The Proton Pump Inhibitor Lansoprazole, but not Rabeprazole, the Increased Blood Concentrations of Calcineurin Inhibitors in Japanese Patients with Connective Tissue Diseases

Kentaro Isoda, Tohru Takeuchi, Takuya Kotani, Suzue Hirano-Kuwata, Takeshi Shoda, Kenichiro Hata, Shuzo Yoshida, Shigeki Makino and Toshiaki Hanafusa

Abstract

Objective  Proton pump inhibitors (PPIs) are frequently coadministered with calcineurin inhibitors (CNIs) such as tacrolimus (TAC) and cyclosporin A (CSA), to treat or prevent upper gastrointestinal complications in Japanese patients with connective tissue diseases (CTDs). The coadministration of PPIs increases the blood concentration of TAC due to drug interaction. We retrospectively investigated the influence of the coadministration of PPIs and CNIs, as well as the influence of the cytochrome P450 (CYP) 2C19 gene polymorphism status, on the blood concentrations of TAC and CSA in patients with CTDs.

Methods  Patients treated with TAC (n=35) or CSA (n=30) were enrolled and divided into three groups according to the PPI they received: lansoprazole (LPZ)-combined, rabeprazole (RPZ)-combined, and non-PPI-combined groups. We compared the blood concentrations of TAC or CSA and the incidences of adverse events among the three groups. CYP2C19 gene polymorphisms were also assessed to investigate its influence on the blood concentration of TAC or CSA.

Results  LPZ significantly increased the blood concentration of TAC 12 hours after TAC administration (p=0.030 and p=0.003, respectively) and CSA (p=0.047 and p=0.014, respectively) in comparison with RPZ and non-PPI-combined treatment. There were no significant differences in the mean CSA blood concentration two hours after administration in patients with or without PPI treatment, in the incidence of adverse events, or in the CYP2C19 gene polymorphism status among the three groups.

Conclusion  Combining agents that are mainly metabolized by CYP3A4 such as LPZ elevates the blood concentrations of TAC and CSA, which could lead to adverse events.

Key words: proton pump inhibitors, calcineurin inhibitors, tacrolimus, cyclosporin A, connective tissue diseases

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Introduction

Calcineurin inhibitors (CNIs) such as tacrolimus (TAC) and cyclosporin A (CSA) are immunosuppressive drugs that inhibit nuclear factor-xB (NF-xB) activity and are used in organ transplantation and to treat connective tissue diseases (CTDs) (1-4). There are remarkable inter- and intra-individual variabilities in their pharmacokinetics of these drugs due to the influence of diet and drug interactions. CNIs are metabolized by cytochrome P450 (CYP) 3A4 in the liver and small intestine. Interactions among CYP3A4-metabolized drugs influences the clinical efficacies and adverse effects of CNIs.

Proton pump inhibitors (PPIs), such as lansoprazole (LPZ) and rabeprazole (RPZ), are empirically administered for the treatment or prevention of upper gastrointestinal complications, including reflux esophagitis and gastrointesti-
nal ulceration. Like CNIs, PPIs are metabolized by the CYP systems (3A4 and 2C19). The coadministration of PPIs increases the blood concentration of TAC in organ transplantation patients (5). To our knowledge, no study has previously investigated drug interactions between CSA and PPIs, but the pharmacokinetic manner of CSA may be similar to that of TAC.

We retrospectively investigated the influence of the coadministration of CNIs and PPIs on the CNI pharmacokinetics in Japanese patients with CTDs and evaluated the influence of PPI coadministration on clinical and laboratory features.

**Materials and Methods**

**Patient and clinical characteristics**

We included Japanese patients with CTDs who were admitted to Osaka Medical College Hospital between January 2008 and December 2011 and who had received TAC (Prograf®, Astellas Co., Ltd., Tokyo, Japan) or CSA (Neoral®, Novartis Pharmaceutical Co., Ltd., Basel, Switzerland). TAC and CSA were administered after dinner and before breakfast, respectively. The dose of CSA was adjusted so that the level at two hours after administration (C2) was 8000 ng/mL. We excluded patients receiving drugs other than PPIs that may modify the 3A4 and 2C19 CYP systems (3A4 and 2C19). The coadministration of PPIs increases the blood concentration of TAC in organ transplantation patients (5). To our knowledge, no study has previously investigated drug interactions between CSA and PPIs, but the pharmacokinetic manner of CSA may be similar to that of TAC.

