의학 박사학위논문

신장이식 환자에서 세포내 면역억제제 농도 측정 기술 개발과 임상 적용

Monitoring the intracellular tacrolimus concentration and its clinical application in kidney transplant recipients

2016 년 8 월

서울대학교 의과대학
의과학과 의과학전공
한 승 석

신장이식 환자에서 세포내 면역억제제 농도 측정 기술 개발과 임상 적용

Monitoring the intracellular tacrolimus concentration and its clinical application in kidney transplant recipients

지도교수 김 연 수

이 논문을 의학 박사 학위논문으로 제출함

2016 년 7 월

서울대학교 의과대학

의과학과 의과학전공

한 승 석

한승석의 박사 학위논문을 인준함

2016 년 7 월

위 원 장	이 동 섭	(인)
부 위 원 장	김 연 수	(인)
위 원	박 수 길	(인)
위 원	조 주 연	(인)
위 원	최 무 림	(인)

Abstract

Monitoring the intracellular tacrolimus concentration and its clinical application in kidney transplant recipients

Seung Seok Han
Department of Biomedical Sciences
The Graduate School
Seoul National University

Although monitoring the intracellular concentration of immunosuppressive agents may be a promising approach to individualizing therapy in patients with organ transplantation, further studies on this issue are needed prior to its clinical application. We aimed to address the relationship between intracellular and whole blood concentrations of tacrolimus (IC-TAC and WB-TAC, respectively), the factors affecting this relationship, and the rejection risk by IC-TAC in kidney recipients with stable graft function. Both

IC-TAC and WB-TAC were measured simultaneously in 213 kidney recipients using liquid chromatography/tandem mass spectrometry. The tacrolimus ratio was defined as IC-TAC per WB-TAC. Analyses for genetic polymorphism in ATP binding cassette subfamily B member 1 (*ABCB1*) gene and flow cytometry of patients' peripheral blood mononuclear cells (PBMCs) were conducted to probe the relationship and the immunoreactivity as a potential risk of rejection, respectively. The relationship between IC-TAC and WB-TAC was relatively linear (r=0.67; P<0.001). The factors affecting the tacrolimus ratio were sex, hematocrit, and the transplant duration, as following characteristics: a high tacrolimus ratio was noted in female patients, patients with low hematocrit, and patients with short transplant period. However, the tacrolimus ratio did not correlate with the prior history of rejection or genetic polymorphism in *ABCB1*. After stimulation of PBMCs with phorbol-12-myristate 13-acetate and ionomycin, the proportion of T cells producing interferon-gamma or interleukin-2 was higher in low-IC-TAC group than in high-IC-TAC group. These results suggest that monitoring intracellular tacrolimus concentrations may be helpful in certain cases of kidney transplantation. Nevertheless, further studies are required to evaluate the value of IC-TAC in several clinical settings, such as rejection and drug toxicity.

.....

Keywords : intracellular concentration, immunosuppressive

agent, kidney transplantation, tacrolimus, acute rejection, peripheral blood mononuclear cell

Student Number : 2013-30619

Table of Contents

LIST of TABLES

List of Figures

Introduction

The annual rate of kidney transplantation has steadily increased due to its proven survival benefit over other treatment options [1]. Although graft rejection is a major complication to recipients, the introduction of new immunosuppressive agents has led to a large reduction in the rejection rate and has increased the long-term utility of grafts [2]. The overall rate of acute rejection is approximately 10% within 1 year under the current immunosuppressive regimen [3]. However, this rate is suboptimal and should be further reduced because acute rejection is directly related to the economic burden of kidney transplantation as well as the worse graft outcome [4,5].

Calcineurin inhibitor is a cornerstone immunosuppressive agent to maintain immune tolerance and prevent rejection episodes. Firstly, cyclosporine was discovered in the 1970s, and its entry into the collection of immunosuppressive agents in the early 1980s was a major breakthrough in the transplant field. In 1994, the Food and Drug Administration approved one more calcineurin inhibitor called tacrolimus in liver transplant recipients. Subsequently, tacrolimus has been approved in kidney (1997) and heart (2006) transplant recipients together. Tacrolimus and cyclosporine differ in the chemical structure as follows: cyclosporine is a cyclic endecapeptide, and tacrolimus is a macrocyclic lactone (Figure 1). Tacrolimus and cyclosporine act throughout binding to FK506-binding protein and cyclophylin, respectively, and inhibit the function of calcineurin enzyme.

Consequently, this process leads to the block of interleukin-2 (IL-2) production, which is essential for T cell activation.

Previous evidence has confirmed that tacrolimus is the preferred choice of treatment for increasing graft survival compared with other calcineurin inhibitors [6]. Furthermore, it is recommended that exposure to tacrolimus be reduced to a certain degree for the prevention of calcineurin inhibitor-induced nephrotoxicity [7]. Although there are global guidelines for the use of tacrolimus based on the rejection risk [8], detailed information about how to administer this drug to individual recipients under various circumstances has not been published. Furthermore, current evidence does not support the relationship between blood tacrolimus level and the subsequent risk of acute rejection [9], which means the optimal target concentration of blood tacrolimus has not been defined. To reduce both the rejection and adverse events triggered by tacrolimus, the establishment of personalized guidelines for the use of tacrolimus is an essential step.

To accomplish this objective, several pharmacokinetic and pharmacodynamic methods have been suggested. In current pharmacokinetic monitoring protocols, adjustment of the tacrolimus dose according to the whole blood level of tacrolimus is relatively simple and useful. However, this method alone is not enough to improve the patient's outcome due to inter- and intra-individual differences in physiologic responses. In particular, it cannot be assured that the tacrolimus concentration in whole blood is always linked to the tacrolimus concentration in lymphocytes, which are

tacrolimus targets. Importantly, compared with the blood tacrolimus concentration, the intracellular tacrolimus concentration may be more closely related to T cell responses against grafts [10]. However, data on the intracellular tacrolimus concentrations in kidney recipients are scarce. Moreover, previous studies in this area have focused only on the role of genetic polymorphisms with no regard for other factors [11].

To determine whether the measurements of intracellular tacrolimus concentration may be applicable to actual clinical practice, further studies on this issue are needed. Herein, we aimed to evaluate the relationship between whole blood and intracellular tacrolimus levels, the factors affecting this relationship, and the potential risk of rejection according to the intracellular level in kidney transplant recipients.

Methods

Participants

To the best of our knowledge, no studies have attempted to identify the factors affecting the balance between whole blood and intracellular tacrolimus levels in stable kidney recipients. One study revealed that genetic polymorphisms in the ATP-binding cassette subfamily B member 1 (*ABCB1*) were related to tacrolimus concentrations although no information was provided regarding the patients' graft status or multivariate results [11]. Based on this study result, we attempted to calculate the minimal sample size of the cohort. Using a two-sided 5% significance level, a power of 80%, an anticipated drop-out rate of 10%, and the minor allele frequency of rs1045642 (0.459), it was determined that a total of 210 patients was required. We screened patients prior to 4 weeks of tacrolimus measurement. The inclusion criteria were as follows: age ≥18 years, use of tacrolimus, stable graft function (change in serum creatinine of less than 0.3 mg/dL within 3 months), stable concentration of blood tacrolimus compared to the last concentration (i.e., there was no reason to alter the tacrolimus dose), and the ability and willingness to provide written informed consent. The exclusion criteria were as follows: multiple organ transplantation, change in the tacrolimus dose within 1 month of the inclusion assessment for any reason (e.g., rejection, infection, or decreased graft function), and the use of

medications known to interact with tacrolimus and change the tacrolimus level (e.g., anti-fungal and anti-viral agents) [12]. Overall, 220 patients provided written informed consent. However, before or at the time of tacrolimus measurement, 6 patients were dropped out [withdrawal of consent (n=4); change in graft function (n=1); and no follow-up (n=1)]. Accordingly, 214 patients were enrolled between January 2014 and February 2015.

Data collection

The clinical parameters recorded included the following: age; sex; donor source (living relative, living non-relative, or deceased); previous history of transplantation; diabetes mellitus; histories of delayed graft function, acute rejection, recurrent original disease, and calcineurin inhibitor-induced nephrotoxicity; and the duration of transplantation. Delayed graft function was defined as the need for dialysis within 1 week of transplantation. Other episodes (i.e., acute rejection, recurrence, and tacrolimus-induced nephrotoxicity) were defined as the occurrence of events within 6 months of transplantation. The patients used triple immunosuppressive agents such as prednisolone, mycophenolate mofetil, and tacrolimus. The target range of WB-TAC in stable kidney recipients of our institute was between 2 and 8 ng/mL, although seven patients' WB-TACs were higher than 8 ng/mL. However, their tacrolimus doses were not changed before and after the time of study enrollment because of stable graft functions confirmed. Serum hematocrit, lymphocyte

proportion, albumin, and creatinine were measured, and proteinuria was semi-quantitatively scored from negative to +4 with a dipstick test and defined as a score ≥+1. All of these baseline parameters were assessed when the tacrolimus level was examined.

