Differences in Peripheral Blood Lymphocytes between Brand-Name and Generic Tacrolimus Used in Stable Liver Transplant Recipients

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Significance of the Study

- In this study, peripheral blood lymphocytes were compared between stable liver transplant recipients receiving brand-name and generic tacrolimus. The level of CD4+Foxp3+ T cells was higher in the brand-name than in the generic tacrolimus group after transplantation. This study found that brand-name tacrolimus could have more potential immunosuppressive activity than generic tacrolimus regarding the contribution of CD4+Foxp3+ T cells to graft tolerance in liver transplant recipients.

Keywords

Liver transplantation · $\gamma\delta$ T cells · Regulatory T cells · Stable graft function · Living donors

Abstract

Objectives: In this study, peripheral blood lymphocytes were compared between a brand-name and a generic tacrolimus group in stable liver transplant recipients. **Subjects and Methods:** Sixteen patients who underwent ABO-compatible living donor liver transplants between 2012 and 2013 and had stable graft function were included in this study. Ten patients received brand-name tacrolimus and 6 patients received generic tacrolimus. CD3, CD4, CD8, $\gamma\delta$, CD4+FoxP3+, and CD3–CD56+ T cells were analyzed in peripheral blood obtained preoperatively and 4, 8, 12, and 24 weeks after liver transplantation. Categorical variables were compared using a χ^2 test or Fisher exact test, and continuous variables were

compared using the Mann-Whitney U test. **Results:** Regarding the baseline and perioperative characteristics, there were no statistically significant differences between the 2 groups. Immunosuppression also was not different. Subtype analysis of T-cell populations carried out in parallel showed similar levels of CD3, CD4, CD8, and $\gamma\delta$ T cells with brand-name tacrolimus and generic tacrolimus in stable liver transplant recipients. However, the levels of CD4+Foxp3+ and CD3–CD56+ T cells were higher in the brand-name tacrolimus group than in the generic tacrolimus group 8 weeks after transplantation ($p < 0.05$). **Conclusions:** The level of CD4+Foxp3+ T cells was higher in the brand-name tacrolimus group than in the generic tacrolimus group after transplantation. This finding showed that brand-name tacrolimus could have more potential immunosuppressive activity than generic tacrolimus regarding the contribution of CD4+Foxp3+ T cells to graft tolerance in liver transplant recipients.

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Introduction

Tacrolimus is one of the most commonly used medications for the prevention of liver allograft rejection. Tacrolimus acts as an immunomodulatory agent by blocking the transcription of the gene encoding interleukin-2 (IL-2) that is essential for T-cell-mediated immune response [1, 2].

Most countries have tried to reduce the cost of care for transplant patients, but the financial burdens of immunosuppressive therapy remain high [3]. This situation has led to the development and wide usage of generic tacrolimus, which has met all standards for bioequivalence and is therapeutically equivalent to brand-name tacrolimus [4, 5]. Many recipients had received generic tacrolimus after the expiration of the tacrolimus patent. Generic drugs offer significant cost savings for liver transplant programs and recipients; however, there has been considerable concern among transplant hepatologists and patients about the equivalence of the generic and brand-name forms [3, 6]. Given the lack of adverse events reported and the associated cost savings, conversion from brand-name to generic tacrolimus has been encouraged in organ transplantation [4].

$\alpha\beta$ T lymphocytes play a central role in several experimental models of allograft rejection and tolerance, acting as both effector and suppressor T cells [7]. Many studies have demonstrated that the T helper (Th)1/Th2 cell balance [8], natural killer (NK) cells [9], NKT cells [10], Foxp3⁺ regulatory T cells (Tregs) [11], and Th17 cells [12] are involved in graft function such as rejection or tolerance. Recently, $\gamma\delta$ T cells were also reported to be associated with liver allograft tolerance [13]. Human $\gamma\delta$ T cells are composed of 2 main subsets, such as V δ 1 and V δ 2, according to the rearranged V δ chain. V δ 1 $\gamma\delta$ T cells, which are predominant in the spleen, intestine, and liver, possess regulatory and effector properties, but V δ 2 $\gamma\delta$ T cells, which are the main subset of $\gamma\delta$ T cells in the peripheral blood, possess defense properties against pathogens [7]. Several studies have reported that a preponderance of V δ 1 $\gamma\delta$ T cells is associated with liver allograft acceptance [14–16].

Some studies have described the safety and efficacy of generic tacrolimus in liver transplant recipients [5, 17]. However, the analysis of peripheral lymphocytes in blood between patients treated with brand-name and generic tacrolimus has not been done. Hence, in this study, the peripheral blood lymphocytes in brand-name and generic tacrolimus were compared in stable liver transplant recipients.

