Impact of intestinal CYP2C19 genotypes on the interaction between tacrolimus and omeprazole, but not lansoprazole, in adult living-donor liver transplant patients

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Running title: Intestinal CYP2C19 in tacrolimus-PPI interaction

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Abbreviations: CYP, cytochrome P450; PPI, proton pump inhibitor; LDLT, living-donor liver transplantation; C/D, concentration/dose; EMs, extensive metabolizers; IMs, intermediate metabolizers; PMs, poor metabolizers.
Abstract

To assess the effects of intestinal cytochrome P450 (CYP) 2C19 on the interaction between tacrolimus and proton pump inhibitors, we examined the concentration/dose ratio [(ng/mL)/(mg/day)] of tacrolimus coadministered with omeprazole (20 mg) or lansoprazole (30 mg) to 89 adult living-donor liver transplant patients on postoperative days 22-28, considering the CYP2C19 genotypes of the native intestine and the graft liver, separately. The concentration/dose ratio of tacrolimus coadministered with omeprazole was significantly higher in patients with two variants (*2 or *3) for intestinal CYP2C19 (median, 6.38; range, 1.55-22.9) than intestinal wild-type homozygotes (median, 2.11; range, 1.04-2.54) and heterozygotes (median, 2.11; range, 0.52-4.33) (P=0.010), but the extent of the increase was attenuated by carrying the wild-type allele in the graft liver even when patients were CYP3A5*1 non-carriers. Conversely, the CYP2C19 polymorphisms both in the native intestine and in the graft liver little influenced the interaction between tacrolimus and lansoprazole, but CYP3A5*1 non-carriers showed higher tacrolimus concentration/dose ratio than CYP3A5*1 carriers. Furthermore, our experiments in vitro revealed that lansoprazole had a stronger inhibitory effect on the CYP3A5-mediated metabolism of tacrolimus than omeprazole, although not significantly (IC_{50}=19.9 ± 13.8 μM for lansoprazole, 53.7 ± 6.1 μM for omeprazole). Our findings suggest that intestinal as well as graft liver CYP2C19 play a
relatively greater role in the metabolism of omeprazole than it does for lansoprazole, so that the effects of CYP3A5 on the metabolism of tacrolimus might be masked by the interaction with omeprazole associated with the CYP2C19 genotype.
Introduction

The immunosuppressant tacrolimus is characterized by a narrow therapeutic index and remarkable intra- and inter-individual variability in its pharmacokinetics (Venkataramanan et al., 1995; Kahan et al., 2002). This variability can be attributed to factors such as poor absorption (Masuda and Inui, 2006), extensive first-pass metabolism (Lampen et al., 1995; Wilkinson, 2005) and drug-drug interactions (Christians et al., 2002). Tacrolimus is mainly metabolized by cytochrome P450 (CYP) 3A4 and CYP3A5 in the small intestine and liver (Shiraga et al., 1994; Hesselink et al., 2003). Especially, CYP3A5 plays a key role in the pharmacokinetics of tacrolimus (Kamdem et al., 2005; Dai et al., 2006). Several studies of heart (Zheng et al., 2003), lung (Wang et al., 2006), kidney (Hauflord et al., 2004; Macphee et al., 2005; Hauflord et al., 2006; Kuypers et al., 2007), and liver (Goto et al., 2004; Masuda et al., 2006; Uesugi et al., 2006; Fukudo et al., 2008) transplant patients treated with tacrolimus have shown a significant association between the CYP3A5 polymorphisms and tacrolimus dose-adjusted trough blood levels.

