Regular Article

Larger Dosage Required for Everolimus than Sirolimus to Maintain Same Blood Concentration in Two Pancreatic Islet Transplant Patients with Tacrolimus

Eriko SATO1, Ikuko YANO1, Masahiro SHIMOMURA1, Satohiro MASUDA1, Toshiya KATSURA1, Shin-ichi MATSUMOTO2, Teru OKITSU2, Yasuhiro IWANAGA2, Shinji UEMOTO2 and Ken-ichi INUI1,*

1Department of Pharmacy, Kyoto University Hospital, Faculty of Medicine, Kyoto University, Kyoto, Japan
2Transplantation Unit, Kyoto University Hospital, Faculty of Medicine, Kyoto University, Kyoto, Japan
3Department of Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan

Summary: We attempted a switch of mammalian target of rapamycin (mTOR) inhibitors from sirolimus to everolimus, a derivative of sirolimus and now on the market in Japan, in two pancreatic islet transplant patients. Both patients were administered tacrolimus with sirolimus or everolimus. They had been administered 5 or 9 mg sirolimus once a day and had maintained a trough concentration of about 15 ng/mL as measured by high performance liquid chromatography with ultraviolet detection. After the switch from sirolimus to everolimus, they were given 10 or 12 mg/day of everolimus twice a day to maintain a trough concentration of 12-15 ng/mL as measured by a fluorescence polarization immunoassay (FPIA) method. Afterward, the blood concentrations of everolimus and sirolimus after the conversion were measured by high performance liquid chromatography with mass spectrometry and everolimus concentrations were found to be 5-10 ng/mL. These data show that a larger dosage is needed for everolimus than sirolimus to maintain the same trough blood concentration. Data obtained by the FPIA for everolimus should be carefully evaluated after switching from sirolimus to everolimus because of the cross-reactivity of the antibody with sirolimus.

Keywords: everolimus; sirolimus; tacrolimus; pancreatic islet transplantation

Introduction

Pancreatic islet transplantation is a critical treatment for type 1 diabetes when it is difficult to control blood glucose levels despite an optimal insulin regimen and less invasive than pancreatic transplantation. With the Edmonton protocol,1) results of pancreatic islet transplantation improved markedly. According to the Edmonton protocol, Kyoto University Hospital performed 17 transplantations from non-heart-beating donors for 9 patients as of the end of 2006. The first successful living-donor islet transplantation was carried out on January 19, 2005.2) The Edmonton protocol consists of high-dose sirolimus (rapamycin) and low-dose tacrolimus for immunosuppression.3) Sirolimus suppresses the proliferation of lymphocytes by blocking growth factor-driven signal transduction through the inhibition of mammalian target of rapamycin (mTOR).3) In Japan, however, sirolimus is not approved by the Japanese government as an immunosuppressant. Everolimus, a derivative of sirolimus, has a shorter elimination half-life than sirolimus,4–6) and is expected to achieve a steady-state more quickly and adjust blood concentrations more easily. Everolimus has already been approved as an immunosuppressant in Europe and in March 2007, was approved as an immunosuppressant for heart transplant patients in Japan. Hence, we conducted a switch of mTOR inhibitors from sirolimus to everolimus in pancreatic islet transplant patients. Generally, clinical studies on everolimus in organ transplant patients have been performed with the concomitant administration of cyclosporine and steroids. There are a few reports on everolimus using tacrolimus.
Since everolimus as well as cyclosporine and tacrolimus are metabolized by cytochrome P450 (CYP) 3A and also transported via P-glycoprotein,7–9 pharmacokinetic interactions may vary between everolimus and tacrolimus or cyclosporine.

Here, we report pharmacokinetic differences between sirolimus and everolimus in two pancreatic islet transplant patients concomitantly administered tacrolimus. The blood concentration of everolimus was measured by fluorescence polarization immunoassay (FPIA) method as well as high performance liquid chromatography with mass spectrometry (LC/MS).

