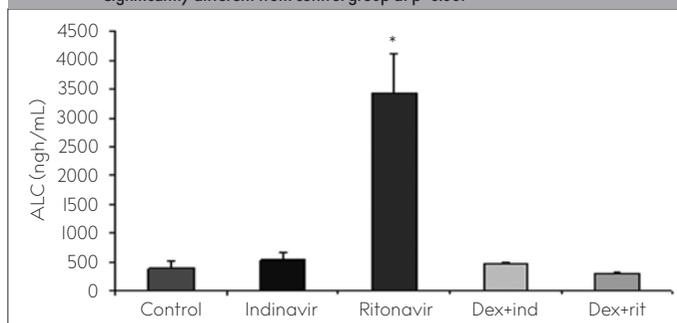


Figure 2. The AUC values of tacrolimus of all the groups. Each column with bar represents the mean±SD. Only the AUC of ritonavir group was significantly higher than the control group. *significantly different from control group at p<0.001



Statistical Analysis

Values were expressed as the mean±SD. Differences among the AUC values of groups were analyzed by ANOVA and Bonferroni test. P values less than 0.05 were considered to be significant.

RESULTS

The time courses of blood concentration of tacrolimus after i.v. administration of tacrolimus (1 mg/kg) to all the groups are shown in Figure 1. The two-compartment model of tacrolimus was clearly observed in group 2 (indinavir-pretreated group) and group 3 (ritonavir-pretreated group).

The AUCs of tacrolimus of group 2 (given 10 mg/kg i.v. indinavir) and group 3 (given 5 mg/kg i.v. ritonavir) were determined as 533.5±139.85 and 3428±683 ng.h/mL, respectively. Although both values were higher than control group (394.56±127.51 ng.h/mL), only ritonavir group was found to be statistically significant from control group (Figure 2, p<0.001).

In group 4 (indinavir group) and group 5 (ritonavir group) that were pretreated with dexamethasone, the AUC values of tacrolimus were calculated as 462.98±27 and 282.6±32.82 ng.h/mL, respectively. There were no statistically significant differences found between the AUCs of tacrolimus of dexamethasone pretreated groups and control group (394.56±127.51 ng.h/mL).

DISCUSSION

In this study, tacrolimus blood levels and AUC values of the groups which were pretreated with indinavir and ritonavir were found to be higher than the control group, whereas the values of ritonavir group were significant.

Ritonavir and indinavir are protease inhibitors which are substrates and inhibitors of CYP 3A enzymes. Koudriakova et al. (13) have reported that ritonavir was similarly biotransformed by microsomes containing expressed CYP 3A4 or CYP 3A5 isoenzymes, whereas indinavir was metabolized more by CYP 3A4 than by CYP 3A5. Both ritonavir and indinavir could inhibit CYP3A enzymes and also they were shown to induce CYP3A expression (14, 15). Due to the fact that they cause induction and elevated enzyme levels, the inhibitory effects of these drugs show a diminishing pattern. Despite of this, the AUC values were found to be high but not as high as they were predicted. This shows that the present inducing effect is not as powerful as the inhibitory effect.

Due to the immunosuppressant effect that it owes, tacrolimus is used rather frequently in organ transplantation patients, and its pharmacokinetic interactions with the protease inhibitors such as indinavir and ritonavir are reported in experimental animals and human subjects (16). In accordance with our results, other studies have shown that protease inhibitors could increase the tacrolimus blood levels. Although studies reporting no association between elevated tacrolimus blood levels and toxicity are present (16), there are also studies emphasizing concern between the level of free fraction of tacrolimus in blood and toxic effects or transplant rejection (17).

In the study conducted by Ernest et al. (15) that investigated the effects of the protease inhibitors on CYP3A family, ritonavir was found to be a potent inhibitor of both CYP3A4 and CYP3A5, whereas indinavir showed no time- or concentration-dependent effect on the activity of these two enzymes. In concordance with the study of Ernest et al. (15), the higher results that we found in the ritonavir group could be explained with the suggestion that ritonavir has a potent inhibitory effect both on CYP3A4 and on CYP3A5. The fact that AUC values of indinavir group are not significantly higher than those of control group may support the claim that indinavir lacks a significant inhibitory effect on CYP3A family as Ernest et al. (15) asserted in their study.

Although it has been suggested that dexamethasone induced P-gp and CYP 3A enzymes, Yumoto et al. (8) have reported that dexamethasone pretreatment increased P-gp level in the intestine 1.9-fold but not in the liver and increased CYP 3A activity in the liver 9.7-fold but not in the intestine. In the ritonavir group of our study which is pretreated by dexamethasone, the fact that tacrolimus AUC values in which only ritonavir was administered tended to regress to those of the control group and indinavir group values following a similar pattern reveal that CYP3A enzyme family in the liver can be sufficiently induced by dexamethasone. We predict that protease inhibitors in groups which were pretreated with dexamethasone did not have an effect on tacrolimus AUC values due to enzyme induction.

It has also been reported that the protease inhibitors have P-gp inhibitory effect which may cause an increase in intestinal absorption of tacrolimus as well as changing its tissue distribution (16, 17). However, in our study, intravenously administered tacrolimus was not expected to have an effect on AUC values. Moreover, the fact that dexamethasone, which is a well-known P-gp inducer, does not have an effect on P-gp expression in liver while it is inducing intestinal P-gp may also explain the lack of effect of intravenously administered tacrolimus on AUC values.

It has been shown in many studies that the principal enzyme which is responsible for tacrolimus metabolism in liver and small intestines is the CYP3A4 that constitutes more than 90% of its whole metabolism. However, in the recent years, there have been studies suggesting that the polymorphism of CYP3A5 may also affect the bioavailability of tacrolimus (18-20). It has also been reported that the patients who exhibit CYP 3A5*1/*1 genotype may have an increased metabolism of tacrolimus in their liver and intestines, and therefore to obtain and maintain the appropriate blood levels of tacrolimus in these patients, an elevation of oral dose may be required. The complications which are seen in some renal transplant patients may be explained to a certain extent with the probable alteration in tacrolimus metabolism due to the genetic polymorphism of CYP3A5, and it has been reported that because of the difficulties regarding to proper dose arrangement, these patients' clinical status had worsened gradually. The interindividual variations of tacrolimus bioavailability among the subjects may also be explained with this genetic polymorphism fact (18).

These results suggest that the role of CYP3A5 enzyme has to be taken into account for probable drug interactions and sufficient immunosuppression in patients who are treated with tacrolimus.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Dokuz Eylül University Animal Care Committee (28.03.2012)

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