Erythroid Recovery Affects Tacrolimus Levels after Engraftment during Stem Cell Transplantation

Noriyasu Fukuoka,*a Osamu Imataki,a Hiroaki Tanaka,a Kumiko Tani,a Hiroaki Ohnishi,b and Hitoshi Houchi a

*Department of Pharmacy, Kagawa University Hospital; and b Division of Hematology, Department of Internal Medicine, Faculty of Medicine, Kagawa University; 1750–1 Ikenobe, Miki-cho, Kita-gun, Kagawa 761–0793, Japan.

Received December 7, 2011; accepted July 31, 2012

Tacrolimus is commonly used in stem-cell transplants (SCT) for prophylaxis of graft-versus-host disease and is continuously administered throughout transplantation. The dose of tacrolimus is frequently decreased to maintain a desired concentration during the recovery of hemocytes after engraftment. If parameters which affect tacrolimus clearance are identified, it is of clinical use to estimate concentrations and aid dosing. The objective of this study was to identify which hematologic parameters affect tacrolimus clearance. Seventeen consecutive Japanese patients with hematological malignancies who received allogeneic SCT between March 2004 and January 2007 were enrolled in this study. Their steady-state concentrations were routinely measured and standardized as the concentration/dose (C/D) ratio ((ng/mL)/(mg/kg/d)). Multivariate analysis was performed to identify which hemocyte parameters affected the C/D ratio. Of the 13 patients, gradual dose reduction was required to combat elevated tacrolimus concentrations. The mean post-engraftment C/D ratio was higher than the pre-engraftment C/D ratio in each patient. The mean C/D ratio for all patients after engraftment was 1.56-fold higher (p=0.00004, range: 1.04–3.03) than that before engraftment. The variation ratio was calculated by dividing the C/D ratio by that on the engraftment day. Multivariate analysis revealed that the reticulocyte (RET) level (×10^3 count/µL) was the sole parameter influencing this ratio, and both parameters were expressed as: Variation ratio=0.004×RET+1.0. RET recovery of patients could influence the C/D ratio and tacrolimus clearance was affected by recipient original red blood cells, but not that of transfused red blood cells.

Key words tacrolimus; concentration–dose ratio; reticulocyte; engraftment; clearance; allogeneic stem cell transplantation

Tacrolimus is a compound produced by Streptomyces tsukubaensis that has potent immunosuppressive properties.1) In stem cell transplants (SCT), tacrolimus is used for prophylaxis of graft-versus-host disease (GVHD) because it exerts stronger immunosuppression than cyclosporine by specifically suppressing T cell functions.2) Tacrolimus has a narrow therapeutic window, which is affected by inter-/intra-individual variability, and so regular monitoring of concentrations is essential for the management of transplant patients in order to achieve optimal efficacy and minimal toxicity.3,4) Although tacrolimus can be constantly infused to maintain a target concentration upon transplantation initiation, its concentration in whole blood; plasma and red blood cells (RBC), which are the major components of blood. It was speculated that the concentration measured in whole blood was affected by factors such as the reticulocyte (RET) level, RBC count, hemoglobin (Hb) level, and hematocrit (Hct) level. As the corpuscular blood component, including RBC and/or RET, recovers along with the clinical stage after transplantation, it is necessary to evaluate any associations between the tacrolimus concentration and these parameters before and after engraftment. The objective of this study was to establish which hemocyte level affected the pharmacokinetics of tacrolimus in SCT patients. The relationship between hemocytes and tacrolimus pharmacokinetics was investigated. Dosage tailoring of tacrolimus during transplantation was also examined.

MATERIALS AND METHODS

Patients Seventeen consecutive Japanese patients with hematological malignancies who received allogeneic SCT in our hospital between March 2004 and January 2007 were enrolled in this study. Informed written consent was obtained from eligible patients. The Institutional Review Board of our hospital approved this study. Patients harboring the following conditions were excluded; (1) clinically apparent liver damage identified with T. Bil. >1.3 mg/dL, (2) renal impairment emerging with Cr >1.3 mg/dL, and (3) patients treated with antifungal agents, such as voriconazole and itraconazole, that affect the concentration of tacrolimus.