Subjects and Methods

Patients

Sixty-four patients underwent ABO-compatible liver transplantation between 2012 and 2013. All patients were cytomegalovirus seropositive and received a right lobe graft from a living donor. All recipients received tacrolimus as a calcineurin inhibitor. The study was approved by the Samsung Medical Center's Institutional Review Board in Seoul. The exclusion criteria were recipients of cyclosporine, sirolimus, or everolimus, as well as patients with bile duct complications, cytomegalovirus infection, biopsy-proven acute rejection, hepatocellular carcinoma recurrence, graft failure, or who died. Sixteen patients were selected because they had stable graft function without complications. Ten patients received brand-name tacrolimus (Prograf®; Astellas Pharma, Tokyo, Japan), while 6 patients received generic tacrolimus (Tacrobell®; Chong Kun Dang Pharma, Seoul, Korea). One surgeon used brand-name tacrolimus and the other surgeon used generic tacrolimus because they prescribed their favorite medication. All demographic and clinical data were obtained from medical records. All patients were followed for the first 24 weeks after living donor liver transplantation (LDLT).

Immunosuppression

The immunosuppressive protocol of our center was used as described previously [18]. Basiliximab (20 mg) was used as an induction agent. All patients were infused with prostaglandin E₁, gabexate mesilate, and methylprednisolone. Maintenance immunosuppressive therapy consisted of corticosteroids, tacrolimus, and mycophenolate mofetil (MMF). Corticosteroids were withdrawn 3 months after transplantation.

Peripheral Blood Lymphocyte Subpopulations

Lymphocytes were analyzed in fresh whole blood samples obtained preoperatively and 4, 8, 12, and 24 weeks after liver transplantation. For lymphocyte subset analysis using multicolor flow cytometry, whole blood was incubated with various monoclonal antibodies specific for lineage CD markers according to the manufacturer's instructions [19]. All monoclonal antibodies were purchased from eBioscience (San Diego, CA, USA), BD Biosciences (Franklin Lakes, NJ, USA), or Thermo Fisher Scientific (Rockford, IL, USA). The cocktail for $\gamma\delta$ T cells consisted of CD3-PerCP Cy5.5 (SK7, eBioscience), CD4-APC-eFluor780 (RPA-T4, eBioscience), CD8-PE-Cy7 (SK1, eBioscience), TCR V delta 1-FITC (TS8.2, Thermo Fisher Scientific), and V δ 2 TCR-PE (B6, BD Biosciences). The cocktails for Tregs and NK cells were as follows: CD4-PerCP Cy5.5 (RPA-T4, eBioscience), CD25-APC (BC96, eBioscience), and FOXP3-PE (PCH101, eBioscience) for Tregs, and CD3-PerCP Cy5.5 (SK7, eBioscience), CD16-APC-H7 (3G8, BD Biosciences), and CD56-FITC (MEM188, eBioscience) for NK cells. Briefly, 100 μ L of whole blood containing different combinations of antibodies was incubated for 15 min at room temperature in the dark. This was followed by red blood cell lysis and washing in phosphate-buffered saline (PBS). Cells were reconstituted with PBS containing 0.5% albumin, and FACS acquisition for cell surface staining analysis was performed. For intracellular staining, surface stained cells were fixed and permeabilized with Fix/Perm reagent (eBioscience) and then incubated with anti-Foxp3 antibody. Various lymphocyte subsets were defined as follows: CD3⁺CD4⁺ for helper T cells,

Table 1. Recipient characteristics of the brand-name and generic tacrolimus groups in living donor liver transplantation

	Brand-name tacrolimus (<i>n</i> = 10)	Generic tacrolimus (<i>n</i> = 6)	<i>p</i> value
<i>Baseline</i>			
Sex (male)	9 (90%)	5 (83.3%)	0.696
Age, years	56 (44–68)	48 (40–67)	0.368
Diagnosis			
Alcoholic	0 (0%)	1 (16.7%)	
HBV	2 (20%)	1 (16.7%)	
HBV, HCC	8 (80%)	2 (33.3%)	
HCV, HCC	0 (0%)	1 (16.7%)	
NBNC	0 (0%)	1 (16.7%)	0.176
History			
Hypertension	1 (10%)	0 (0%)	0.424
Diabetes	2 (20%)	1 (16.7%)	0.869
Child-Pugh class			0.801
A	6 (60%)	3 (50%)	
B	2 (20%)	2 (33.3%)	
C	2 (20%)	1 (16.7%)	
MELD score	8 (7–42)	9 (7–19)	0.875
<i>Perioperative</i>			
Macrosteatosis, %	5 (1–5)	5 (5–10)	0.313
GRWR	0.96 (0.79–1.26)	1.25 (0.89–1.26)	0.127
Operative time, min	532 (424–725)	539 (441–604)	0.833
Cold ischemic time, min	76 (47–117)	95 (57–106)	0.366
Warm ischemic time, min	33 (20–50)	26 (14–55)	0.445
Intensive care unit stay after transplantation, n (days)	6 (4–7)	6 (5–7)	0.755
Hospitalization, n (days)	22 (21–28)	22 (17–24)	0.755
<i>Immunosuppression</i>			
Tacrolimus concentration at 3 months	5.5 (2.8–13.9)	5.3 (2.3–12.4)	0.788
MMF at 3 months	7 (70%)	4 (66.7%)	0.889
Steroids at 3 months	7 (70%)	5 (83.3%)	0.551