The trough level of blood tacrolimus was measured using liquid chromatography/tandem mass spectrometry (LC-MS/MS) [Agilent 1260 Infinity Binary LC (Agilent Technologies, Santa Clara, CA, USA) and API 4000 QTRAP system (AB Sciex, Framingham, MA, USA)]. At the same time, the intracellular tacrolimus level was measured as follows. First, when the patients arrived at the center, peripheral blood mononuclear cells (PBMCs) were readily isolated from 8 mL of whole blood (collected in a heparinized tube) using a Ficoll gradient. The blood samples were centrifuged at 400×g for 40 minutes at 4°C. After collecting the PBMC layer, two wash steps were performed. Next, the viable cells were counted after excluding dead cells with trypan blue, using the automated cell counter (Invitrogen, Carlsbad, CA, USA). Cells were suspended in 1 mL of phosphate-buffered saline (PBS) and stored at -80°C until sample preparation for LC-MS/MS. All steps were conducted at 4°C to prevent the efflux of tacrolimus from the cells.

For LC-MS/MS sample preparation, 200 μL of suspended cells were mixed with 50 μL of internal standard (50 ng/mL ascomycin in 50% methanol) and 1 mL of methyl tertiary butyl ether. After vortexing and centrifugation (14,000 rpm, 5 minutes, 4°C), the organic solvent layer was dried using a nitrogen concentrator. The extract was

reconstituted in 75 μL of 50% methanol and 0.1% formic acid. After vortexing and centrifugation (14,000 rpm, 5 minutes, 4°C), 5 μL of the reconstituted sample was injected into the LC-MS/MS. The LC-MS/MS instrumentation is described in the appendix section. In addition to the whole blood and intracellular concentrations of tacrolimus (WB-TAC and IC-TAC, respectively), we defined the tacrolimus ratio as IC-TAC/WB-TAC (unit: $pg \cdot 10^{-6}$ cells/$ng \cdot mL^{-1}$) to identify the cases with unbalanced distribution between whole blood and intracellular compartments. The assay development and validation processes were conducted based on the previous study results [11,13].

Genotyping analysis

Genomic DNA was extracted from whole blood samples using the QIAamp DNA blood kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol. The determination of single nucleotide polymorphisms (SNPs) in *ABCB1* was performed with the SNaPshot Multiplex kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instruction. The analysis was carried out using GeneMapper software (version 4.0; Applied Biosystems, Foster City, CA, USA).

IFN-γ and IL-2 analysis

Baseline plasma levels of interferon-gamma (IFN-γ) and IL-2 were

measured to determine whether the patients were systemically stable using a kit for enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN, USA). To evaluate the changes in pharmacodynamic profiles caused by intracellular tacrolimus (i.e., immune reactivity), we performed flow cytometric assessment of IFN-ɣ and IL-2 in 39 patients. PBMCs were cultured at a density of 1×10^6 cells per 200 μL in 96-well round-bottom plates. The medium used was RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum and penicillin-streptomycin. Samples were activated with 50 ng/mL phorbol-12-myristate13-acetate and 1 μg/mL ionomycin (Sigma-Aldrich, St. Louis, MO, USA) for 6 hours at 37°C with 5% CO_2. To retain cytokines within the cells, the protein transport inhibitor (BD GolgiStop^{TM}; BD Biosciences, San Jose, CA, USA) was used beginning at 2 hours after activation. After stimulation, the cells were fixed and permeabilized with PBS and 0.5% Triton X-100. Subsequently, surface and intracellular staining of CD3-FITC, CD4-PE-Cyanine5, CD8-PE, and IFN-ɣ-APC (or IL-2-APC) (BD Biosciences, San Jose, CA, USA; eBioscience, San Diego, CA, USA) was performed. Isotype control and fluorescence minus one control tubes were included as appropriate.

The study protocol complies with the Declaration of Helsinki and received full approval from the institutional review boards at Seoul National University Hospital (H-1307-141-507).

Statistical analysis

All of the analyses and calculations were performed using SPSS (version 21.0; IBM, Armonk, NY, USA), STATA (version 12.0; StataCorp LP, College Station, TX, USA), and FlowJo (version 10.0.7; FlowJo LLC, Ashland, OR, USA). The data are presented as the mean ± standard deviation for continuous variables and as proportions for categorical variables. Based on variable distributions using histograms, the variables with non-normal distributions are expressed as medians (interquartile ranges). The comparisons were evaluated using the chi-square test for categorical variables, analysis of variance (ANOVA) for normally distributed continuous variables (least significant difference analysis between two groups), and the Kruskal-Wallis test for non-normally distributed continuous variables (Mann-Whitney U test between two groups). The correlation coefficient between normally distributed continuous variables was measured using Pearson's correlation test or the linear regression model. The fractional polynomial method was also applied to account for a possible nonlinear relationship [14]. Analysis of covariance (ANCOVA) was used to determine the most significant parameter among multiple variables based on the hypothesis that all relationships were linear. The difference between paired samples with non-normal distributions was evaluated using the Wilcoxon signed-rank test. A P value of less than 0.05 was considered significant.

Results

Baseline characteristics

A total of 214 patients were initially enrolled, but 1 patient had an IC-TAC less than the lower limit of quantification. Accordingly, 213 patients were finally analyzed. Table 1 shows the baseline characteristics of the study participants. All patients were of Asian descent. The WB-TAC, IC-TAC, and tacrolimus ratio were 4.6 ± 1.83 ng/mL, 43.4 ± 30.10 pg/10^6 cells, and 9.3 ± 4.25, respectively.

We analyzed three SNPs in the *ABCB1* gene (i.e., rs1045642, rs2032582, and rs1128503). Genotyping of *ABCB1* revealed that the major alleles of rs1045642, rs2032582, and rs1128503 were the C allele, G allele, and T allele, respectively, in the present cohort. The allele and genotype frequencies are described in Table 2. All of these SNPs were within Hardy-Weinberg equilibrium bounds (Ps>0.05).

We also measured the baseline plasma IFN-ɣ and IL-2 simultaneously, and we found that most of the patients had undetectable levels of both cytokines (Figure 2).

Relationship between whole blood and intracellular tacrolimus concentrations

Figure 3 shows a scatter plot illustrating the distribution of the WB-TAC and IC-TAC. Pearson's correlation coefficient (r) was 0.67 (P<0.001). When the non-linear method was applied (red line in the

figure), the relationship also seemed to be linear. Nevertheless, we wanted to focus on the cases outside this correlation (i.e., outlier points of Figure 3). Accordingly, we defined the tacrolimus ratio (IC-TAC/WB-TAC) and sought to determine which cases were outside of this linear relationship (e.g., which characteristics do the circled or squared cases in Figure 3 have?). Patients were divided into three groups based on the quintiles of the tacrolimus ratio (Table 1). As a result, several factors such as sex, hematocrit, and albumin, were associated with the tacrolimus ratio. Table 3 shows the relationships after considering the tacrolimus ratio as a continuous variable: sex, hematocrit, and transplant duration were significantly correlated to the tacrolimus ratio. The correlation coefficient and corresponding *P* value for hematocrit were -0.251 and <0.001, respectively. For transplant duration, the correlation coefficient and corresponding *P* value for hematocrit were 0.183 and 0.008, respectively. However, the SNPs in the *ABCB1* gene were not associated with the tacrolimus ratio except rs1045642 (Table 4). Furthermore, most of the SNPs were not associated with the WB-TAC or IC-TAC, except for rs2032582 (considering the T allele as the major allele), which was associated with the WB-TAC. Histories of graft rejection, recurrence, or calcineurin inhibitor-induced nephrotoxicity were also not related with the tacrolimus ratio, even though we set up the timeframe to <2 weeks. The multivariate model revealed that sex, hematocrit, and transplant duration were significant factors related to the tacrolimus ratio. However, other factors such as

albumin and SNPs in the *ABCB1* gene were not independent factors. Table 5 provides detailed data obtained with the multivariate model (e.g., F and P values). Additionally, Figure 4 presents the trends of WB-TAC, IC-TAC, and tacrolimus ratio in each timeframe of transplantation.

