Impact of Intestinal CYP2C19 Genotypes on the Interaction between Tacrolimus and Omeprazole, but Not Lansoprazole, in Adult Living-Donor Liver Transplant Patients

Keiko Hosohata, Satohiro Masuda, Toshiya Katsura, Yasutsugu Takada, Toshimi Kaido, Yasuhiro Ogura, Fumitaka Oike, Hiroto Egawa, Shinji Uemoto, and Ken-ichi Inui

Department of Pharmacy, Kyoto University Hospital (K.H., S.M., T.Kat., K.I.), and Department of Surgery, Graduate School of Medicine (Y.T., T.Kai., Y.O., F.O., H.E., S.U.), Kyoto University, Kyoto, Japan

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ABSTRACT:

To assess the effects of intestinal cytochrome P450 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 to 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 CYP2C19 (median, 6.38; range, 1.55–22.9) than intestinal wild-type

The immunosuppressant tacrolimus is characterized by a narrow therapeutic index and remarkable intra-individual 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 3A4 and CYP3A5 in the small intestine and liver (Shiraga et al., 1994; Hesselink et al., 2003). In particular, 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 (Haufroid et al., 2004, 2006; Macphee et al., 2005; 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 with 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. We have recently 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

ABBRVIATIONS: P450, cytochrome P450; PPI, proton pump inhibitor; PM, poor metabolizer; IM, intermediate metabolizer; EM, extensive metabolizer; LDLT, living-donor liver transplantation; C/D, concentration/dose; M-I, 13-O-demethyl tacrolimus.
were examined using recombinant microsomal preparations. Furthermore, the inhibitory effect of tacrolimus and PPIs in LDLT patients, considering the genotypes of the CYP2C19 genotypes in the small intestine on the interaction between tacrolimus and PPIs in liver transplant patients.

In the present study, we examined the impact of the CYP3A5 and 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 to 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 and 5-fluorouracil, 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 to 300 μM and 1 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% zinc sulfate. The reaction mixture consisted of 400 nM tacrolimus, 12 mM glucose 6-phosphate, 0.25 IU of glucose 6-phosphate dehydrogenase, 6 mM MgCl2, and 0.07 mg/ml microsomal protein for P450 (heterologous baculovirus-insect cell system). The concentrations for CYP3A4 and CYP3A5 activities were 5 min at 37°C. The concentration of 13-O-demethyl tacrolimus (M-I) (a gift from Astellas Pharma Inc., Tokyo, Japan), the primary metabolite, was quantified by liquid chromatography/tandem mass spectrometry method (Shinomura et al., 2008). IC50 values were estimated using a nonlinear regression analysis of competition curves with one compartment, and the following equation: V = (100 × IC50/[IC50 + [1]]) + A, where V is production of M-I, which accounts for most of the metabolic clearance of tacrolimus (% of control), [1] is the concentration of each PPI, and A is the nonspecific metabolism of tacrolimus (% of control).

**Statistical Analysis.** The C/D ratio of tacrolimus coadministered with PPIs was compared using the 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 ± S.D., depending on data type. For all the analyses, two-tailed P < 0.05 was considered statistically significant. All the statistical analyses were conducted using GraphPad Prism, version 4 (GraphPad Software Inc., San Diego, CA).

### Table 1

**Characteristics of patients (n = 89)**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Whole (n = 89)</th>
<th>Treatment with PPIs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Omeprazole (n = 35)</td>
</tr>
<tr>
<td>Age, years</td>
<td>51.9 ± 10.3</td>
<td>52.4 ± 8.8</td>
</tr>
<tr>
<td>Gender (male/female), n</td>
<td>46/43</td>
<td>19/16</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>60.0 ± 10.0</td>
<td>59.8 ± 10.2</td>
</tr>
<tr>
<td>Graft-to-recipient weight ratio, %</td>
<td>1.14 ± 0.30</td>
<td>1.15 ± 0.29</td>
</tr>
<tr>
<td>ABO blood group match (identical/compatible/incompatible), n</td>
<td>60/12/17</td>
<td>24/4/7</td>
</tr>
<tr>
<td>Primary disease, n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>70</td>
<td>30</td>
</tr>
<tr>
<td>Primary biliary cirrhosis</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>Fulminant hepatic failure</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Others</td>
<td>9*</td>
<td>2</td>
</tr>
<tr>
<td>Donor age, years</td>
<td>45.0 ± 13.2</td>
<td>46.0 ± 12.6</td>
</tr>
<tr>
<td>Donor gender (male/female), n</td>
<td>50/39</td>
<td>18/17</td>
</tr>
</tbody>
</table>

*The primary disease was biliary atresia, Budd-Chiari syndrome, nonalcoholic steatohepatitis, and primary sclerosing cholangitis.*

(Obach et al., 2001; Laplpe 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. Considering the genotypes of the poor metabolizer phenotype in Japanese subjects (De Moura 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 method (De Moura et al., 1994a,b). 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 three groups based on the CYP2C19 genotype as follows: CYP2C19*1/*1 (EMs), CYP2C19*1/*2 or CYP2C19*1/*3 (IMs), and CYP2C19*2/*2, CYP2C19*3/*3, or CYP2C19*2/*3 (PMs) (Igak et al., 2004). As for the CYP3A5 genotype, patients were allocated to two groups as follows: CYP3A5*1/*1 or CYP3A5*1/*3 (*1 carriers) and CYP3A5*3/*3 (*3 noncarriers).