We retrospectively investigated the influence of the coadministration of CNIs and PPIs on the CNI pharmacokinetics in Japanese patients with CTDs and evaluated the influence of PPI coadministration on clinical and laboratory features.

**Measurement of TAC and CSA blood concentrations**

The TAC blood concentrations were measured with the Dimension® clinical chemistry system (TAC-R Flex reagent cartridge, Siemens Healthcare Diagnostic Inc., New York, USA). Blood samples were collected 12 hours after the administration of TAC (C12). The concentration/dose ratio (C12/D) was calculated to minimize concentration dispersion.

The CSA blood concentrations were measured by a CSA monoclonal whole-blood assay (Cyclosporine-SP-Dynapack, Abbott Laboratories, Chicago, USA) using a fluorescence polarization immunoassay kit. The blood samples were collected before administration (trough level, C0) and at C2. The concentration/dose ratios (C0/D and C2/D) were calculated to minimize concentration dispersion.

**Adverse events related to CNIs**

We evaluated the adverse events related to TAC administration (i.e., kidney dysfunction and impaired glucose tolerance) based on the results of an early phase II study of TAC for rheumatoid arthritis and a phase III study of TAC for lupus nephritis (1, 2). The criteria for kidney dysfunction during the TAC administration period included either 1) a ≥0.3-mg/dL increase in serum creatinine (Cr) level in patients with an initial Cr value >0.5 mg/dL before TAC treatment or 2) a ≥0.2-mg/dL increase in Cr level in patients with a Cr level <0.5 mg/dL. The criteria for glucose intolerance during the TAC administration period included one of the following: 1) an ≥0.5% increase glycated hemoglobin (-Hb) A1c (National Glycohemoglobin Standardization Program: NGSP) level, 2) persistent elevation of fasting blood sugar level of ≥110 mg/dL, or 3) persistent elevation of HbA1c (NGSP) level ≥6.5%. We also evaluated the adverse events related to CSA administration (i.e., kidney dysfunction). CSA-related kidney dysfunction was regarded as ≥30% increase in Cr level within 24 weeks after the start of CSA administration.

**Genotyping for CYP2C19**

Genomic DNA was isolated from peripheral venous blood leukocytes with the NucleoSpin Blood L® kit (Takara Bio Inc., Shiga, Japan). CYP2C19 has the following alleles: wild-type, CYP2C19*1, CYP2C19*2 (G681A in exon 5), and CYP2C19*3 (G636A in exon 4). CYP2C19 genotyping was determined by polymerase chain reaction and restriction fragment length polymorphism methods using Msp 1 or Bam H1 as previously described (6-8). The study subjects were classified into one of three genotype groups as follows: extensive metabolizer (EM: CYP2C19*1/1), intermediate metabolizer (IM: CYP2C19*1/2 and ‘1/3), and poor metabolizer (PM: CYP2C19*2/2, ‘2/3, and ‘3/3).

**Statistical analysis**

Data are presented as the mean ± standard error of the mean (s.e.m.) and were analyzed with the statistical program JMP for the Windows, version 9.0 (SAS Institute Inc., Cary, USA). Differences in the clinical findings and adverse events were evaluated with Kruskal-Wallis tests and chi-square analyses. Pharmacokinetic parameters were calculated by polymerase chain reaction and restriction fragment length polymorphism methods using Msp 1 or Bam H1 as previously described (6-8). The study subjects were classified into one of three genotype groups as follows: extensive metabolizer (EM: CYP2C19*1/1), intermediate metabolizer (IM: CYP2C19*1/2 and ‘1/3), and poor metabolizer (PM: CYP2C19*2/2, ‘2/3, and ‘3/3).

**Results**

**Patient profiles**

The background characteristics of the 35 patients treated with TAC and the 30 patients with CSA are shown in Ta-
Table 1. Clinical Characteristics of Patient Treated with Tacrolimus