Clinically relevant drug-drug interactions have been observed between tacrolimus and proton pump inhibitors (PPIs) in those with CYP2C19 gene variants, poor metabolizers (PMs) and intermediate metabolizers (IMs), compared to those with no variants, extensive metabolizers (EMs) (Itagaki et al., 2004; Miura et al., 2007).
Because CYP2C19 and CYP3A4/5 are mainly responsible for the metabolism of PPIs (Andersson, 1996), PPIs themselves inhibit the metabolism of tacrolimus via CYP3A4/5 in patients carrying variant alleles of CYP2C19, thereby increasing the blood concentrations of tacrolimus. Furthermore, the magnitude of CYP2C19-mediated metabolism of omeprazole is greater than that of lansoprazole. Recently, we have reported the interaction between tacrolimus and lansoprazole in a living-donor liver transplant (LDLT) patient with the CYP2C19*2/*3 and CYP3A5*3/*3 genotypes both in the native intestine and in the graft liver (Hosohata et al., 2008). In liver transplantation, the genetic backgrounds of the native intestine (recipient) and the graft liver (donor) are different in many cases. Furthermore, several studies have assessed the expression or catalytic activity of intestinal CYP2C19 (Obach et al., 2001; Lapple et al., 2003; Paine et al., 2006). Based on these backgrounds, we hypothesized that intestinal CYP2C19 would affect the interaction between tacrolimus and PPIs in liver transplant patients.

In the present study, we examined the impact of the CYP3A5 as well as CYP2C19 genotypes in the small intestine on the interaction between tacrolimus and PPIs in LDLT patients, considering the genotypes of the native intestine and the graft liver, separately. Furthermore, the inhibitory effects of the PPIs on the CYP3A5-mediated metabolism of tacrolimus were examined using recombinant microsomal preparations.
Materials and Methods

Patients.

Between February 2004 and January 2008, 89 de novo adult LDLT patients and their 89 corresponding donors were enrolled in this study, having first provided their written informed consent. Patients (all Japanese), who were receiving tacrolimus with either omeprazole (n=35) (Omepral®, AstraZeneca Co. Ltd., Osaka, Japan) at 20 mg/day or lansoprazole (n=54) (Takepron®, Takeda Pharmaceutical Co. Ltd., Osaka, Japan) at 30 mg/day, were studied on days 22-28 post transplantation (Table 1). This study was conducted in accordance with the Declaration of Helsinki and its amendments, and was approved by the Kyoto University Graduate School and Faculty of Medicine, Ethics Committee.

Dosage regimen of tacrolimus and measurement of tacrolimus concentrations.

The basic immunosuppression regimen consisted of tacrolimus with low-dose steroids (Inomata et al., 1996). Tacrolimus was administered orally at a dose of 0.075 mg/kg every 12 hours from the evening of postoperative day 1 (Yasuhara et al., 1995; Inomata et al., 1996). The target of the whole-blood trough concentration of tacrolimus was set at between 10 and 15 ng/mL during the first 2 weeks. Steroid treatment was started at graft reperfusion at a dose of 10 mg/kg, with a gradual reduction from 2 mg ·
kg⁻¹ ⋅ day⁻¹ to 0.3 mg ⋅ kg⁻¹ ⋅ day⁻¹ during the first 2 weeks after surgery. The dosage of tacrolimus was adjusted on the basis of whole-blood trough concentrations measured about 12 hours after the evening dosage every day, using a semiautomated microparticle enzyme immunoassay (IMx®; Abbott, Tokyo, Japan) (Yasuhara et al., 1995).

**Evaluation of drug interactions between tacrolimus and PPIs.**

Because the oral administration of PPIs started approximately two weeks after surgery, we evaluated data on postoperative days 22-28. The clinical course of all patients enrolled in this study was stable. Then, the average of dose-normalized blood concentration of tacrolimus during this observation period was assessed as concentration/dose (C/D) ratio [(ng/mL)/(mg/day)] of tacrolimus for each patient and used for the analysis.

We excluded data obtained during treatment with a temporal high-dose steroid injection against acute cellular rejection due to induction of the intestinal expression of CYP3A4 (Masuda et al., 2004).

**Genotyping.**

Genomic DNA was extracted from the peripheral blood of transplant patients or
donors with a Wizard® Genomic DNA Purification kit (Promega Corporation, Madison, WI, USA). Because two CYP2C19 variant alleles, CYP2C19*2 and CYP2C19*3, account for the poor metabolizer phenotype in Japanese (De Morais et al., 1994a), the detection of the wild-type allele (*1) and these two variant alleles was performed using a polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method (De Morais et al., 1994a; De Morais et al., 1994b). The genotyping of CYP3A5 was performed as described previously (Goto et al., 2004; Uesugi et al., 2006; Fukudo et al., 2008).