**Methods**

**Ethics:** These studies were conducted in accordance with the Declaration of Helsinki and its amendments and were approved by the Kyoto University Graduate School and Faculty of Medicine Ethics Committee. Written informed consent was obtained from each patient.

**Monitoring of blood concentrations for immunosuppressants:** Whole blood concentrations of sirolimus (Rapamune®, Wyeth, Madison, NJ) were measured by high performance liquid chromatography with ultraviolet detection (HPLC-UV) as described previously.10 The whole blood concentration of everolimus (Certican®, Novartis Pharma AG, Basel, Switzerland) was determined by a FPIA (Innofluor® Certican® Assay, Seradyn, Inc., Indianapolis, IN) using a TDxFLx® analyzer (Abbott Japan Co. Ltd., Tokyo, Japan).

Remnant blood samples after measurement of everolimus by FPIA were stored at −80°C. Everolimus and sirolimus whole blood concentrations were determined by a liquid-liquid extraction procedure and analysis of the extract by LC/MS in selected ion monitoring mode using atmospheric pressure chemical ionization as an interface at the laboratory of Novartis Pharma S. A. S. (Rueil Malmaison, France). Assay quantification limits were 0.3 ng/mL for everolimus and 0.5 ng/mL for sirolimus.

**Cross-reactivity of sirolimus with the antibody for everolimus:** To evaluate the cross-reactivity of sirolimus with the antibody for everolimus used in the assay, sirolimus was spiked in control human whole blood and sirolimus concentration was measured using FPIA for everolimus. Sirolimus concentrations were prepared at 5, 10, 20 and 50 ng/mL and tested in triplicate.

**Time course study of everolimus in islet transplant patients:** On the day immediately before the discharge of each patient, a time course study of everolimus was conducted. Blood samples were collected just before and 1, 2, 4, and 8 hrs after the morning administration. Whole blood concentrations of everolimus were determined using LC/MS at the laboratory of Novartis.

**Results**

**Case report:** Patient 1, a 48-year-old Japanese woman, had been treated with sirolimus and tacrolimus (Prograf®, Astellas Pharma Inc., Tokyo, Japan) after islet transplantation, according to the Edmonton protocol.1) Thirty-six days after the transplantation, the mTOR inhibitor was converted. We called the day of conversion day 0. Both everolimus and sirolimus were administered on day 0 and only everolimus was administered after that. She kept taking tacrolimus as before (3–4 mg/day). Sirolimus was administered once a day. Everolimus and tacrolimus were administered twice daily. Blood sampling was performed once a day in the morning before the next administration of drugs. Before day 0, the whole blood concentration of sirolimus was quantified by HPLC-UV to adjust the trough concentration of sirolimus to 12–15 ng/mL. After day 0, the dosage of everolimus was adjusted to achieve a target trough blood concentration of 12–15 ng/mL as determined by FPIA. On day 0, the administration of everolimus was started at 4 mg/day, which was less than the dosage of sirolimus on day −1 (5 mg/day). Since the trough concentration of everolimus gradually decreased, the everolimus dosage was increased to 10 mg/day and the blood concentration reached the target level (Fig. 1, upper panel).

Patient 2, a 41-year-old Japanese woman, started the administration of everolimus 63 days after transplantation. Based on experience with patient 1, from the start, she was administered 12 mg/day of everolimus, this being greater than the dosage of sirolimus on day −1 (9 mg/day). As a result she did not experience a remarkable fall in the trough concentration of everolimus (Fig. 1, lower panel). During the switch from sirolimus to everolimus, she was concomitantly administered 4–6 mg/day of tacrolimus.

Neither patient showed remarkable change in tacrolimus trough concentration, which remained at 3–6 ng/mL, or had clinical complications during the study period. Neither patient was treated with potent inducers or inhibitors of CYP3A and P-glycoprotein.