<table>
<thead>
<tr>
<th></th>
<th>TAC-ctrl</th>
<th>TAC+LPZ</th>
<th>TAC+RPZ</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>13</td>
<td>11</td>
<td>11</td>
<td>-</td>
</tr>
<tr>
<td>Diseases</td>
<td>RA 8, SLE 5</td>
<td>RA 6, SLE 3, SSc 1, SjS 1</td>
<td>RA 3, SLE 6, SSc 1, AOSD 1</td>
<td>0.358</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>54.2 ± 18.6</td>
<td>56.7 ± 19.7</td>
<td>49.8 ± 23.0</td>
<td>0.720</td>
</tr>
<tr>
<td>Female/Male</td>
<td>10/3</td>
<td>7/4</td>
<td>6/5</td>
<td>0.552</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>52.6 ± 6.9</td>
<td>52.1 ± 16.5</td>
<td>53.9 ± 9.2</td>
<td>0.667</td>
</tr>
<tr>
<td>CYP2C19 genotype</td>
<td>4/9</td>
<td>5/6</td>
<td>5/6</td>
<td>0.750</td>
</tr>
<tr>
<td>PDN dose (mg/day)</td>
<td>15.8 ± 20.0</td>
<td>12.3 ± 8.5</td>
<td>29.6 ± 15.2</td>
<td>0.038</td>
</tr>
<tr>
<td>TAC dose (mg/day)</td>
<td>2.2 ± 1.2</td>
<td>2.3 ± 0.9</td>
<td>2.6 ± 0.7</td>
<td>0.558</td>
</tr>
</tbody>
</table>


Data are presented as the mean ± standard deviation (SD) or the number of subjects.

Table 2. Clinical Characteristics of Patient Treated with Cyclosporin A

<table>
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<tr>
<th></th>
<th>CSA-ctrl</th>
<th>CSA+LPZ</th>
<th>CSA+RPZ</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>11</td>
<td>10</td>
<td>9</td>
<td>-</td>
</tr>
<tr>
<td>Diseases</td>
<td>DM 6, RA 1, SLE 2, SSc 1, IP 1</td>
<td>DM 6, SLE 1, SSc 1, AOSD 1, IP 1</td>
<td>DM 7, SLE 1, MCTD 1</td>
<td>1.000</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>49.4 ± 8.6</td>
<td>51.5 ± 17.4</td>
<td>49.7 ± 5.5</td>
<td>0.268</td>
</tr>
<tr>
<td>Female/Male</td>
<td>7/4</td>
<td>10/0</td>
<td>8/1</td>
<td>0.075</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>49.4 ± 8.6</td>
<td>52.3 ± 8.5</td>
<td>49.2 ± 7.0</td>
<td>0.692</td>
</tr>
<tr>
<td>CYP2C19 genotype</td>
<td>3/8</td>
<td>4/6</td>
<td>2/7</td>
<td>0.784</td>
</tr>
<tr>
<td>PDN dose (mg/day)</td>
<td>30.8 ± 13.3</td>
<td>27.9 ± 18.1</td>
<td>35.0 ± 15.1</td>
<td>0.555</td>
</tr>
<tr>
<td>CSA dose (mg/day)</td>
<td>182.3 ± 43.4</td>
<td>215.0 ± 41.2</td>
<td>197.2 ± 44.1</td>
<td>0.235</td>
</tr>
</tbody>
</table>


Data are presented as the mean ± standard deviation (SD) or the number of subjects.

Table 1 and Table 2, respectively. In the TAC+RPZ group, the mean dose of prednisolone was higher than that in the other groups (p=0.038). There were no significant differences in the other clinical features among the LPZ-, RPZ-, and non-PPIs-combined groups.

**Comparison of C12 and C12/D among the TAC+LPZ, TAC+RPZ, and TAC-ctrl groups**

The trough C12 level in TAC-treated patients is shown in Fig. 1A. The mean C12 values of the TAC+LPZ, TAC+RPZ, and TAC-ctrl groups were 7.9±4.6, 4.4±2.0, and 3.1±2.2 ng/mL, respectively. The C12 value in the TAC+LPZ group was significantly higher than that in the TAC+RPZ and TAC-ctrl groups (p=0.030 and p=0.003, respectively). There was no significant difference with regard to the C12 value between the TAC+RPZ and TAC-ctrl groups.

To minimize concentration dispersion, the C12/D values were also compared in the patients treated with TAC (Fig. 1B). The mean C12/D values of the TAC+LPZ, TAC+RPZ, and TAC-ctrl groups were 4.2±3.3, 1.7±0.9, and 1.6±1.1, respectively. In the TAC+LPZ group, the C12/D value was also significantly higher than that in the TAC+RPZ and TAC-ctrl groups (p=0.024 and p=0.013, respectively), whereas there was no significant difference between the TAC+RPZ and TAC-ctrl groups.