Additionally, we explored the baseline characteristics according to the quintiles of IC-TAC (Table 6). As a result, the transplant duration was the strongest factor affecting the IC-TAC levels. When multivariate analyses were performed (Table 7), the transplant duration and the lymphocyte proportion remained significant in the relationship with IC-TAC.

Subsequently, we assumed a potential non-linear relationship between these continuous parameters and the tacrolimus ratio (Figure 5). As a result, the relationship between hematocrit and the tacrolimus ratio appeared to be linear, but the relationship between transplant duration and the tacrolimus ratio appeared to be non-linear: the tacrolimus ratio decreased more abruptly in the early transplant period than in the later transplant period. With this in mind, we verified the change of tacrolimus ratio in 14 patients whose primary samples were obtained within 1 year of transplantation (Figure 6). After 1 year, secondary samples were obtained, and their tacrolimus ratios were significantly lower than those of the primary samples: 10.7 ± 3.30 in the primary samples vs. 8.4 ± 2.60 in the secondary samples; P=0.041.

Activation of T cells according to the intracellular tacrolimus concentration

We hypothesized that IC-TAC may have a clinical implication by predicting the immune reactivity as a potential risk of rejection [15]. With this in mind, we conducted flow cytometry of IFN-γ$^+$ and IL-2$^+$ T cells and compared the proportions of these cells with the IC-TAC tertiles (Figure 7). We found that the proportion of CD3$^+$CD4$^+$IFN-γ$^+$ T cells was higher in the low-IC-TAC group than in the high-IC-TAC group. Similarly, CD3$^+$CD4$^+$IL-2$^+$ and CD3$^+$CD8$^+$IL-2$^+$ T cells were more common in the low-IC-TAC group than in the high-IC-TAC group. In contrast, the production of IFN-γ by CD8$^+$ T cells was not associated with the IC-TAC. The overall trend in the WB-TAC was similar to that of the IC-TAC (Figure 8). However, the correlations with CD3$^+$CD8$^+$IFN-γ$^+$ and CD3$^+$CD8$^+$IL-2$^+$ T cells were not statistically significant for the WB-TAC.

Discussion

A personalized approach to the use of tacrolimus is essential for improving graft outcome and reducing adverse events induced by tacrolimus. Recent studies have focused on this issue; however, the role of the intracellular tacrolimus concentration has not been thoroughly investigated. In the present study, we obtained three novel findings. First, we found that the correlation between blood and intracellular tacrolimus concentrations was relatively linear if the patients were stable. Second, sex, hematocrit, and transplant duration had significant effects on the balance between blood and intracellular tacrolimus concentrations, whereas SNPs in the *ABCB1* gene did not. Third, the IC-TAC determined the degree of T cell activation as a potential risk of rejection. In particular, the latter finding suggests that intracellular tacrolimus concentration monitoring may be reserved for a particular group of patients with an unbalance between IC-TAC and WB-TAC.

A few studies have been performed to examine the correlation between the WB-TAC and IC-TAC in transplant recipients [11,16,17]. The data on liver transplantation (n=90) showed that the IC-TAC was not associated with the WB-TAC at 7 days [16]. Unlike the WB-TAC, the IC-TAC had a significant association with quantitative scores related to hepatic histology and clinical rejection. Based on the heart transplant data (28 samples from 24 patients) [17], the IC-TAC was not related to the WB-TAC, and the median duration of

transplantation was 1255 days (range: 65 days to 9556 days). The previous kidney transplant data (from 96 patients) also revealed that there was no correlation between the IC-TAC and WB-TAC at 7 days or at the steady state (detailed descriptions of the sample number in each timeframe were not provided, and there were no data on the timing of the steady state) [11]. In contrast to those studies, our data showed that the IC-TAC had a relatively linear relationship with the WB-TAC, although the relationship was not perfect. These conflicting results may be due to the fact that all of the previous studies had small or modest sample sizes and did not evaluate whether the patients were systemically stable. The examination of patients for only 7 days after transplantation is insufficient to guarantee that both the graft and patient are stable. We only included patients with stable graft functions and further confirmed that they had low levels of inflammatory cytokines. Nevertheless, we cannot suggest that the IC-TAC completely corresponded with the WB-TAC. As shown in the markers of Figure 3, our primary focus was outlier cases beyond the linear relationship. Accordingly, we attempted to identify the factors related to the balance between the two compartments (i.e., blood and lymphocyte) by defining the tacrolimus ratio.

Sex-dependent differences in the pharmacokinetics of tacrolimus have been reported [18]. These differences may be attributable to complex interactions among genetic, hormonal, and socio-economic factors [19]. However, the currently available information is limited to

the blood level, as studies have not been performed to examine the cellular level. We initially found that female patients had higher IC-TACs than male patients, even when their blood tacrolimus concentrations were the same, although we could not determine the mechanism underlying this result.

Additionally, we found that both the hematocrit and the transplant duration were negatively associated with the tacrolimus ratio. The hematocrit determines the amount of tacrolimus that exists in unbound form because of saturable binding to red blood cells [20]. With this in mind, a large unbound fraction of tacrolimus due to a low hematocrit can induce influx to the intracellular compartment and increase the tacrolimus ratio. Regarding transplant duration, the tacrolimus ratio in the early transplant period was significantly higher than that in the later period. Furthermore, the relationship appeared to be non-linear: changes in the tacrolimus ratio were observed primarily in the early transplant period. Our paired sample test for the subsequent samples (i.e., one year apart) verified this result. This finding indicates that the decrease in the IC-TAC would be largely non-proportional to the decrease in the WB-TAC during the early period. We cannot suggest a potential underlying mechanism; however, this finding has clinical implications, particularly when tacrolimus must be tapered. We revealed that the IC-TAC had a larger impact on the activation of T cells than the WB-TAC, and this information suggests that monitoring the WB-TAC and IC-TAC together, especially in the early period, can be helpful to prevent

rejection during the tapering period compared than monitoring the WB-TAC alone.

Tacrolimus is a substrate of *ABCB1*, which has an excretory function [21]. This protein is also expressed in lymphocytes and may play a role in viral resistance and in trafficking cytokines and enveloped viruses [22]. It is plausible that the expression or function of *ABCB1* affects the IC-TAC. However, in contrast to previous study results [10], we could not identify an independent association between any SNP in *ABCB1* and the tacrolimus ratio (or the IC-TAC). This result may be due to several factors. Primarily, the associations between SNPs of *ABCB1* and molecular or clinical phenotypes have been largely inconsistent across several studies [23], and SNPs do not necessarily affect the expression or function of the *ABCB1* gene. Furthermore, various external stresses, such as recipient and donor factors, could alter the expression or function of *ABCB1*. Additionally, previous studies focused mainly on short-term periods and did not adjust for other covariates such as sex, hematocrit, and transplant duration [11]. Compared with the previous studies, the present study considered many factors on this issue.

Although the present results are informative, this study has a limitation. The cross-sectional study design did not provide information on the clinical outcomes (e.g., rejection) according to the IC-TAC, and thus the clinical implication of IC-TAC may not be supported by our results alone. However, the present study measured patients' T cell activation as a marker of their immune status,

because the monitoring of immune reactivity is known to be related with the risk of rejection [15]. Accordingly, IC-TAC was well correlated with the degree of T cell reactivity. For the WB-TAC, the correlation was also consistent, but modest compared with IC-TAC. Nevertheless, the current study design restricted further exploration of the significance of IC-TAC because each patient had a different transplant timeframe. To solve these problems, studies including patients with the same transplant period are recommended. Additionally, we did not measure T cell responses according to the T cell subtype. It has been revealed that the expression and activity of *ABCB1* are different between T cell subtypes [10], and thus, IC-TAC and its corresponding results (e.g., IFN-γ and IL-2 production) may change depending on the subtypes. Lastly, we focused on ABCB1 in terms of genetic polymorphism, but did not examine other potential factors, such as cytochrome P450 [24], which may contribute to the tacrolimus ratio.

Appropriate use of tacrolimus in kidney transplant recipients is essential because it can reduce the current risks of rejection and adverse events triggered by tacrolimus. We may need information about the intracellular tacrolimus concentrations in particular cases affecting the balance of tacrolimus between the blood and intracellular compartments. Nevertheless, the present results addressed this issue only in the patients with stable graft functions. Accordingly, future studies will determine the issues, such as values in the rejection state or other unstable conditions before a clinical application of

intracellular tacrolimus monitoring could become available.