**Pharmacokinetic analysis:** Whole blood concentrations of everolimus and sirolimus after the conversion were determined using LC/MS. After discontinuance of administration, sirolimus remained in the blood for several days (Fig. 1). The concentration of everolimus measured by FPIA was greater than that obtained by LC/MS, especially immediately after the conversion. To evaluate the cross-reactivity of sirolimus with the antibody for everolimus in the assay, we measured concentrations of sirolimus spiked in control human whole blood using FPIA for everolimus. As shown in Figure 2, the antibody for everolimus showed extensive cross-reactivity with sirolimus ([Detected as everolimus] = 1.43 + 0.47 × [Sirolimus concentration], \( r^2 = 0.992 \)).
Fig. 1. Trough blood concentrations of sirolimus measured by HPLC-UV (open circles) and LC-MS (open triangles) and those of everolimus measured by FPIA (closed circles) and LC-MS (closed triangles) are plotted for each patient. Dark and light shaded areas show daily dosages of sirolimus and everolimus, respectively.

Fig. 2. Sirolimus blood concentrations measured by the FPIA method for everolimus. Each point represents the mean ± SD (n = 3). The solid line shows the fitting line. The dotted line represents the line of identity (i.e., slope = 1).

Figure 3 shows the trough concentration per dose (C/D) ratio profiles of sirolimus and everolimus. C/D ratios of everolimus were calculated from concentrations determined by LC/MS and the dosage administered on the previous day. In patient 1, C/D ratios of sirolimus and everolimus were 3.26 ± 0.35 (ng/mL)/(mg/day) (mean ± standard deviation, n = 4) and 0.87 ± 0.12 (n = 22, except day 1), respectively. In patient 2, the ratios were 1.67 ± 0.03 (n = 3) and 0.52 ± 0.09 (n = 13, except day 1), respectively. In each patient, the C/D ratio of everolimus was approximately three times less that of sirolimus. C/D ratios of everolimus and sirolimus in patient 1 were twice those in patient 2.

We performed a time course study on everolimus. On day 23 for patient 1 and day 13 for patient 2. Everolimus concentration profiles measured by LC/MS are shown in Figure 4. Patient 1 was administered 4.5 mg everolimus and the peak concentration (17.1 ng/mL) was obtained at
2 h after the administration. Patient 2 was administered 7 mg everolimus and the peak concentration (31.8 ng/mL) was obtained at 1 h. The areas under the concentration-time curve from 0 to 8 h (AUC<sub>0–8</sub>) calculated by the trapezoidal method were 94 and 142 ng·h/mL in patient 1 and patient 2, respectively, while the concentrations at pre-dose and 8 h in patient 1 were nearly the same as those in patient 2, respectively.

Discussion

As shown in Figure 1, our patients were administered 8–14 mg/day of everolimus (with tacrolimus), and achieved trough concentrations of 5–10 ng/mL as measured by the LC/MS. Compared with other reports in which 1.5 or 3 mg/day of everolimus with cyclosporine were administered to renal transplant patients to maintain trough concentrations in a similar range,<sup>11,12</sup> our doses were quite large. We consider that this discrepancy mainly resulted from the difference in calcineurin inhibitor used, namely tacrolimus or cyclosporine. Everolimus as well as tacrolimus and cyclosporine are substrates of CYP3A and P-glycoprotein,<sup>7–9</sup> but lower blood concentrations of tacrolimus than cyclosporine in the clinical situation compared with each affinity value may have little influence on the pharmacokinetics of everolimus. Recently, Kovarik et al.<sup>13</sup> reported that the level of exposure to everolimus was 2.5 fold higher with cyclosporine than tacrolimus. It has been reported that average everolimus predose blood concentrations were significantly lower by 2.9 fold in the absence compared with the presence of cyclosporine.<sup>15</sup> The trough concentrations of sirolimus with cyclosporine are reported to be 1.42 times higher than those with tacrolimus.<sup>16</sup> Taking these findings into consideration, cyclosporine has a more profound effect on everolimus than sirolimus pharmacokinetics and our patients may need a considerably larger dosage of everolimus due to the lack of pharmacokinetic interaction with tacrolimus.