**Classification of patients.**

The patients themselves and their corresponding donors were separately classified into 3 groups on the basis of the CYP2C19 genotype as follows: CYP2C19*1/*1 (extensive metabolizers, EMs), CYP2C19*1/*2 or CYP2C19*1/*3 (intermediate metabolizers, IMs), and CYP2C19*2/*2, CYP2C19*3/*3, or CYP2C19*2/*3 (poor metabolizers, PMs) (Itagaki et al., 2004). As for the CYP3A5 genotype, patients were allocated to 2 groups as follows: CYP3A5*1/*1 or CYP3A5*1/*3 (*1 carriers) and CYP3A5*3/*3 (*1 non-carriers).

**In vitro inhibition of CYP3A4/5-dependent tacrolimus metabolism by PPIs.**
To evaluate the inhibitory effects of omeprazole and lansoprazole on the metabolism of tacrolimus, we performed experiments in vitro using recombinant microsomes, as reported previously with slight modifications (Li et al., 2004; Dai et al., 2006).

In brief, omeprazole and lansoprazole were serially diluted with methanol to yield final concentrations ranging from 1 μM to 300 μM and 1 μM to 200 μM, respectively. The reaction was started with 50 μL of 1.5 mM NADP (Nacalai Tesque, Kyoto, Japan), and was stopped with 1 mL of 6.25% ZnSO₄. The reaction mixture consisted of 400 nM tacrolimus, 12 mM glucose-6-phosphate, 0.25 IU glucose 6-phosphate dehydrogenase, 6 mM MgCl₂, and 0.07 mg/mL microsomal protein for P450 (heterologous baculovirus-insect cell-expressed human CYP3A4 or human CYP3A5 in P450 reductase and cytochrome b₅) (BD Gentest, Woburn, MA, USA) in 100 mM potassium phosphate buffer (pH 7.4) with or without PPIs, in a final volume of 500 μL. The final methanol concentration in the incubation mixture was less than 1%, with minimal impact on enzymatic activity (Li et al., 2004). The incubation conditions for CYP3A4 and CYP3A5 activities were 5 min at 37°C. The concentration of 13-O-demethyl tacrolimus (M-I) (kindly gifted by Astellas Pharma Inc.), the primary metabolite, was quantified by liquid chromatography-tandem mass spectrometry (LC-MS/MS) method (Shimomura et al., 2008). IC₅₀ values were estimated using a
non-linear regression analysis of competition curves with one compartment, and the following equation:

\[ V = \frac{100 \times IC_{50}}{IC_{50} + [I]} + A \]

where \( V \) is production of M-I, which accounts for most of the metabolic clearance of tacrolimus (% of control), \([I]\) is the concentration of each PPI and \( A \) is the non-specific metabolism of tacrolimus (% of control).

**Statistical analysis.**

The C/D ratio of tacrolimus coadministred with PPIs were compared using the Mann-Whitney \( U \) test for two genotype groups, or the Kruskal–Wallis test, followed by the Dunn post-hoc test for multiple comparisons for more than two genotype groups. Data are expressed as the median and range or mean ± SD, depending on data type. For all analyses, two-tailed \( P<0.05 \) was considered statistically significant. All statistical analyses were conducted using GraphPad PRISM, version 4 (GraphPad Software, San Diego, CA, USA).
Results

Effects of intestinal as well as graft liver CYP2C19 genotypes on the interaction between tacrolimus and PPIs.

For the CYP2C19 genotype, *1, *2, and *3 alleles were found in 52.2%, 32.6%, and 15.2% in the graft liver, and 53.9%, 35.4%, and 10.7% in the native intestine, respectively. Then, the EMs (*1/*1), IMs (*1/*2 and *1/*3) and PMs (*2/*2, *2/*3, and *3/*3) of CYP2C19 was found in 28.1% (n=25), 48.3% (n=43), and 23.6% (n=21) in the graft liver, and 30.3% (n=27), 47.2% (n=42), and 22.5% (n=20) in the native intestine, respectively. For the CYP3A5 genotype, *1 and *3 alleles were found in 19.1% and 80.9% in the graft liver, and 20.8% and 79.2% in the native intestine, respectively. Then, the frequencies of CYP3A5*1 carriers (*1/*1 and *1/*3) and CYP3A5*1 non-carriers (*3/*3) were 33.7% (n=30) and 66.3% (n=59) in the graft liver, and 32.6% (n=29) and 67.4% (n=60) in the native intestine, respectively.