Preparation Regimen All patients were treated with a conventional regimen (BU/CY; busulfan 4mg/d for 4d and...
cyclophosphamide 60 mg/kg/d for 2 d, or CY/TBI; cyclophosphamide 60 mg/kg/d for 2 d and 12 Gy total body irradiation) or a reduced-intensity regimen (RIST) (Flu/Mel; fludarabine and melphalan, at doses of 25 mg/m² for 5 d and 70 mg/m² for 2 d, respectively, or CdA/BU; cladribine and busulfan, at dose of 0.11 mg/kg for 6 d and 3.2 mg/kg for 2 d, respectively), and received tacrolimus for GVHD prophylaxis at 0.01 mg/kg from 1 d prior to transplantation. We routinely used 200 mg hydrocortisone as a preparation for stem cell transfusion on the transplantation day. The RIST regimen was administered to patients who were not candidates for conventional transplantation due to high age or organ dysfunction. Results of therapeutic drug monitoring were used to adjust the tacrolimus dose within several hours after the concentration had been measured. Patients with anemia were treated with irradiated red cell concentrates for blood cell transfusions in order to maintain a safe level of RBC (Hb >70 g/dL and Hct >20%).

Dosing Regimen  Patients initially received 24 h of continuous intravenous tacrolimus administration at 0.01 mg/kg, and the therapeutic window was set at 10 to 15 ng/mL. Diets of all patients were strictly controlled and consisted of well-cooked and bacteria-free foods, and grapefruit was prohibited.

Clinical Data Monitoring  Clinical laboratory data were examined three times a week during the study period. Laboratory data including RET level (count/µL), RBC (count/µL), Hb (g/dL), total protein (g/dL), albumin level (ALB) (g/dL), and Hct (%) were collected for data analysis.

Tacrolimus Assay  Blood samples were collected three times a week under steady-state conditions during continuous intravenous administration of tacrolimus. A steady-state was defined as when the tacrolimus dose had not changed for at least 48 h. It was estimated, based on the half-life of this drug (approximately 12 h), that the tacrolimus concentration reached almost 90% of the actual steady-state concentration within 48 h.

Tacrolimus concentrations were measured using an IMx Tacrolimus II analyzer (Abbott Diagnostics, Abbott Park, IL, U.S.A.) according to a micro particle enzyme immunoassay method (MEIA). The range of detection was from 1.5 to 30 ng/mL. Two hundred µL precipitation reagents were added to an equivalent whole blood sample to conduct hemolysis. After centrifugation at room temperature (9000 × g for 6 min), MEIA measured the amount of liberated tacrolimus in the supernatant as the total of free and bound tacrolimus. Concentrations were evaluated before allogeneic SCT until tacrolimus was orally administered (about 30 d after engraftment).

Statistical Analysis  Blood concentrations were normalized to the daily dose per body weight as the concentration/dose (C/D) ratio (ng/mL/mg/kg/d) to evaluate fluctuations in clearance and to remove the influence of variations in individual drug distributions.

A paired t-test or an analysis of variance was used to compare parameters and elucidate significant differences. Multivariate correlation analysis was conducted in order to identify factors that contributed to the C/D ratio with backward stepwise linear regression using SPSS®14.0 software for Windows®. All p-values were two-tailed, and $p < 0.05$ was considered to be significant.

RESULTS

Patients’ Characteristics  As summarized in Table 1,
disease status at transplant was first remission in 6 patients, second remission in 1 patient, first relapse in 4 patients, second relapse in 3 patients, remission failure in 2 patients, and complete cellular response in 1 patient, which included 7 standard-risk and 10 high-risk patients. Four patients underwent the conventional regimen and 13 patients received the RIST regimen, three of which were additionally treated with low-dose TBI (2 Gy) accompanied with the RIST conditioning regimen. All patients received RIST, three of which were low-dose TBI (2 Gy).

Pre-/Post-Engraftment Dose and C/D Ratio

C/D ratios were compared as mean values between pre- and post-engraftment. In each patient, C/D ratios were averaged in pre- and post-engraftment stages. In all patients, the mean C/D ratio was significantly higher (p=0.00004) after engraftment than that before engraftment (Fig. 1a). The mean of all patients’ C/D ratios in the post-engraftment stage was 1.56-fold higher than that before engraftment. In 13 patients, the dose was gradually decreased as the C/D ratio increased in order to maintain the desired concentration.

Figure 1b shows the time profiles of dose, concentration, and C/D ratio in patient 6 as a representative patient. No severe liver injury (total bilirubin >2.0 mg/mL) was observed in the clinical course of any patient during the study period.