Values are presented as *n* (%) or medians (range). HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; NBNC, non-B non-C; MELD, Model for End-Stage Liver Disease; GRWR, graft-to-recipient weight ratio; MMF, mycophenolate mofetil.

CD3+CD8+ for cytotoxic T cells, CD3–CD56+CD16+ for NK cells, and TCR Vδ1 and Vδ2 for CD3+CD4–CD8– γδT cells. Cells were analyzed on a FACSCanto II using FACSDIVA software (BD Biosciences).

Statistical Analysis

Categorical variables were compared using a χ^2 test or Fisher exact test and were expressed as percentages. Continuous variables were compared using the Mann-Whitney U test and were expressed as medians and ranges. Repeated measures of lymphocyte populations after LDLT were analyzed using a mixed model. Absolute counts at each point after transplantation were compared using the Mann-Whitney U test with Bonferroni correction. Statistical significance was defined as $p < 0.05$. Data analysis was performed using SPSS 21.0 (IBM Corp., Armonk, NY, USA).

Results

Baseline Characteristics

The baseline characteristics of the patients are summarized in Table 1. There were no statistically significant differences in diagnosis, gender, age, history of hypertension, or diabetes, in Child-Pugh class, or in Model for End-Stage Liver Disease (MELD) scores between the 2 groups. Operative time, macrosteatosis, cold and warm ischemic time, graft-to-recipient weight ratio, stay in intensive care unit after LDLT, and hospitalization did not reach significance in the perioperative period. The proportions of MMF and steroids in the brand-name and generic tacrolimus groups at 3 months after LDLT were

Fig. 1. Changes in $\alpha\beta$ T cells after living donor liver transplantation: CD3+ T cells (a), CD4+ T cells (b), CD8+ T cells (c), and CD4/CD8 ratio (d).

Fig. 2. Changes in $\gamma\delta$ T cells after living donor liver transplantation: $\gamma\delta$ T cells (**a**), V δ 1 $\gamma\delta$ T cells (**b**), V δ 2 $\gamma\delta$ T cells (**c**), and V δ 1/V δ 2 ratio (**d**).

Fig. 3. Changes in CD3-CD56+ T cells (a) and CD4+FoxP3+ T cells (b) after living donor liver transplantation.

similar (70 vs. 66.7% for MMF and 70 vs. 83.3% for steroids, respectively). In addition, the concentration of tacrolimus in the brand-name and generic tacrolimus groups was 5.5 and 5.3 ng/dL, respectively ($p = 0.8$)

Peripheral Blood T-Cell Subsets between Brand-Name and Generic Tacrolimus

The absolute lymphocyte counts and absolute cell counts of CD3+ T cells after LDLT were increased after 8 weeks (Fig. 1). The absolute lymphocyte counts and absolute cell counts of CD3+ T cells in the brand-name tacrolimus group did not vary from those in the generic tacrolimus group. In subgroup analysis, absolute cell counts of CD4+ T cells in the brand-name tacrolimus group were generally higher than in the generic tacrolimus group after 8 weeks, but absolute cell counts of CD8+ T cells in the generic tacrolimus group were higher than in the brand-name tacrolimus group (Fig. 1). The CD4/CD8 ratio in the brand-name tacrolimus group was higher than in the generic tacrolimus group after LDLT.

Absolute cell counts of $\gamma\delta$ T cells in the brand-name tacrolimus group were higher than in the generic tacrolimus group (Fig. 2). Absolute cell counts of $\gamma\delta$ T cells in the generic tacrolimus group remained the same after LDLT,

but absolute cell counts of $\gamma\delta$ T cells in the brand-name tacrolimus group increased after 8 weeks. In subgroup analysis, absolute cell counts of V δ 1 and V δ 2 $\gamma\delta$ T cells in the brand-name tacrolimus group were increased after 8 weeks. However, V δ 1/V δ 2 ratio in the generic tacrolimus group was higher than in the brand-name tacrolimus group (Fig. 2).