To investigate whether intestinal CYP2C19 polymorphisms affected the interaction between tacrolimus and PPIs, patients were divided on the basis of the CYP2C19 genotype of transplant recipients (Table 2). The C/D ratio of tacrolimus coadministered with omeprazole was significantly higher in patients with two variant alleles for intestinal CYP2C19 than those with the wild-type homozygote (CYP2C19*1/*1) or heterozygote (CYP2C19*1/*2 or CYP2C19*1/*3) (P=0.010). Similarly, patients with an engrafted
liver carrying two variant alleles for CYP2C19 showed significantly higher C/D ratio than the other groups ($P=0.022$). Furthermore, the distribution of CYP3A5*1 non-carriers did not vary between the different CYP2C19 genotype groups (Table 2).

In contrast, the C/D ratio of tacrolimus coadministered with lansoprazole was not associated with CYP2C19 polymorphisms in the native intestine ($P=0.52$) or the graft liver ($P=0.82$).

The tacrolimus C/D ratio between CYP2C19 EMs and IMs were found comparable (Table 2), so that the analyses were carried out between the EMs/IMs versus PMs. In patients receiving omeprazole, the C/D ratio of tacrolimus was significantly increased in PMs compared with EMs/IMs ($P=0.005$ for native intestine, $P=0.018$ for graft liver).

**Effects of intestinal as well as graft liver CYP3A5 genotypes on the interaction between tacrolimus and PPIs.**

Because we have reported that the CYP3A5 genotypes of both recipients and donors are important for the oral clearance of tacrolimus in liver transplant recipients (Goto et al., 2004; Fukudo et al., 2006; Uesugi et al., 2006; Fukudo et al., 2008; Hosohata et al., 2008), we examined the effects of CYP3A5 on the interaction between tacrolimus and PPIs (Table 3). In patients receiving omeprazole, the C/D ratio of tacrolimus was significantly higher in patients with an engrafted liver carrying the
CYP3A5*3/*3 genotype (*1 non-carriers) than the other group (*1 carriers) (P=0.034).

Similar trends were observed, although not statistically significant, for the intestinal CYP3A5 genotype (P=0.47). In patients receiving lansoprazole, *1 non-carriers conferred significantly higher C/D ratio of tacrolimus than *1 carriers (P=0.015 for native intestine, P=0.049 for graft liver).

Effects of the combination of intestinal and graft liver genotypes on the interaction between tacrolimus and PPIs.

As a feature of liver transplantation, the genotypes of recipients (native intestine) are different from those of donors (graft liver) in many cases. Focusing on this feature, we assessed the effects of the combination of intestinal and graft liver CYP2C19 genotypes on the interaction between tacrolimus and PPIs.

As shown in Fig. 1A, carriers of at least one CYP2C19 wild-type allele in both the native intestine and the graft liver (EMs/IMs for native intestine and graft liver) showed the lowest values for the tacrolimus C/D ratio (reference group). Compared with the reference group, carriers of at least one CYP2C19 wild-type allele either in the native intestine or in the graft liver showed almost the same values for the C/D ratio of tacrolimus. However, two CYP2C19 variant alleles both in the native intestine and in the graft liver (PMs for native intestine and graft liver) conferred a significantly higher
(6.9-fold) C/D ratio of tacrolimus than the other groups ($P=0.0032$). Furthermore, within CYP3A5*1 non-carriers both in the native intestine and in the graft liver (closed circles, $n=18$), the C/D ratio was significantly higher in those who also carried two CYP2C19 variant alleles than any other group ($P=0.017$), whilst there was no difference among the genotypes carrying at least one CYP2C19 wild-type allele either in the native intestine or in the graft liver. Conversely, the combination of intestinal and graft liver CYP2C19 genotypes little affected the C/D ratio of tacrolimus in patients receiving lansoprazole (Fig. 1B).

Experiments in vitro.