Table 1. Baseline characteristics of the study participants according to the tacrolimus ratio levels

	Total	Tacrolimus ratio (IC-TAC/WB-TAC)		
		1st quintile	2nd - 4th quintile	5th quintile
	(n=213)	(n=42)	(n=129)	(n=42)
Tacrolimus ratio	9.3 ± 4.25	5.1 ± 0.69‡	8.6 ± 1.61	15.7 ± 4.88‡
Age (years)	47.1 ± 13.27	45.9 ± 13.18	47.9 ± 13.54	45.7 ± 13.27
Male sex (%)	59.2	78.6*	60.5	35.7†
Donor source (%)				
Living related donor	41.3	28.6	42.6	50.0
Living unrelated donor	19.7	23.8	21.7	9.5
Deceased donor	39.0	47.6	35.7	40.5
Previous history of transplantation (%)	6.1	9.5	3.9	9.5
Diabetes mellitus (%)	16.9	19.0	19.4	7.1
Combined immunosuppressive agents				
Prednisolone (mg/day)	4.2 ± 1.57	4.3 ± 1.53	4.1 ± 1.63	4.5 ± 1.41
Mycophenolate mofetil (mg/day)	692.6 ± 464.84	701.9 ± 422.58	727.0 ± 472.02	577.6 ± 475.17
Blood findings				
Hematocrit (%)	40.9 ± 5.33	42.2 ± 6.72	41.1 ± 4.77	38.9 ± 4.97*
Lymphocyte (%)	29.8 ± 8.16	30.2 ± 7.42	30.0 ± 8.49	28.8 ± 7.96
Albumin (g/dL)	4.4 ± 0.30	4.5 ± 0.27*	4.3 ± 0.30	4.4 ± 0.32
Creatinine (mg/dL)	1.3 ± 0.45	1.27 ± 0.56	1.26 ± 0.39	1.25 ± 0.51
Proteinuria (%)	24.9	26.2	26.4	19.0
Delayed graft function (%)	3.8	7.1	3.9	0

Acute rejection (%)	15.6	9.5	18.0	14.3	0.410
Recurrence (%)	2.4	2.4	2.3	2.4	1.000
CIN (%)	7.5	4.8	8.5	7.1	0.720
Transplant duration (months)	58 (32 - 88)	63 (36 - 83)	59 (32 - 93)	50 (16 - 71)	0.140

Comparisons were evaluated using the chi-squared test for categorical variables, ANOVA for normally distributed continuous variables (LSD post hoc analysis between two groups), and the Kruskal-Wallis test for non-normally distributed continuous variables (Mann-Whitney U test between two groups). The 2nd to 4th quintile group served as a reference for comparison between two groups.

*P<0.05; † P<0.01; ‡ P<0.001.

IC-TAC, intracellular concentration of tacrolimus; WB-TAC, whole blood concentration of tacrolimus; CIN, calcineurin inhibitor-induced nephrotoxicity.

Table 2. SNP frequencies in kidney transplant recipients

SNP position	Allele frequency		Genotype frequency		*P*
	Allele	n (%)	Genotype	n (%)	
rs1045642	C	280 (65.7)	C/C	89 (41.8)	0.656
	T	146 (34.3)	C/T	102 (47.9)	
			T/T	22 (10.3)	
rs2032582	G	214 (50.2)	G/G	46 (21.6)	0.105
	A+T	212 (49.8)	G/A+T	122 (57.3)	
			A+T/A+T	45 (21.1)	
	A	70 (16.4)	A/A	4 (1.9)	0.683
	G+T	356 (83.6)	A/G+T	62 (29.1)	
			G+T/G+T	147 (69.0)	
	T	142 (33.3)	T/T	20 (9.4)	0.528
	A+G	284 (66.7)	T/A+G	102 (47.9)	
			A+G/A+G	91 (42.7)	
rs1128503	C	186 (43.7)	C/C	39 (18.3)	0.905
	T	240 (56.3)	C/T	108 (50.7)	
			T/T	66 (31.0)	

SNP, single nucleotide polymorphism.

Table 3. Baseline parameters associated with the ratio of intracellular tacrolimus level to blood tacrolimus level

	Tacrolimus ratio (IC-TAC/WB-TAC)	Correlation coefficient	*P*
Age (years)[1]		-0.073	0.288
Sex[2]			
Male	8.3 ± 3.10		Reference
Female	10.7 ± 5.21		<0.001
Donor type[2]			
Living related donor	9.9 ± 4.61		Reference
Living unrelated donor	8.5 ± 2.81		0.083
Deceased donor	9.1 ± 4.41		0.216
Previous history of transplantation[2]			
No	9.3 ± 4.29		Reference
Yes	9.0 ± 3.75		0.757
Diabetes mellitus[2]			
No	9.5 ± 4.49		Reference
Yes	8.4 ± 2.69		0.171
Immunosuppressive agents[1]			
Prednisolone (mg/day)		0.015	0.829
Mycophenolate mofetil (mg/day)		-0.070	0.311
Blood findings[1]			
Hematocrit (%)		-0.251	<0.001
Lymphocyte (%)		-0.095	0.169
Albumin (g/dL)		-0.118	0.087
Creatinine (mg/dL)		-0.015	0.830
Proteinuria[2]			
No	9.2 ± 3.86		Reference
Yes	9.6 ± 5.30		0.587
Delayed graft function[2]			

No	9.2 ± 4.28	Reference
Yes	9.7 ± 4.16	0.539
Recurrence[2]		
No	9.3 ± 4.24	Reference
Yes	10.0 ± 5.66	0.729
CIN[2]		
No	9.3 ± 4.34	Reference
Yes	9.2 ± 3.01	0.891
Transplant duration (years)[2]		
≤1	11.2 ± 3.57	Reference
1 - 2	10.4 ± 3.27	0.583
2 - 5	8.9 ± 3.75	0.031
>5	9.1 ± 4.74	0.039

[1]Pearson's correlation was used.

[2]Student's t-test or post-hoc analysis (LSD) of ANOVA was used.

IC-TAC, intracellular concentration of tacrolimus; WB-TAC, whole blood concentration of tacrolimus; CIN, calcineurin inhibitor-induced nephrotoxicity.

Table 4. Association between SNP of *ABCB1* and tacrolimus concentration

SNP position	Genotype	WB-TAC Concentration (ng/mL)	P	IC-TAC Concentration (pg/10^6 cells)	*P*	IC-TAC/WB-TAC Ratio (pg.10^{-6} cells/ng.mL^{-1})
rs1045642	C/C[1]	4.5 ± 1.82	0.464	42.1 ± 27.04	0.345	8.9 ± 3.39
	C/T	4.5 ± 1.70		42.5 ± 30.89		9.3 ± 3.99
	T/T	5.0 ± 2.39		52.2 ± 37.45		11.1 ± 7.35*
rs2032582	G/G[1]	4.6 ± 2.07	0.204	45.7 ± 31.28	0.164	9.4 ± 3.34
	G/A+T	4.4 ± 1.60		40.2 ± 28.74		9.0 ± 4.01
	A+T/A+T	5.0 ± 2.09		49.7 ± 31.93		10.1 ± 5.52
	A/A[1]	4.3 ± 1.01	0.820	41.8 ± 11.91	0.903	10.0 ± 2.69
	A/G+T	4.7 ± 1.67		42.0 ± 23.36		8.9 ± 3.36
	G+T/G+T	4.6 ± 1.83		43.4 ± 30.10		9.5 ± 4.61
	T/T[1]	5.5 ± 2.35	0.032	54.5 ± 38.61	0.215	10.6 ± 7.63
	T/A+G	4.3 ± 1.69*		41.7 ± 31.20		9.4 ± 4.08
	A+G/A+G	4.6 ± 1.80		43.4 ± 30.10		9.0 ± 3.34
rs1128503	C/C[1]	4.4 ± 1.48	0.429	40.1 ± 21.39	0.164	9.1 ± 3.69
	C/T	4.5 ± 1.71		41.0 ± 25.23		8.9 ± 3.35
	T/T	4.8 ± 2.17		49.2 ± 39.88		10.1 ± 5.62

[1]This group served as a reference for comparison between two groups.

*$P<0.05$.

Table 5. Analysis of covariance* to find the significant parameters related with the tacrolimus ratio

Parameter	df	Mean square	*F*	*P*
Age	1	20.932	1.275	0.260
Sex	1	83.933	5.111	0.025
Donor type	2	16.550	1.008	0.367
History of transplantation	1	10.824	0.659	0.418
Diabetes mellitus	1	28.693	1.747	0.188
Prednisolone	1	26.474	1.612	0.206
Mycophenolate mofetil	1	0.636	0.039	0.844
Hematocrit	1	75.193	4.579	0.034
Lymphocyte	1	54.688	3.330	0.070
Albumin	1	20.836	1.269	0.261
Creatinine	1	9.184	0.559	0.456
Proteinuria	1	1.714	0.104	0.747
Delayed graft function	1	34.265	2.087	0.150
Acute rejection	1	19.438	1.184	0.278
Recurrence	1	8.402	0.512	0.475
CIN	1	0.368	0.022	0.881
Transplant duration	1	118.780	7.233	0.008
rs1045642	2	3.211	0.196	0.823
rs2032582	2	3.456	0.210	0.810
rs1128503	2	0.330	0.020	0.980

*R^2=0.197.