**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 to 300 μM and 1 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% zinc sulfate. The reaction mixture consisted of 400 nM tacrolimus, 12 mM glucose 6-phosphate, 0.25 IU of glucose 6-phosphate dehydrogenase, 6 mM MgCl2, and 0.07 mg/ml microsomal protein for P450 (heterologous baculovirus-insect cell system) expressed in human CYP3A4 or human CYP3A5 in P450 reductase and cytochrome b5 (BD Gentest, Woburn, MA) 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 whole-blood trough concentration of tacrolimus was set at between 10 and 15 ng/ml day 1 (Yasuhara et al., 1995; Inomata et al., 1996). The target of the whole-blood trough concentrations measured approximately 12 h after the evening dosage every day using a semiautomated microparticle enzyme immunoassay (Ixm; Abbott, Tokyo, Japan) (Yasuha et al., 1995).

**Evaluation of Drug Interactions between Tacrolimus and PPIs.** Because the oral administration of PPIs started approximately 2 weeks after surgery, we evaluated data on postoperative days 22 to 28. The clinical course of all the patients enrolled in this study was stable. The average of dose-normalized blood concentration of tacrolimus during this observation period then 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 because of induction of the intestinal expression of CYP3A4 (Masuda et al., 2004).
**Results**

**Effects of Intestinal and Graft Liver CYP2C19 Genotypes on the Interaction between Tacrolimus and PPIs.** For the CYP2C19 genotype, *I*, *S*, and *S* 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. The EMs (*I/*I), IMs (*I/*S and *S/*S), and PMs (*S/*S) of CYP2C19 then were found in 28.1% (*I; n = 25), 48.3% (*I; n = 43), and 23.6% (*I; n = 21) in the graft liver and 30.3% (*I; n = 27), 47.2% (*I; n = 42), and 22.5% (*I; n = 20) in the native intestine, respectively. The CYP3A5 genotype, *I* and *S* 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 (*I/*I) and CYP3A5*1 noncarriers (*S/*S) 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 based on 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*2/*2) (P = 0.010). Likewise, 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 noncarriers 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 was 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 and 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, 2008; Uesugi et al., 2006; 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*1/*1 genotype (*I noncarriers) than the other group (*I carriers) (P = 0.034). Similar trends were observed, although not statistically significant, for the intestinal CYP3A5 genotype (P = 0.47). In patients receiving lansoprazole, *I* noncarriers conferred significantly higher C/D ratio of tacrolimus than *I* 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 both in the native intestine and in 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 noncarriers 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), whereas 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. Figure 2 shows representative data among three separate experiments. The apparent inhibition constant (IC<sub>50</sub>) values (mean ± S.D.) 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 (P > 0.05, omeprazole versus lansoprazole for CYP3A4 and CYP3A5).
pared with EMs might be partly because of the different magnitudes of CYP2C19-mediated metabolism among PPIs. 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 P450s could regulate the clearance of tacrolimus. In the present study, the C/D ratio of tacrolimus coadministered with omeprazole was significantly high only in cases in which the recipients were CYP2C19 wild-type allele either in the native intestine and in the graft liver, indicating that intestinal and graft liver CYP2C19 genotypes (EMs, CYP2C19*1/*1; IMs, CYP2C19*1/*2, and *1/*3; PMs, CYP2C19*2/*2, *2/*3, and *3/*3) both in the native intestine and in the graft liver could regulate the clearance of tacrolimus. In the present study, the C/D ratio of tacrolimus coadministered with omeprazole was significantly high only in cases in which the recipients themselves and their corresponding donors had two variant alleles for CYP2C19 (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 Caucasian populations. However, this study has clarified that there is a low probability of a strong tacrolimus-omeprazole interaction in liver transplant pa-

### Data are expressed as median (range).

* P < 0.05, *1 carriers versus *1 noncarriers (U test).

<table>
<thead>
<tr>
<th>PPI Variables</th>
<th>CYP3A5 Genotype</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>*1 Carriers</td>
</tr>
<tr>
<td><strong>Omeprazole</strong></td>
<td></td>
</tr>
<tr>
<td>Native intestine</td>
<td>n=11</td>
</tr>
<tr>
<td>Graft liver</td>
<td>n=14</td>
</tr>
<tr>
<td><strong>Lansoprazole</strong></td>
<td></td>
</tr>
<tr>
<td>Native intestine</td>
<td>n=18</td>
</tr>
<tr>
<td>Graft liver</td>
<td>n=16</td>
</tr>
</tbody>
</table>

**FIG. 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; IMs, CYP2C19*1/*2, and *1/*3; PMs, CYP2C19*2/*2, *2/*3, and *3/*3). The closed circles indicate the CYP3A5*1 carriers (CYP3A5*1/*1 or *1/*2), and open circles indicate the CYP3A5*3 carriers (CYP3A5*3/*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.
patients because of the extremely small concordance rate of PM genotypes of the recipients with those of their corresponding donors.

We previously 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 and CYP3A5 (Fig. 2). Our findings suggest that intestinal and 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 has 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 noncarriers conferred a higher tacrolimus C/D ratio than CYP3A5*/1 carriers (Table 3). Our experiments in vitro showed that lansoprazole had a stronger inhibitory effect on the CYP3A4-mediated metabolism of tacrolimus than omeprazole, although not significantly (IC50 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 are 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 a 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 showed that the CYP2C19 defective genotype in the native intestine affected the interaction between tacrolimus and omeprazole 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 CYP2C19 and 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.

References


Address correspondence to: Ken-ichi Inui, Department of Pharmacy, Kyoto University Hospital, Sakyo-ku, Kyoto 606-8507, Japan. E-mail: inui@kuhp.kyoto-u.ac.jp