Next, we examined the inhibitory effects of PPIs on the metabolism of tacrolimus by CYP3A4 and CYP3A5 using recombinant microsomal preparations. Fig. 2 shows representative data among three separate experiments. The apparent inhibition constant (IC$_{50}$) values (mean ± SD) for omeprazole and lansoprazole were 51.9 ± 15.9 μM and 44.5 ± 18.0 μM for CYP3A4, and 53.7 ± 6.1 μM and 19.9 ± 13.8 μM for CYP3A5, respectively ($P>0.05$, omeprazole vs lansoprazole for CYP3A4 and CYP3A5).
Discussion

There has been growing recognition that intestinal as well as hepatic metabolism of orally administered drugs can play a significant role in the first-pass effects (Floren et al., 1997; Hebert, 1997). Despite evaluation of the expressions or catalytic activity of intestinal CYP2C19 (Obach et al., 2001; Lapple et al., 2003; Paine et al., 2006), no studies have assessed the effects of intestinal CYP2C19 on the oral clearance of drugs.

The interaction between tacrolimus and PPIs in CYP2C19 PMs are based on indirect effects of PPIs as CYP3A4/5 inhibitors because the main metabolic pathway of PPIs is CYP3A4/5 in CYP2C19 PMs. Furthermore, the direct effects of CYP2C19 on tacrolimus biotransformation can be negligible because tacrolimus never interacts with any CYP drug metabolism enzymes in addition to CYP3A4/5 (Lecointre et al., 2002).

Therefore, we focused on the indirect interaction between tacrolimus and PPIs via CYP3A4/5 in patients with the CYP2C19 polymorphisms, considering the CYP2C19 genotypes of the native intestine and the graft liver, separately. Our results revealed that LDLT patients with two CYP2C19 variants in the native intestine as well as graft liver seemed susceptible to the inhibitory effects of omeprazole, rather than lansoprazole, on the metabolism of tacrolimus (Table 2, Fig. 1). This varying degree of interaction of omeprazole and lansoprazole with tacrolimus in CYP2C19 PMs compared with EMs might be partly due to the different magnitudes of CYP2C19-mediated
metabolism among PPIs (Sakai et al., 2001). The contribution of CYP2C19 in the metabolism of omeprazole is greater than that of lansoprazole (Ishizaki and Horai, 1999). To the best of our knowledge, this is the first study indicating the pharmacokinetic significance of intestinal CYP2C19 in humans in vivo.

In liver transplantation, the genotypes of drug metabolism enzymes between recipients (native intestine) and donors (graft liver) are generally different. Thus, the different genotypes of intestinal and hepatic CYPs could regulate the clearance of tacrolimus. In the present study, only in cases where the recipients themselves and their corresponding donors had two variant alleles for CYP2C19, was the C/D ratio of tacrolimus coadministered with omeprazole significantly high (Fig. 1A). However, the extent of the increase was attenuated by carrying at least one CYP2C19 wild-type allele either in the native intestine or in the graft liver, indicating that intestinal and hepatic CYP2C19 could compensate for the functional loss caused by the CYP2C19 variants in the interaction between tacrolimus and omeprazole in LDLT patients. Considering the relatively high frequency of CYP2C19 PMs in the Japanese population (approximately 20%) (De Morais et al., 1994a), the interaction between tacrolimus and omeprazole is more relevant in Japanese than Caucasians. However, this study has clarified that there is a low probability of a strong tacrolimus-omeprazole interaction in liver transplant patients because of the extremely small concordance rate of PM genotypes of the
recipients with those of their corresponding donors.