CIN, calcineurin inhibitor-induced nephrotoxicity.

Table 6. Baseline characteristics according to the intracellular tacrolimus concentrations

	Intracellular tacrolimus concentrations			
	1st quintile (n=42)	2nd - 4th quintile (n=129)	5th quintile (n=42)	*P*
Tacrolimus ratio	16.8 ± 3.98	37.5 ± 10.31‡	87.8 ± 38.52‡	<0.001
Age (years)	47.4 ± 14.70	47.3 ± 13.42	46.0 ± 11.47	0.856
Male sex (%)	69.0	59.7	47.6	0.133
Donor source (%)				0.080
Living related donor	31.0	42.6	47.6	
Living unrelated donor	16.7	24.0	9.5	
Deceased donor	52.4	33.3	42.9	
Previous history of transplantation (%)	4.8	5.4	9.5	0.579
Diabetes mellitus (%)	19.0	16.3	16.7	0.916
Combined immunosuppressive agents				
Prednisolone (mg/day)	4.4 ± 1.56	3.9 ± 1.53	4.8 ± 1.51†	0.002
Mycophenolate mofetil (mg/day)	667.6 ± 482.70	737.4 ± 447.91	579.8 ± 457.88	0.150
Blood findings				
Hematocrit (%)	40.4 ± 5.55	41.3 ± 5.57	40.0 ± 4.19	0.369
Lymphocyte (%)	29.3 ± 8.06	30.6 ± 7.95	27.7 ± 8.67*	0.114
Albumin (g/dL)	4.4 ± 0.31	4.4 ± 0.27	4.4 ± 0.38	0.883
Creatinine (mg/dL)	1.30 ± 0.57	1.26 ± 0.40	1.23 ± 0.46	0.771
Proteinuria (%)	35.7	24.0	16.7	0.122
Delayed graft function (%)	4.8	4.7	0	0.360

Acute rejection (%)	9.5	20.2	7.3
Recurrence (%)	2.4	1.6	4.9
CIN (%)	4.8	7.8	9.5
Transplant duration (months)	79 (48 - 97)	59 (34 - 87)*	27 (10 - 53)‡

Comparisons were evaluated using the chi-squared test for categorical variables, ANOVA for normally distributed continuous variables (LSD post hoc analysis between two groups), and the Kruskal-Wallis test for non-normally distributed continuous variables (Mann-Whitney U test between two groups). The 2nd to 4th quintile group served as a reference for comparison between two groups.

*P<0.05; † P<0.01; ‡ P<0.001.

IC-TAC, intracellular concentration of tacrolimus; WB-TAC, whole blood concentration of tacrolimus; CIN, calcineurin inhibitor-induced nephrotoxicity.

Table 7. Analysis of covariance* to find the significant parameters related with the intracellular tacrolimus concentration

Parameter	df	Mean square	*F*	*P*
Age	1	1053..451	1.380	0.242
Sex	1	1639.650	2.147	0.145
Donor type	2	140.026	0.183	0.833
History of transplantation	1	909.172	1.191	0.277
Diabetes mellitus	1	867.214	1.136	0.288
Prednisolone	1	149.093	0.195	0.659
Mycophenolate mofetil	1	1051.419	1.377	0.242
Hematocrit	1	935.636	1.225	0.270
Lymphocyte	1	3051.414	3.996	0.047
Albumin	1	864.261	1.132	0.289
Creatinine	1	1629.428	2.134	0.146
Proteinuria	1	374.748	0.491	0.484
Delayed graft function	1	1238.454	1.622	0.204
Acute rejection	1	20.876	0.027	0.869
Recurrence	1	1.280	0.002	0.967
CIN	1	3.918	0.005	0.943
Transplant duration	1	16277.977	21.318	<0.001
rs1045642	2	44.769	0.059	0.943
rs2032582	2	63.005	0.083	0.921
rs1128503	2	352.437	0.462	0.631

*R^2=0.223.

CIN, calcineurin inhibitor-induced nephrotoxicity.

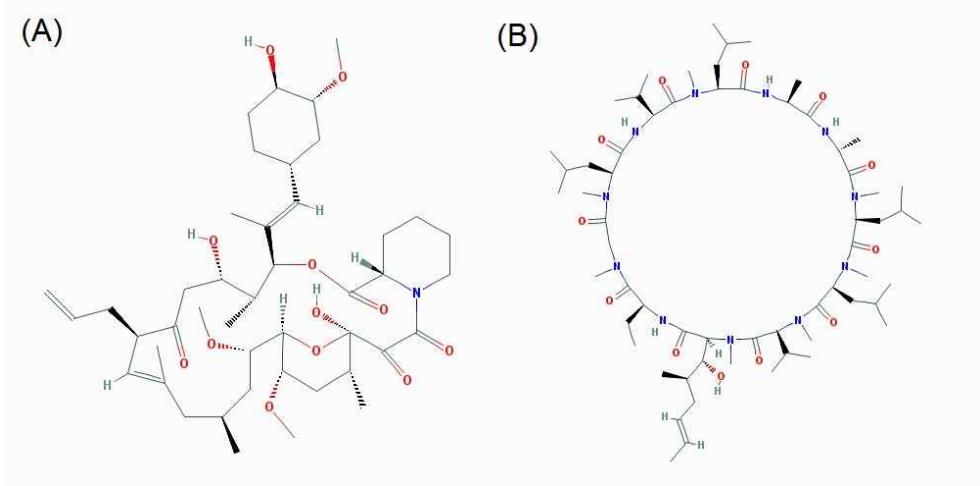
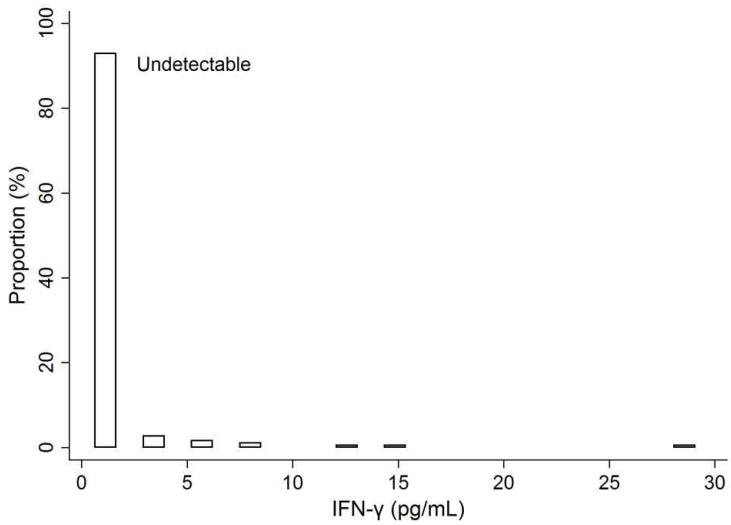


Figure 1. Structure of tacrolimus (A) and cyclosporine (B). Images were obtained from the open database for chemicals (Pubchem: https://www.ncbi.nlm.nih.gov/pccompound).

(A)



(B)

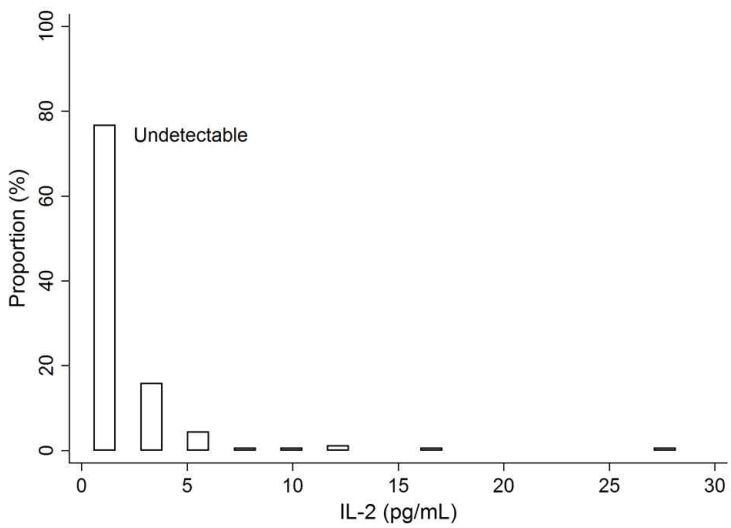


Figure 2. Plasma levels of interferon-γ (A) and interleukin-2 (B) in the study participants at the time of tacrolimus concentration measurement. Most of the patients had low levels of these cytokines.