Influence of Hemocytes on the Change in C/D Ratio

For multiple regression analysis, the following laboratory data were considered to contribute to the C/D ratio of tacrolimus: RET level, RBC level, Hct, Hb, total protein, and ALB. Transfusion volume was also included to these hematologic parameters as a contributing factor. Contributing factors, such as hemocyte level, that influenced the variation ratio are shown in Table 2. According to multivariate analysis, the factor that influenced this ratio was only the RET level (p=0.002).

RET levels were converted into logarithmic values (log
October 2012 1651

(RET level)) and compared as mean values between pre- and post-engraftment. In each patient, log(RET level) values were averaged in pre- and post-engraftment stages. In all patients, the mean log(RET level) was significantly higher \((p=0.0009)\) after engraftment than that before engraftment (Fig. 2).

To standardize the \(C/D\) ratio and to define a baseline on the engraftment day, changes in the \(C/D\) ratio were calculated by dividing the measured \(C/D\) ratio by that on the engraftment day for each patient and this value was evaluated as the variation ratio. These ratios were significantly increased one week after the engraftment day. The log(RET level) was also assessed as the variation ratio in the same way. RET level increased exponentially after engraftment in each patient (Fig. 3).

Furthermore, RET was significantly correlated with the variation ratio as shown by the following equation: variation ratio\(=0.004\times\text{RET}+1.0\) (Fig. 4).

**DISCUSSION**

Tacrolimus is primarily metabolized in the liver by CYP3A7 and eliminated through biliary excretion. Therefore, clinical events that impair hepatic function may reduce tacrolimus clearance and lead to an increased concentration of tacrolimus via diminished CYP3A4 activity. In fact, tacrolimus is a substrate for both CYP3A4 and CYP3A5. It has been reported that elevated levels of total bilirubin \((>2.0\ mg/dL)\) resulted in an impairment of hepatic clearance using population pharmacokinetic clearance estimation. However, we do not consider that the CYP3A4 activity of the study participants declined after engraftment in the present study because no such elevation in total bilirubin was observed in any patient. Severe acute GVHD (grades III to IV) was also reported to cause a moderate decrease in tacrolimus clearance. In the study patients, five of seventeen patients (29.4%) exhibited severe acute GVHD. This incidence of severe acute GVHD is similar to that found among Japanese patients undergoing SCT from HLA matched or 1-locus mismatched related donors. This indicates that our study population did not display a tendency to suffer from GVHD. Based on the assessment of clinical data throughout engraftment, no organ failure, which occasionally accompanies GVHD, was detected. Clinical symptoms of acute GVHD were observed after transplantation; however, these events occurred outside of the study period. Therefore, we believe that the present results were not influenced by GVHD.

In addition to elevated total bilirubin levels and GVHD, the effects of drug interactions should also be considered. SCT patients also receive multiple agents that are known inhibitors or inducers of CYP3A4 including anti-emetics, corticosteroids, antibiotics, and anti-fungals. All patients were treated with the same supportive care regimen and hydrocortisone was routinely used at 200 mg/d on the transplantation day. In addition, corticosteroid equivalent to 0–4400 mg prednisolone dose was taken around the engraftment day according to clinical conditions. Corticosteroids may have an induction effect on CYP3A4 expression and reduce tacrolimus concentrations in experimental settings. Though little data are available on the clinical impact of pharmacokinetic interactions between tacrolimus and corticosteroids, the present study may have been affected by concomitant corticosteroids. Corticosteroid
doses were varied in each patient, but they were constantly administered throughout the study period. As the influence on tacrolimus was constant and/or negligible, the results seem not to have been affected by corticosteroids.

The absence of CYP3A5 expression in approximately 60% of Japanese people is correlated to a genetic polymorphism (CYP3A5*3). Trough levels after tacrolimus administration and area under the concentration–time curve values were lower in patients who express CYP3A5*1/*1 and CYP3A5*1/*3 than those in CYP3A5*3/*3. These reports indicate that the C/D ratios of tacrolimus are correlated with the expression of the CYP3A5 genotype in transplanted patients and tacrolimus requires therapeutic drug monitoring. As the present study compared C/D ratios in the same patients, results were not affected by the CYP3A5 genotype.

Regarding Hct, there was a possibility of it confounding the MEIA data. MEIA tends to overestimate concentrations in samples with Hct <25%. As the present patients with anemia were managed by blood transfusions to maintain their level of Hct at >20%, it may be necessary to take Hct fluctuations into consideration when assessing C/D ratios. Hct levels were stable at 26.7±6.6% and 25.7±4.0% during pre- to post-engraftment, respectively, and we had a good correlation between concentrations and clinical conditions. In addition, concentrations were assessed as C/D ratios and were compared between pre- and post-stages as variation ratios. From above reasons, an overestimation of MEIA was assumed to be negligible.