Previously, we reported that intestinal CYP3A5 was significantly associated with the oral clearance of tacrolimus in liver transplant patients (Uesugi et al., 2006). In the present study, CYP3A5 also affected the interaction between tacrolimus and PPIs (Table 3), which is consistent with our experiments in vitro using recombinant microsomes, showing that omeprazole and lansoprazole inhibited the metabolism of tacrolimus via CYP3A4 as well as CYP3A5 (Fig. 2). Our findings suggest that intestinal as well as hepatic CYP3A5 is responsible for the interaction between tacrolimus and PPIs. However, even if patients had the CYP3A5*3/*3 genotype both in the native intestine and in the graft liver (Fig. 1A, closed circles), the C/D ratio of tacrolimus coadministered with omeprazole showed low values when carrying at least one CYP2C19 wild-type allele either in the native intestine or in the graft liver. These results suggest that the CYP2C19 have a greater effect on overall metabolism of omeprazole than that of CYP3A5. Therefore, in patients receiving omeprazole, carriers of two CYP2C19 variant alleles both in the native intestine and in the graft liver (PMs/PMs) showed the greatest increased C/D ratio of tacrolimus, and the variability in the metabolism of omeprazole caused by CYP2C19 polymorphisms is more likely to mask the effects of the CYP3A5 genotype on the metabolism of tacrolimus. Conversely, in patients receiving lansoprazole, there was no significant difference
among the CYP2C19 genotypes (Table 2), but the CYP3A5*1 non-carriers conferred a high tacrolimus C/D ratio than CYP3A5*1 carriers (Table 3). Our experiments in vitro showed that lansoprazole had a stronger inhibitory effect on the CYP3A5-mediated metabolism of tacrolimus than omeprazole, although not significantly (IC_{50}=19.9 ± 13.8 μM for lansoprazole, 53.7 ± 6.1 μM for omeprazole) (Fig. 2). The present results are in good agreement with the previous reports that the relative contribution of CYP2C19 against CYP3A4/5 in omeprazole is greater than that in lansoprazole (Ishizaki and Horai, 1999). In addition, our data is also consistent with lansoprazole being a poorer inhibitor of CYP3A4, as suggested by Li et al (2004).

The present study must be interpreted within the context of its potential limitations. First, we did not have control group (patients treated with tacrolimus not receiving PPIs). Second, medications including inducers or inhibitors of CYP3A4 were not strictly controlled in both transplant patients and their corresponding donors.

In conclusion, we first demonstrated that the CYP2C19 defective genotype in the native intestine affected the interaction between tacrolimus and omeplazole in LDLT patients, but the effect was attenuated by the wild-type genotype in the graft liver even when patients had the CYP3A5*3/*3 genotype in both the native intestine and the graft liver. On the other hand, CYP3A5 rather than CYP2C19 was associated with the
interaction between tacrolimus and lansoprazole in liver transplantation. The present findings suggest that the genotyping of \textit{CYP2C19} and \textit{CYP3A5} both in the native intestine and in the graft liver might contribute to safer dosing and monitoring of tacrolimus coadministered with omeprazole and lansoprazole early on after liver transplantation.
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Footnotes

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Legends for Figures

**Figure 1.** Effects of the combination of intestinal and graft liver CYP2C19 genotypes on the C/D ratio of tacrolimus coadministered with omeprazole (A) or lansoprazole (B). Patients were categorized based on the intestinal and graft liver CYP2C19 genotypes (EMs, CYP2C19*1/*1, *1/*2, and *1/*3; IMs, CYP2C19*1/*2 and *1/*3; PMs, CYP2C19*2/*2, *2/*3, and *3/*3). The closed circles indicate the CYP3A5*1 non-carriers (CYP3A5*3/*3) both in the native intestine and in the graft liver, and open circles indicate the CYP3A5*1 carriers (CYP3A5*1/*1 or *1/*3). Each bar indicates the median values. *P* values were determined by the Kruskal–Wallis test, followed by the Dunn post-hoc test for multiple comparisons.

**Figure 2.** Concentration-dependent inhibition of the formation of 13-O-demethyl tacrolimus (M-I) by omeprazole and lansoprazole for CYP3A4 (A) and CYP3A5 (B). Each symbol represents the mean ± SE for 3 independent experiments. The apparent inhibition constant (IC₅₀) values for omeprazole and lansoprazole were 51.9 ± 15.9 and 44.5 ± 18.0 µM for CYP3A4, and 53.7 ± 6.1 and 19.9 ± 13.8 µM for CYP3A5, respectively.
TABLE 1. Characteristics of patients (n=89)

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Data are expressed as number or mean ± SD.