IFN-γ, interferon-γ; IL-2, interleukin-2.

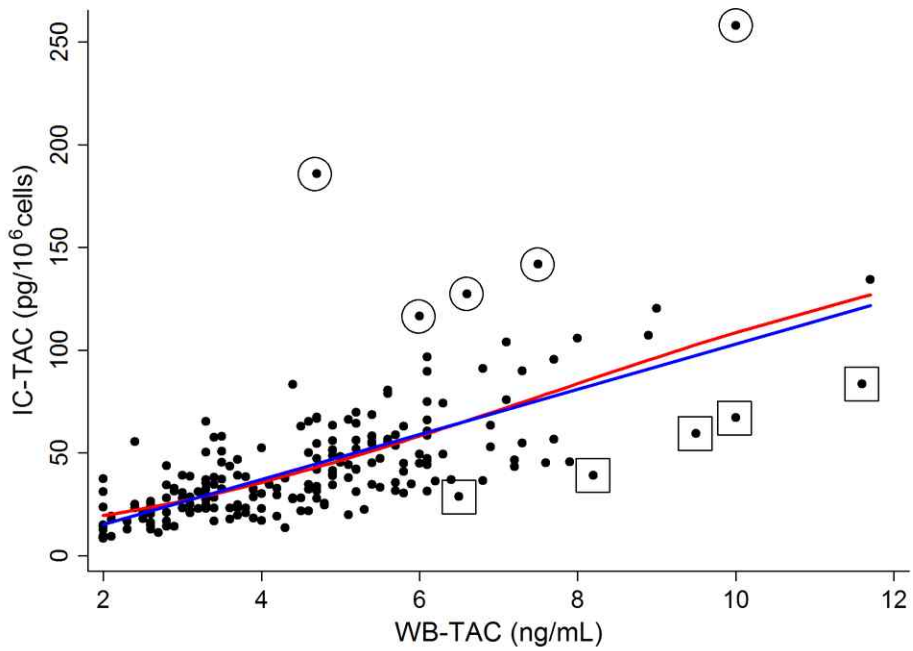
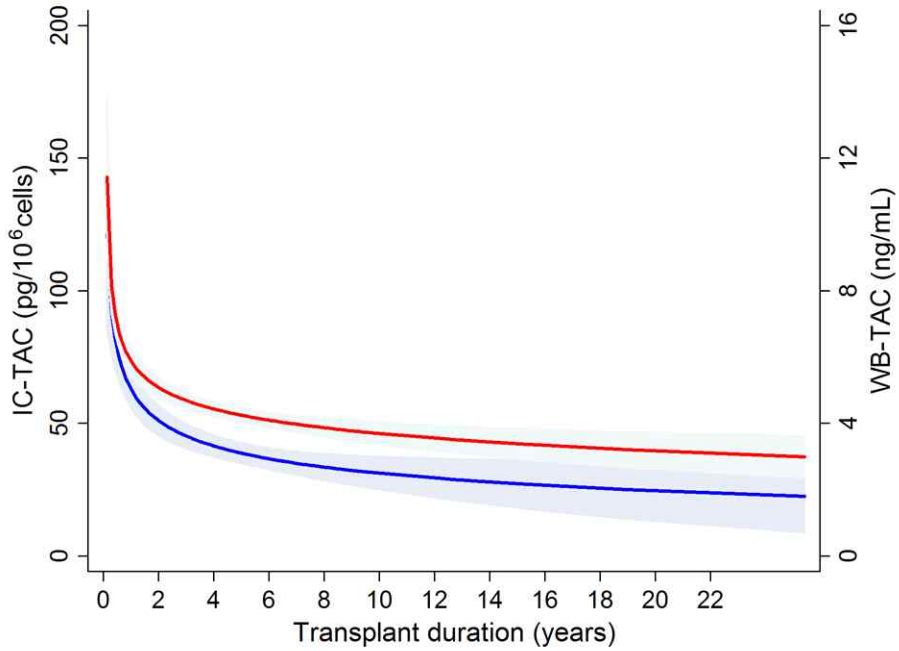


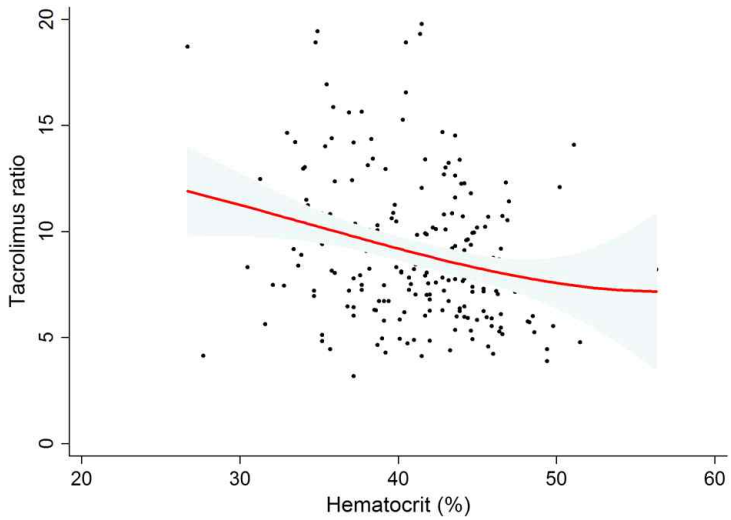
Figure 3. Scatter plot illustrating blood and intracellular tacrolimus concentrations. Blue and red lines represent linear and non-linear relationships between two variables, respectively. Circled dots indicate representative cases with high tacrolimus ratio; and squared dots indicate representative cases with low tacrolimus ratio. IC-TAC, intracellular concentration of tacrolimus; WB-TAC, whole blood concentration of tacrolimus.



Timeframe (yrs)	≤1	1–2	2–4	4–6	6–10	>10
Patient no.	21	16	55	46	66	9
WB-TAC	6.8 ± 2.06	5.5 ± 1.13	4.7 ± 1.74	4.4 ± 1.48	3.8 ± 1.49	3.4 ± 1.50
IC-TAC	75.2 ± 33.32	61.2 ± 25.28	43.3 ± 36.76	42.7 ± 25.84	31.4 ± 16.72	29.2 ± 13.86
Tacrolimus ratio	11.0 ± 3.67	10.7 ± 3.15	8.8 ± 3.95	10.2 ± 5.69	8.4 ± 3.46	8.9 ± 4.10
Creatinine	1.2 ± 0.20	1.3 ± 0.29	1.2 ± 0.33	1.2 ± 0.35	1.3 ± 0.45	1.9 ± 1.25

Figure 4. Dependence of the changes of IC-TAC (red line) and WB-TAC (blue line) on the transplant duration. The table below indicates the changes of tacrolimus parameters and graft function.

(A)



(B)

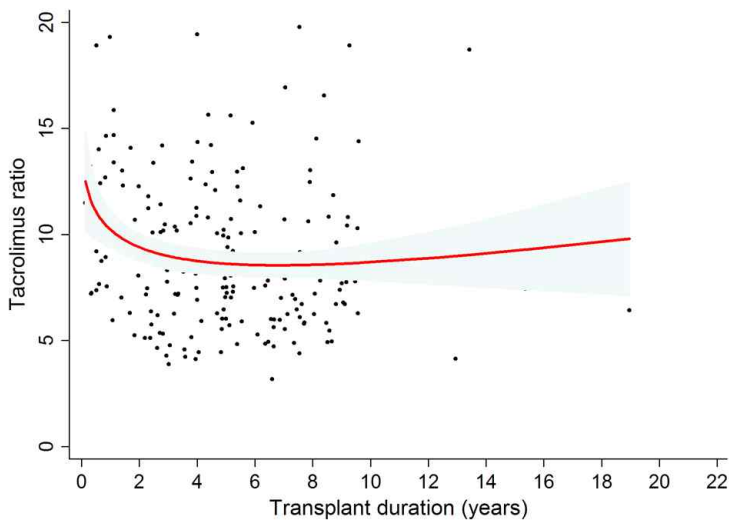


Figure 5. Fitted curves between the tacrolimus ratio and the hematocrit (A) or transplant duration (B). The range area indicates the 95% confidence interval.