Several clinical conditions can also affect drug concentrations, especially in patients with hematologic malignancies, and an unstable hemocyte status influences the results of studies such as ours; therefore, we speculated that the C/D ratio was affected by the levels of hemocytes such as RBC, RET, Hb, and Hct. In liver transplant patients, hepatic clearance varies as a function of post operative days, but the hepatic CYP system does not change much during SCT. Hepatic extraction to metabolize tacrolimus depends on parameters associated with hemocytes, such as RBC, ALB, and protein, which vary greatly with engraftment stages. Once tacrolimus has saturated plasma proteins such as ALB, a decrease in hepatic extraction could be reasonably explained by an increase in the C/D ratio. As the C/D ratio is indirectly affected by ALB but not by Hb or Hct, ALB has a negligible influence on liver extraction of the drug. Except for ALB, the major factor...
controlling the clearance of the drug is its binding properties with RBC, which are dependent on the RBC level. Since proteins in RBC form the major protein component of the blood, the tacrolimus concentration of whole blood may be significantly affected by parameters related to hemocytes. Both the biological and pharmacological properties of tacrolimus are crucial for these findings. First, tacrolimus in the blood is mainly retained with the erythrocyte fraction, which serves as a reservoir of this drug. Second, as tacrolimus is extremely lipophilic and also binds strongly to proteins with a molecular weight of 10–11 kDa, the binding of tacrolimus to erythrocytes prevents its metabolism. The RBC level shows a better correlation with the weight/amount of tacrolimus binding protein than Hct and Hb levels.

The results demonstrated that transfused RBC dose not affect clearance (Table 2). Therefore, the RBC level is small in the pre-engraftment stage and transfused RBC binds weakly to tacrolimus, unbound tacrolimus increases apparently and hepatic metabolic clearance increases, resulting in a lowered C/D ratio. The change of RET level was similar to that of C/D ratio in each patient (Figs. 1a, 2), and the time-dependent change of variation ratio in C/D was also similar to that in RET level (Fig. 3). Furthermore, the RET level is the sole parameter involved in tacrolimus clearance (Table 2). As RET is a good marker of erythroid recovery following transplantation, these observations are supported by the fact that hepatic metabolism of tacrolimus clinically depends on blood cell counts, and plasma clearance of tacrolimus in vivo is correlated with RBC binding capacity.

In other words, RET is the productive activity of the recipient “original” RBC, we speculate that the RBC would be expected to show a strong correlation with the amount of tacrolimus binding protein according to the turnover of RBC. Although RET has a little relevance to tacrolimus clearance because RET counts a few of RBC and they only act as a minor reservoir, RET immediately increases and acts as matured RBC which binds strongly to tacrolimus after engraftment. Therefore, recipient “original” RBC is accelerated to produce from increased RET and tacrolimus is also to be bound to the produced RBC. These are considered to result in the increase of C/D.

Nevertheless, the metabolic ratio of tacrolimus with the major metabolite of 13-O-desmethylate was not changed in either engraftment stages. We thus speculated that tacrolimus clearance was affected by recipient original RBC, but not that of transfused RBC. We concluded that “fresh RBC” recovery could influence the C/D ratio. These results show the inverse relationship between clearance and RET, where dosage was gradually decreased during the time course after engraftment to maintain the same concentration in a practical management (Fig. 1b). These findings suggest that the dose should be modified to decrease from one week after the engraftment. It may be helpful to estimate dosage adjustment based on our equation (Fig. 4).

The limitations of this study would be retrospective and the fact that our results were obtained from a limited number of patients with cytopenia and an unstable hemocyte status who were recovering following transplantation. This instability may reflect other factors influencing the tacrolimus concentration including unknown confounding factors. In addition to concentrations, the RET level also may be helpful for dose adjustments of tacrolimus throughout the care of transplantation patients. The tacrolimus concentration is often not monitored as frequently as it is in our hospital, and these findings may be used as reference criteria for clinical monitoring and for estimating of dose adjustments in hospitals in which monitoring is less frequent. Nevertheless, as the hemocyte status of transplant patients is unstable, the dose must be tailored according to the concentrations observed in each patient.

REFERENCES