Interestingly, the C/D ratio of everolimus was three times smaller than that of sirolimus in the same patients (Fig. 3). Coadministration of inhibitors or inducers of CYP3A or P-glycoprotein would be expected to alter sirolimus or everolimus pharmacokinetics, but comedications in the two patients did not change during the study period. Hepatic impairment would decrease the oral clearance of sirolimus,<sup>17</sup> but neither patient had clinical complications such as hepatic dysfunction. Actually, the trough concentrations of tacrolimus, also metabolized by CYP3A and transported via P-glycoprotein, remained in a similar range during the conversion from sirolimus to everolimus in these patients. Therefore, we consider that a larger dosage is needed for everolimus than sirolimus to maintain the same trough blood concentration in the same patients with tacrolimus. As discussed in the previous paragraph, in the case of concomitant administration of cyclosporine, dosage of everolimus might not be so different from that of sirolimus, because of the more profound pharmacokinetic interaction of cyclosporine with everolimus compared to sirolimus. Pharmacokinetic differences between sirolimus and everolimus with cyclosporine in the same patient should be clarified in future study.

Everolimus has been reported to have a large inter-individual variability in the pharmacokinetics,<sup>16</sup> as also found in our cases. In the time course study, the trough concentrations of everolimus in patients 1 and 2 were similar and peak concentrations and AUC<sub>0–8</sub> in patient 2 were approximately twice those in patient 1 at dosage of 7 mg and 4.5 mg, respectively (Fig. 4). Apparent clearance of everolimus approximately estimated by the dose-normalized AUC<sub>0–8</sub> seems similar in these patients. In contrast, dose-normalized trough concentrations for everolimus and sirolimus were different as also shown in Figure 3. One possible reason for these findings is that the patients had different absorption profiles. In general, the recommended therapeutic range for everolimus is reported as a trough concentration of 3 to 8 ng/mL<sup>17</sup> and the clinical significance of AUC monitoring for everolimus remains to be elucidated.

FPIA is easy and convenient to determine whole blood concentrations of everolimus, but it is known to overestimate everolimus concentrations due to cross-reactivity of the antibody with metabolites of everolimus.<sup>18</sup> Actually, the everolimus concentration measured by FPIA was greater than that obtained by LC/MS over the study period (Fig. 1). This finding is consistent with a report using samples from renal transplant recipients.<sup>19</sup> In a recent report,<sup>20</sup> FPIA gave a positive bias of 1.2 ng/mL compared with HPLC-UV. The antibody for everolimus may cross-react with sirolimus because of the similarity in chemical structure between everolimus and sirolimus. Immediately after switching of the mTOR inhibitors, it was considered that few metabolites of everolimus were present in blood, but the values obtained were greater...
with FPIA than LC/MS (Fig. 1). We consider the difference between the two methods to be caused by cross-reactivity with sirolimus and clarified the cross-reactivity of sirolimus with the antibody used in FPIA for everolimus (Fig. 2), as consistent with recent reports. However, since the values measured by FPIA exceeded those by LC/MS immediately after the conversion (Fig. 1), we consider that metabolites of sirolimus may also cross-react with the antibody of FPIA. These results indicate that the values of everolimus by the FPIA method should be carefully evaluated especially when transplant patients are switched from sirolimus to everolimus.

In conclusion, we report two cases of changing mTOR inhibitors from sirolimus to everolimus with tacrolimus after pancreatic islet transplantation. Each patient needed a considerably larger dosage of everolimus compared to sirolimus to maintain the same trough blood concentrations, which may be explained by lack of pharmacokinetic interaction between tacrolimus and mTOR inhibitors. The concentrations of everolimus measured by FPIA were considerably greater than those by LC/MS. These findings should provide useful information regarding the replacement of sirolimus with everolimus in transplant patients.

References