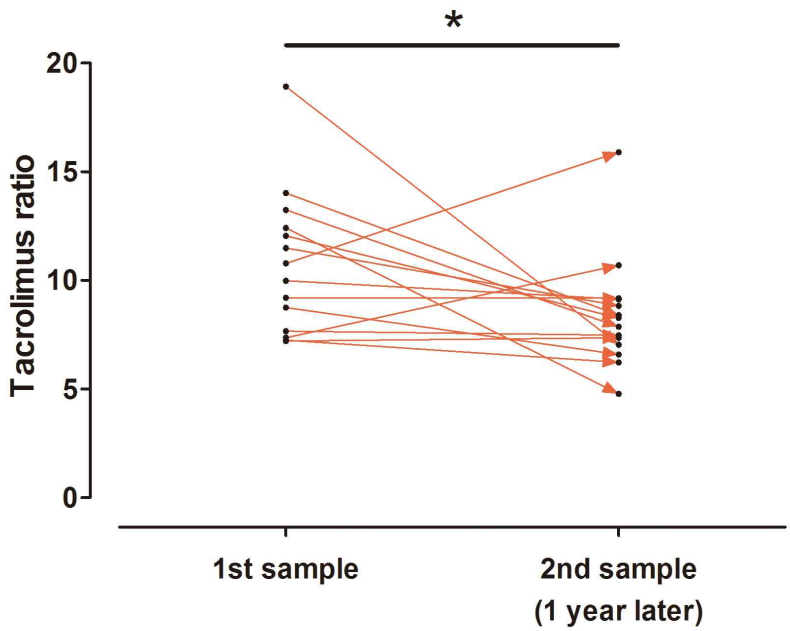


Figure 6. Change in the tacrolimus ratio between measurements taken one year apart. $*P<0.05$.

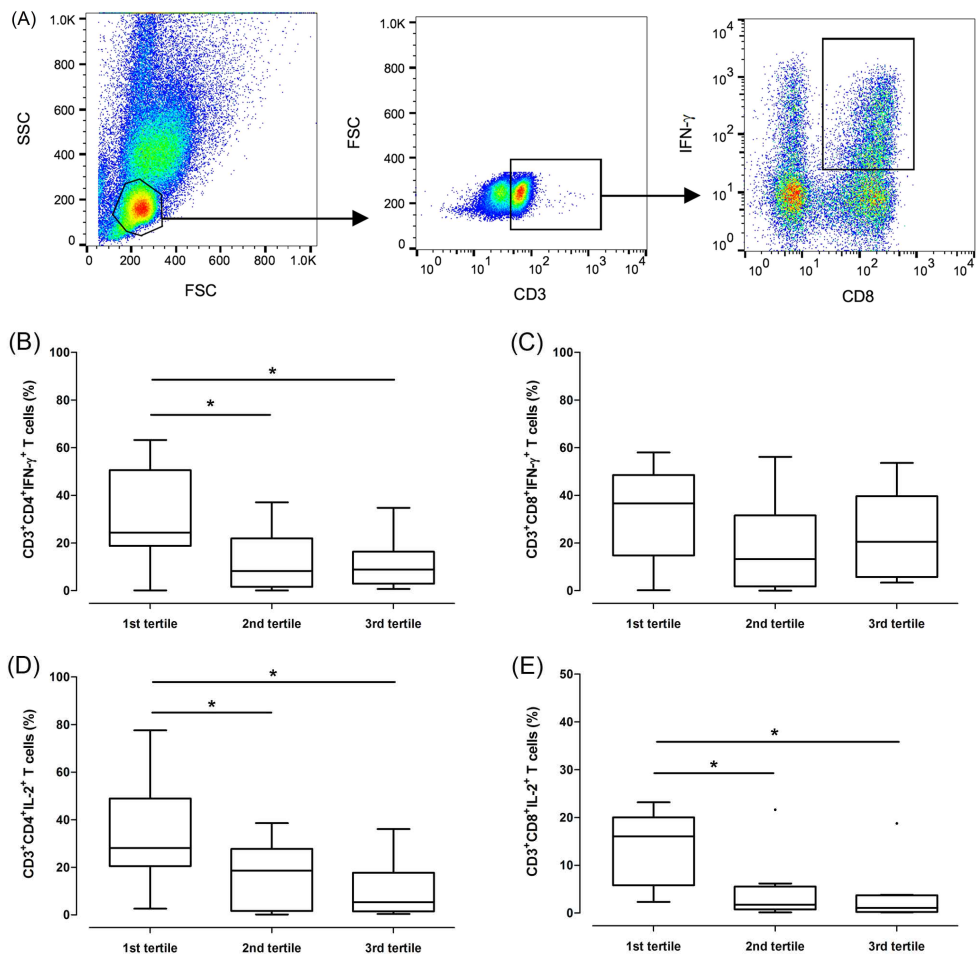


Figure 7. Activation of $CD4^+$ (B and D) and $CD8^+$ (C and E) T cells according to tertiles of intracellular tacrolimus concentrations. (A), Example of the dot plot gating strategy used to calculate the proportion of interferon-ɣ-producing $CD3^+CD8^+$ T cells. (B) and (C), Flow cytometry to identify T cells producing interferon-ɣ. (D) and (E), Flow cytometry to identify T cells producing interleukin-2. IFN-ɣ, interferon-ɣ; IL-2, interleukin-2.

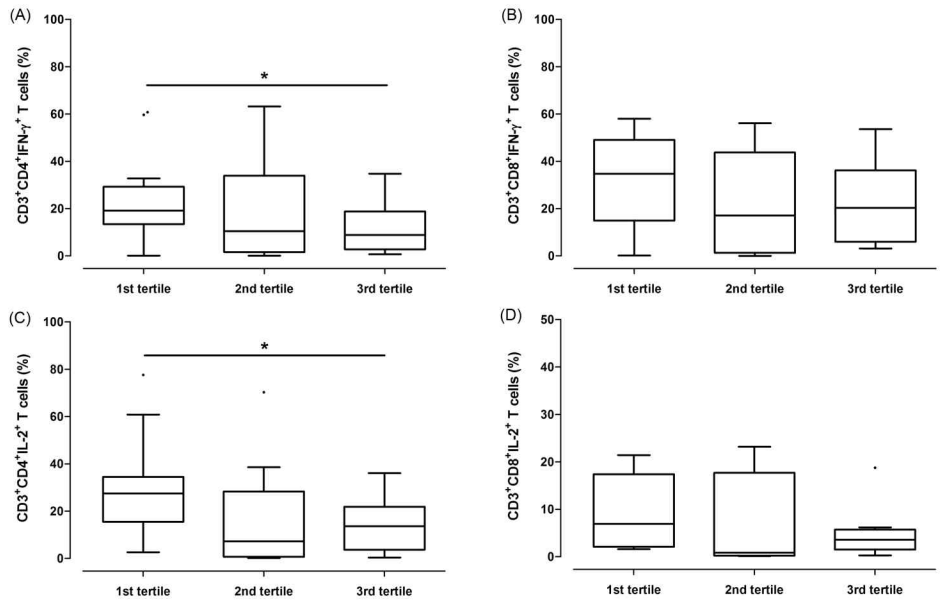


Figure 8. Activation of $CD4^+$ (A and C) or $CD8^+$ (B and D) T cells according to tertiles of whole blood tacrolimus concentrations. (A) and (B), Flow cytometry to identify T cells producing interferon-ɣ. (C) and (D), Flow cytometry to identify T cells producing interleukin-2. IFN-ɣ, interferon-ɣ; IL-2, interleukin-2.

References

1. Wolfe RA, Ashby VB, Milford EL, Ojo AO, Ettenger RE, et al. (1999) Comparison of mortality in all patients on dialysis, patients on dialysis awaiting transplantation, and recipients of a first cadaveric transplant. N Engl J Med 341: 1725-1730.

2. Mayer AD, Dmitrewski J, Squifflet JP, Besse T, Grabensee B, et al. (1997) Multicenter randomized trial comparing tacrolimus (FK506) and cyclosporine in the prevention of renal allograft rejection: a report of the European Tacrolimus Multicenter Renal Study Group. Transplantation 64: 436-443.

3. Matas AJ, Smith JM, Skeans MA, Thompson B, Gustafson SK, et al. (2014) OPTN/SRTR 2012 Annual Data Report: kidney. Am J Transplant 14 Suppl 1: 11-44.

4. Gheorghian A, Schnitzler MA, Axelrod DA, Kalsekar A, L'Italien G, et al. (2012) The implications of acute rejection and reduced allograft function on health care expenditures in contemporary US kidney transplantation. Transplantation 94: 241-249.