**Comparison of C0, C2, C0/D, and C2/D among the CSA+LPZ, CSA+RPZ, and CSA-ctrl groups**

We compared the mean C0 and C2 values of CSA in the patients with and without PPIs treatment (Fig. 2). The mean C0 values of the CSA+LPZ, CSA+RPZ, and CSA-ctrl groups were 193.2±74.6, 131.0±47.6, and 119.9±47.9 ng/mL, respectively. The C0 value in the CSA+LPZ group was significantly higher than that in the other two groups (p=0.047 and p=0.014, respectively). There were no significant differences in the mean C0 values of CSA between the CSA+RPZ and CSA-ctrl groups or the mean C2 values of CSA among the three groups.

To minimize concentration dispersion, the C0/D and C2/D values were also compared in CSA-treated patients (Fig. 3). The mean C0/D values of the CSA+LPZ, CSA+RPZ, and CSA-ctrl groups were 5.3±2.0, 2.3±1.0, and 1.7±0.9, respectively. In the CSA+LPZ group, the C0/D value was significantly higher than that in the CSA-ctrl group (p=0.045) and higher than that in the CSA+RPZ group, although not significantly (p=0.069). There was no significant difference between the CSA+RPZ and CSA-ctrl groups. The mean C2/D values of the CSA+LPZ, CSA+RPZ, and CSA-
among the three groups receiving CSA.

There were no significant differences in the incidences of kidney dysfunction or glucose intolerance among the three groups receiving TAC or in the incidence of kidney dysfunction among the three groups receiving CSA.

**Adverse events**

Adverse events associated with the TAC and CSA administration are shown in Table 3, 4, respectively. The mean observation periods of TAC or CSA administration were 21.1±12.3 and 12.2±11.0 months, respectively. There were no significant differences in the incidences of kidney dysfunction or glucose intolerance among the three groups receiving TAC or in the incidence of kidney dysfunction among the three groups receiving CSA.

**CYP2C19 genotyping**

The results of CYP2C19 gene polymorphism analyses of CSA among the three groups.

There were no significant differences in the mean C2/D values of CSA among the three groups.

**Adverse events**

Adverse events associated with the TAC and CSA administration are shown in Table 3, 4, respectively. The mean observation periods of TAC or CSA administration were 21.1±12.3 and 12.2±11.0 months, respectively. There were no significant differences in the incidences of kidney dysfunction or glucose intolerance among the three groups receiving TAC or in the incidence of kidney dysfunction among the three groups receiving CSA.

**CYP2C19 genotyping**

The results of CYP2C19 gene polymorphism analyses of CSA among the three groups.

There were no significant differences in the mean C2/D values of CSA among the three groups.

**Discussion**

We investigated the influence of coadministration of CNIs and PPIs on the CNI pharmacokinetics and the clinical features of Japanese patients with CTDs. Although there were no differences in the dosage of TAC or CSA in patients with or without PPIs, LPZ increased the C12 of TAC and the trough level of CSA. In contrast, RPZ had no influence on the blood concentrations of TAC or CSA. These TAC results are similar to those of previous renal transplantation studies (9). To the best of our knowledge, this is the first report to describe a drug interaction between CSA and PPIs.

The immunosuppressive effects of and incidence of adverse events with TAC correlate with the area under the curve (AUC) (10). The blood trough concentration (C0) of TAC correlates with the AUC and is used for monitoring re-
nal transplant recipients as an alternative to the AUC (11). Previous reports and the results of the present study showed that the C0 value of TAC significantly increased by the co-administration of LPZ in comparison with RPZ or no PPI (5, 12). TAC is administered twice a day in transplantation patients and the C0 of TAC corresponds to C12. Our study results are in accordance with those of previous studies. The incidence of adverse events related to TAC administration in CTDs patients, such as nephrotoxicity and glucose metabolism disturbances, increases significantly when the C12 value exceeds 10 ng/mL (2). In the present study, there were no differences in the incidence of adverse events between patients with or without PPIs. This may have been because the observation period was too short to evaluate the occurrence of adverse events. The two patients with C12 values of TAC exceeding 10 ng/mL received LPZ. Repeated therapeutic drug monitoring of TAC should be performed in patients treated with PPIs, especially those concomitantly taking LPZ.