aThe primary disease was Biliary atresia, Budd-Chiari syndrome, Nonalcoholic steatohepatitis, or Primary sclerosing cholangitis.
TABLE 2. Effects of intestinal and graft liver CYP2C19 genotypes on the C/D ratio of tacrolimus coadministered with omeprazole (n=35) or lansoprazole (n=54)

<table>
<thead>
<tr>
<th>PPI variables</th>
<th>CYP2C19 genotype (CYP3A5*1 non-carriers)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>EMs</td>
<td>IMs</td>
<td>PMs</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td>Native intestine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Omeprazole</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>11 (9)</td>
<td>15 (9)</td>
<td>9 (9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tacrolimus C/D ratio</td>
<td>2.11 (1.04-2.54)</td>
<td>2.11 (0.52-4.33)</td>
<td>6.38*† (1.55-22.9)</td>
<td>0.010</td>
<td></td>
</tr>
<tr>
<td>Graft liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>10 (6)</td>
<td>13 (8)</td>
<td>12 (7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tacrolimus C/D ratio</td>
<td>1.83 (1.04-6.38)</td>
<td>2.31 (0.52-7.10)</td>
<td>3.27* (1.9-22.9)</td>
<td>0.022</td>
<td></td>
</tr>
<tr>
<td>Lansoprazole</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Native intestine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>16 (11)</td>
<td>27 (21)</td>
<td>11 (4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tacrolimus C/D ratio</td>
<td>2.34 (1.16-12.8)</td>
<td>2.83 (0.72-13.4)</td>
<td>2.27 (0.98-11.7)</td>
<td>0.52</td>
<td></td>
</tr>
<tr>
<td>Graft liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>15 (9)</td>
<td>30 (22)</td>
<td>9 (7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tacrolimus C/D ratio</td>
<td>2.47 (1.13-10.6)</td>
<td>2.76 (1.10-12.8)</td>
<td>2.20 (0.72-13.4)</td>
<td>0.82</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as median (range). CYP3A5*1 non-carriers, CYP3A5*3/*3.

P-values are for the differences among the genotype groups, using the Kruskal–Wallis test, followed by the Dunn post-hoc test for multiple comparisons. *P<0.05, EMs vs. PMs; †P< 0.05, IMs vs. PMs.
**TABLE 3.** Effects of intestinal and graft liver CYP3A5 genotypes on the C/D ratio of tacrolimus coadministered with omeprazole (n=35) or lansoprazole (n=54)

<table>
<thead>
<tr>
<th>PPI variables</th>
<th>CYP3A5 genotype</th>
<th></th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>*1 carriers</td>
<td>*1 non-carriers</td>
<td></td>
</tr>
<tr>
<td>Omeprazole</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Native intestine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>11</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Tacrolimus C/D ratio</td>
<td>1.96 (1.04-7.10)</td>
<td>2.34 (0.52-22.9)</td>
<td>0.47</td>
</tr>
<tr>
<td>Graft liver</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>14</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Tacrolimus C/D ratio</td>
<td>1.93 (0.52-7.10)</td>
<td>2.54* (1.55-22.9)</td>
<td>0.034</td>
</tr>
<tr>
<td>Lansoprazole</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Native intestine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>18</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>Tacrolimus C/D ratio</td>
<td>1.97 (0.98-11.7)</td>
<td>3.85* (0.72-13.4)</td>
<td>0.015</td>
</tr>
<tr>
<td>Graft liver</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>16</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Tacrolimus C/D ratio</td>
<td>1.73 (0.72-11.1)</td>
<td>2.85* (0.98-113.4)</td>
<td>0.049</td>
</tr>
</tbody>
</table>

Data are expressed as median (range). *1 carriers, CYP3A5*1/*1 or *1/*3; *1 non-carriers, CYP3A5*3/*3.

*P < 0.05, *1 carriers vs. *1 non-carriers (Mann-Whitney U test).
Figure 1

A  Omeprazole

C/D ratio of tacrolimus [(ng/mL)/(mg/day)]

B  Lansoprazole

Intestinal CYP2C19

Graft liver CYP2C19

EMs/IMs (n=17)

EMs/IMs (n=9)

PMs (n=6)

PMs (n=3)

EMs/IMs (n=37)

EMs/IMs (n=6)

PMs (n=8)

PMs (n=3)

P=0.0032