5. Meier-Kriesche HU, Ojo AO, Hanson JA, Cibrik DM, Punch JD, et al. (2000) Increased impact of acute rejection on chronic allograft failure in recent era. Transplantation 70: 1098-1100.

6. Webster A, Woodroffe RC, Taylor RS, Chapman JR, Craig JC. (2005) Tacrolimus versus cyclosporin as primary immunosuppression for kidney transplant recipients. Cochrane Database Syst Rev CD003961.

7. Ekberg H, Tedesco-Silva H, Demirbas A, Vitko S, Nashan B, et al. (2007) Reduced exposure to calcineurin inhibitors in renal transplantation. N Engl J Med 357: 2562-2575.

8. Kidney Disease: Improving Global Outcomes (KDIGO) Transplant Work Group. (2009) KDIGO clinical practice guideline for the care of kidney transplant recipients. Am J Transplant 9 Suppl 3: S1-155.

9. Bouamar R, Shuker N, Hesselink DA, Weimar W, Ekberg H, et al. (2013) Tacrolimus predose concentrations do not predict the risk of acute rejection after renal transplantation: a pooled analysis from three randomized-controlled clinical trials. Am J Transplant 13: 1253-1261.

10. Vafadari R, Bouamar R, Hesselink DA, Kraaijeveld R, van Schaik RH, et al. (2013) Genetic polymorphisms in ABCB1 influence the pharmacodynamics of tacrolimus. Ther Drug Monit 35: 459-465.

11. Capron A, Mourad M, De Meyer M, De Pauw L, Eddour DC, et al. (2010) CYP3A5 and ABCB1 polymorphisms influence tacrolimus concentrations in peripheral blood mononuclear cells after renal transplantation. Pharmacogenomics 11: 703-714.

12. van Gelder T. (2002) Drug interactions with tacrolimus. Drug safety 25: 707-712.

13. Capron A, Musuamba F, Latinne D, Mourad M, Lerut J, et al. (2009) Validation of a liquid chromatography-mass spectrometric assay for tacrolimus in peripheral blood mononuclear cells. Ther Drug Monit 31: 178-186.

14. Royston P, Ambler G, Sauerbrei W. (1999) The use of fractional

polynomials to model continuous risk variables in epidemiology. Int J Epidemiol 28: 964-974.

15. Najafian N, Albin MJ, Newell KA. (2006) How can we measure immunologic tolerance in humans? J Am Soc Nephrol 17: 2652-2663.

16. Capron A, Lerut J, Latinne D, Rahier J, Haufroid V, et al. (2012) Correlation of tacrolimus levels in peripheral blood mononuclear cells with histological staging of rejection after liver transplantation: preliminary results of a prospective study. Transpl Int 25: 41-47.

17. Lemaitre F, Antignac M, Fernandez C. (2013) Monitoring of tacrolimus concentrations in peripheral blood mononuclear cells: Application to cardiac transplant recipients. Clin Biochem 46: 1538-1541.

18. Velickovic-Radovanovic R, Mikov M, Paunovic G, Djordjevic V, Stojanovic M, et al. (2011) Gender differences in pharmacokinetics of tacrolimus and their clinical significance in kidney transplant recipients. Gend Med 8: 23-31.

19. Schwartz JB. (2007) The current state of knowledge on age, sex, and their interactions on clinical pharmacology. Clin Pharmacol Ther 82: 87-96.

20. Jusko WJ, Piekoszewski W, Klintmalm GB, Shaefer MS, Hebert MF, et al. (1995) Pharmacokinetics of tacrolimus in liver transplant patients. Clin Pharmacol Ther 57: 281-290.

21. Brinkmann U, Eichelbaum M. (2001) Polymorphisms in the ABC drug transporter gene MDR1. Pharmacogenomics J 1: 59-64.

22. Raviv Y, Puri A, Blumenthal R. (2000)

P-glycoprotein-overexpressing multidrug-resistant cells are resistant to infection by enveloped viruses that enter via the plasma membrane. FASEB J 14: 511-515.

23. Hodges LM, Markova SM, Chinn LW, Gow JM, Kroetz DL, et al. (2011) Very important pharmacogene summary: ABCB1 (MDR1, P-glycoprotein). Pharmacogenet Genomics 21: 152-161.

24. Haufroid V, Wallemacq P, VanKerckhove V, Elens L, De Meyer M, et al. (2006) CYP3A5 and ABCB1 polymorphisms and tacrolimus pharmacokinetics in renal transplant candidates: guidelines from an experimental study. Am J Transplant 6: 2706-2713.

Appendix

Instrumentation for LC-MS/MS

#1. HPLC: Agilent 1260 Infinity Binary LC system (Agilent Technologies, Santa Clara, CA, USA)

Analytical column	Venusil XBP C_{18} (50×2.1 mm, 5 μm, Agela Technologies, Newark, NJ, USA)
Column temperature	40°C
Mobile phase	A) 0.1% formic acid in 2 mM ammonium acetate; B) 0.1% formic acid in 100% methanol
Flow rate	0.2 mL/min
Injection volume	5 μL
Autosampler temperature	4°C
Run time	5 minutes
Retention time	Tacrolimus, 1.02 minutes; IS, 1.01 minutes

#2. MS/MS: API 4000 Qtrap (AB Sciex, Framingham, MA, USA)

Ion source	ESI, positive
Scan type	MRM mode
Parameter	CAD Medium, CUR 20, GS1 50, GS2 50, IS 5500, TEM 500

요약 (국문초록)

신장이식 환자에서 세포내 면역억제제 농도 측정 기술 개발과 임상 적용

세포내 면역억제제 농도 측정법은 장기 이식 환자에서 아직 정립되지 않은 방법이나, 환자 맞춤형 모니터링 및 치료의 일환으로서 기대되고 있다. 하지만 이와 관련된 세포내 면역억제제 농도 연구가 충분히 이루어지지 않았으므로 임상에서의 적용은 아직 요원하다. 이에 따라, 본 연구진은 혈액내 면역억제제 농도와 세포내 농도와의 관련성 및 이에 영향을 미치는 인자를 살펴보기 위하여, 안정적인 이식신 기능을 가진 신장 이식 환자를 대상으로 연구를 진행하였다. 총 213명의 환자를 전향적으로 모집하였고, 세포내 타크로리무스 농도를 액체크로마토그래프 탠덤질량분석기를 이용하여 측정하였으며, 동시에 혈액 타크로리무스 농도도 측정하여 세포내 농도와 비교하였다. 그 결과 두 농도 사이에는 전반적으로 선형의 관련성을 보였다 (r=0.67; P<0.001). 이러한 선형 관련성을 벗어난 경우를 확인하기 위해, 연구진은 세포내 농도와 혈액 농도와의 비율을 구하고 (세포내 농도/혈액 농도), 이에 미치는 인자를 통계적으로 확인하였다. 그 결과, 여성 환자의 경우, 낮은 헤마토크릿 수치를 갖고 있는 경우, 그리고 이식 기간이 짧은 경우에서 세포내 농도와 혈액 농도와의 비율이 높았다. 그러나 과거력상 급성 거부 반응의 발생 유무와

ATP binding cassette subfamily B member 1 (*ABCB1*) 유전자 다형성은 타크로리무스 농도 비율에 영향을 미치지 않았다. 추가적으로 세포내 타크로리무스 농도가 급성 거부 반응의 위험도를 예측할 수 있는지 확인하기 위하여, 39명의 신장 이식 환자로부터 말초혈액단핵세포를 분리한 후 phorbol-12-myristate 13-acetate와 ionomycin으로 자극하는 생체 밖 연구 (ex vivo study)를 진행하였다. 그 결과, 세포내 농도가 낮을수록 T 림프구에서 interferon-gamma 혹은 interleukin-2를 더 많이 생성하였다. 본 실험 결과는 안정적인 이식신 기능을 가진 환자에서 세포내 농도가 추후 발생 가능한 급성 거부 반응의 위험도를 예측할 수 있다는 점을 시사한다. 세포내 면역억제제 농도 측정법을 임상에서 활용하기 위해서는 아직 더 많은 연구가 필요하고, 특히 본 연구에서 다루지 않은 급성 거부 반응 혹은 약물 부작용 등을 갖고 있는 환자에서의 결과가 필요하나, 본 연구는 이러한 후속 연구들의 토대가 된다는 점에서 의미가 있겠다.

.....

주요어 : 세포내 농도, 면역억제제, 신장 이식, 타크로리무스, 급성 거부 반응, 말초혈액단핵세포

학 번 : 2013-30619