The C2 value of CSA is used to monitor immunosuppressive effects (13, 14), whereas the C0 value of CSA is used to monitor adverse events (15). The incidence of adverse events increases when the C0 value of CSA exceeds 200 ng/mL. In this study, the C2 value of CSA was controlled at ≥800 ng/mL for the treatment of CTDs and was not different in patients with or without PPIs. However, coadministration of LPZ increased the C0 value of CSA to a greater degree than did either the coadministration of RPZ or non-coadministration. There were no differences in the incidence of adverse events in the three groups receiving CSA, possibly because of the short observation period. The longitudinal coadministration of LPZ may induce adverse events in comparison with that of RPZ or non-coadministration despite the equivalent immunosuppressive effect of CSA.

The differences of the influences of LPZ and RPZ on the CNI blood concentrations are related to differences in the main metabolic pathways of PPIs, the CYP system (5, 12, 16). LPZ is mainly metabolized by CYP2C19 and CYP3A4, whereas RPZ is mainly excreted via a non-enzymatic pathway in addition to the CYP system (16). Therefore, the coadministration of LPZ largely increases CNI blood concentration compared with that of RPZ through its interaction with CYP3A4.

Altered metabolic activity related to CYP2C19 gene polymorphisms status influences CNI metabolism by

![Figure 3. Comparison of C0/D and C2/D among the CSA+LPZ, CSA+RPZ, and CSA-ctrl groups. A: The mean C0/D values of CSA+LPZ, CSA+RPZ, and CSA-ctrl groups were 0.91±0.35, 0.66±0.16, and 0.65±0.17, respectively. The C0/D value in the CSA+LPZ group was significantly higher than that in the CSA-ctrl group (p=0.045) and higher than that in the CSA+RPZ group, although not significantly (p=0.069). There was no significant difference in the value between the CSA+RPZ and CSA-ctrl groups. B: There were no significant differences in the mean C2/D values of CSA among the three groups. CSA: cyclosporin A, ctrl: control, LPZ: lansoprazole, RPZ: rabeprazole, EM: extensive metabolizer, IM: intermediate metabolizer, PM: poor metabolizer](image)

### Table 3. Adverse Events with Tacrolimus

<table>
<thead>
<tr>
<th></th>
<th>TAC-ctrl (n = 13)</th>
<th>TAC+LPZ (n = 11)</th>
<th>TAC+RPZ (n = 11)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal dysfunction (%)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (9.1)</td>
<td>0.629</td>
</tr>
<tr>
<td>Glucose intolerance (%)</td>
<td>1 (7.2)</td>
<td>1 (9.1)</td>
<td>2 (18.2)</td>
<td>0.820</td>
</tr>
</tbody>
</table>

TAC: tacrolimus, ctrl: control, LPZ: lansoprazole, RPZ: rabeprazole

Data are presented as the number of subjects.

### Table 4. Adverse Events with Cyclosporin A

<table>
<thead>
<tr>
<th></th>
<th>CSA-ctrl (n = 11)</th>
<th>CSA+LPZ (n = 10)</th>
<th>CSA+RPZ (n = 9)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal dysfunction (%)</td>
<td>3 (27.2)</td>
<td>2 (20.0)</td>
<td>2 (22.2)</td>
<td>1.000</td>
</tr>
</tbody>
</table>

CSA: cyclosporin A, ctrl: control, LPZ: lansoprazole, RPZ: rabeprazole

Data are presented as the number of subjects.
CYP3A4 (12). Patients with IM and PM genotype exhibited elevated blood concentrations of PPIs, resulting in increased CNI concentrations. In the presence of LPZ, the influence of CYP2C19 polymorphisms on CNI blood concentration is reportedly more marked than that in the presence of RPZ (12). Our results confirm this effect, but our data did not show any influence of CYP2C19 gene mutations on the CNI concentrations. This may be due to either the small sample number included in this study or to differences in administration strategies of CNIs and PPIs between this study and others. Further studies in a larger number of patients are needed.

We investigated the influence of the coadministration of PPIs on the blood concentrations of TAC and CSA in Japanese patients with CTDs. Combining agents that are mainly metabolized by CYP3A4, such as LPZ, elevates the blood concentrations of TAC and CSA, which could increase the incidence of adverse events. In such cases, it is necessary to perform repeated therapeutic drug monitoring and/or switch to another regimen. The number of patients in the present study was small, and the observation period was short. In the future, a long-term follow-up should be conducted in a larger number of patients.

The authors state that they have no Conflict of Interest (COI).

Acknowledgement
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References