Absolute cell counts of CD3-CD56+ T cells in the brand-name tacrolimus group were higher than in the generic tacrolimus group after 8 weeks. Similarly, absolute cell counts of CD4+Foxp3+ T cells in the brand-name tacrolimus group were higher than in the generic tacrolimus group after 8 weeks (Fig. 3).

Discussion

In this study, the levels of CD4+Foxp3+ and CD3-CD56+ T cells were higher in the brand-name tacrolimus group than in the generic tacrolimus group 8 weeks after

transplantation. These findings indicate that brand-name tacrolimus could have more potential as an immunosuppressant than generic tacrolimus because CD4+Foxp3+ T cells are related to graft tolerance. However, there were no statistically significant differences in CD3, CD4, CD8, or $\gamma\delta$ T cells between the brand-name and the generic tacrolimus groups.

Our study revealed that the median V δ 1/V δ 2 ratio in the generic tacrolimus group were more than 0.2 at each time, but median V δ 1/V δ 2 ratio in the brand-name tacrolimus group was below 0.2 at those times (Fig. 2). The present study revealed that the V δ 1/V δ 2 ratio in the generic tacrolimus group was a little higher than in the brand-name tacrolimus group, but there was no statistically significant difference between the 2 groups. Although there was no statistically significant difference between the 2 groups, the present study demonstrated that brand-name tacrolimus induced an increase in V δ 1 $\gamma\delta$ T cells after 8 weeks compared to the generic tacrolimus group. $\gamma\delta$ T cells possess immunoregulatory function; thus, increased number of circulating $\gamma\delta$ T cells were observed in patients with stable graft function [7, 14–16]. Tolerant liver recipients show an apparent alteration in the peripheral blood $\gamma\delta$ T cell subsets, thus exhibiting an elevation of V δ 1 $\gamma\delta$ T cells and a distinct increase of the V δ 1/V δ 2 ratio [14, 16, 20]. A recent study demonstrated an elevated V δ 1/V δ 2 ratio in tolerant pediatric semiallogeneic recipients as compared to maintenance immunosuppressive and chronic rejection recipients [21].

Our findings revealed that CD4+Foxp3+ T cells in the brand-name tacrolimus group were higher than in the generic tacrolimus group 8 and 12 weeks after transplantation. These observations suggest that brand-name tacrolimus might protect against liver allograft rejection and induce the effect of tolerance compared to generic tacrolimus. Tregs are a well-characterized T CD4+ cell subpopulation defined on the basis of constitutive expression of high levels of IL-2 receptor- α chain (CD25), cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), and forkhead/winged helix transcription factor P3 (FoxP3) [22, 23]. Tregs play a central role in the induction of immunological tolerance after organ transplantation [22, 23]. Currently, immunosuppressive agents used in clinical practice potentially affect Treg cell populations [22, 24].

Regulatory approval for generic drugs is dependent on the demonstration of bioequivalence [3]. However, the pharmacokinetic properties of tacrolimus are affected by a number of patient-related variables, such as gut function, concomitant medication, and liver function [3]. Although generic tacrolimus may lead to improved adher-

ence to immunosuppression and better health outcomes for transplant recipients, patients and health care providers should monitor tacrolimus trough levels more often if a recipient is switched to generic tacrolimus [3, 25].

The present study had several limitations that included (1) a small patient population that had selection bias and (2) the T effector cells were not analyzed. The T effector cell/Treg balance is crucial for the initiation and progression of the graft rejection response. Therefore, an ideal approach to promote tolerance could be to deplete or minimize alloreactive T effector cells. (3) The T-cell changes in the peripheral blood did not necessarily reflect their respective values in liver tissue. (4) Only absolute cell counts in the T cell population were analyzed. (5) There was a lack of any evidence of molecular-based data; therefore, the mechanism of the effect of T cells by brand-name or generic tacrolimus could not be proved.

Conclusions

In this study the CD3–CD56+ and CD4+Foxp3+ T cells in the brand-name tacrolimus group were higher than in the generic tacrolimus group in the peripheral blood T-cell populations in stable liver transplant recipients. This finding could indicate that brand-name tacrolimus might have more potential immunosuppressive activity than generic tacrolimus. Future studies monitoring the immune status of liver transplant recipients in relation to the type, number, and function of T cells are recommended to elucidate these issues in clinical liver transplantation.

Disclosure Statement

The authors declare no conflicts of interest.

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