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Effectiveness, safety and population pharmacokinetics of calcineurin inhibitors in graft-versus-host disease prophylaxis

이식편대숙주질환 예방에서의 칼시뉴린 억제제 효과, 안전성 및 집단약동학 연구

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Abstract

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1. Introduction

1.1. Graft-versus-host disease (GvHD) after hematopoietic stem cell transplantation (HSCT)

Although anticancer effects are related to dose of anticancer agents, hematologic toxicities are the most common dose-limiting toxicities. HSCT is the transfusion of multipotent hematopoietic stem cell in order to recover hematopoiesis after high intensity chemotherapy. Thus high intensity conditioning regimen which includes high dose chemotherapy followed by HSCT is the only curative therapy of majority of hemato-oncologic disease. The number of HSCT increased for 10 years from 195 cases in 2004 to 529 cases in 2014.[1] HSCT can be classified by sources of hematopoietic stem cells or graft types. Stem cells came collected from the peripheral blood, bone marrow, and umbilical cord blood are used in HSCT. In autologous transplantation, the patient's own stem cells are used, while the stem cells come from other donor are used in allogeneic HSCT.[2,3]

After transfusion of allogeneic hematopoietic stem cells, immune cells especially T-cells derived from the donor recognize the recipients as foreign and attack the host's cells. The reaction is

called GvHD and GvHD is a severe complication of allogeneic HSCT. Transplantation related morbidity and mortality is related to acute GvHD, which occurs within 100 days after transplantation.[4] Acute GvHD commonly attacks the liver, skin, and gastrointestinal tract and the severity of acute GvHD is scored according to signs and symptoms represented in those organs. Several risk factors of GvHD including recipient and donor characteristics, graft properties, conditioning chemotherapy and post-HSCT management were suggested. The risk for acute GvHD rises with increasing recipient age, use of unrelated donor, human leukocyte antigen (HLA) mismatch.[5]

1.2. Strategies for GvHD prophylaxis

Immunosuppression has been the primary pharmacologic strategy to prevent GvHD. Methotrexate has been used since the 1950s as a way of shutting down T cells through inhibition of dihydrofolate reductase and production of thymidylate and purines. The calcineurin inhibitors cyclosporine and tacrolimus inhibit T-cell proliferation; combinations with methotrexate have successfully been used since the 1970s and are the cornerstone of most prophylactic regimens.[6]

Alternate agents were the inosine monophosphate dehydrogenase inhibitor mycophenolate mofetil and the mammalian target of rapamycin (mTOR) inhibitor sirolimus. Furthermore given the central role of T cells in GvHD, T-cell depletion (TCD) has been studied since the 1980s as a preventative strategy. Other drugs attempt to target cytokine/chemokine-receptor interactions that appear integral to development of GvHD. Exciting new success has been reported with maraviroc, a CCR5 antagonist that blocks T-cell chemotaxis and dramatically decreased the incidence of gastrointestinal and liver GvHD.[6]

1.3. Calcineurin inhibitor-based GvHD prophylaxis regimens

The introduction in the 1980s of two new immunosuppressive agents, cyclosporine and tacrolimus, which prevented T-cell activation by inhibiting calcineurin, has dramatically improved allograft survival rates. Furthermore, in 1986, the first studies reporting the superior outcomes of calcineurin inhibitor (CNI)-based regimens with notable reduction in GvHD and improved survival as a result of combination therapy (such as cyclosporine plus methotrexate) compared to either agent alone, were published. CNI-based therapies have, therefore, been considered

the standard-of-care for GvHD prevention. Cyclosporine was originally isolated from fungi and was noted to have immunosuppressive effects. This observation led to its use in the prevention of allograft solid organ rejection and GvHD after allogeneic HSCT. Although cyclosporine and tacrolimus are structurally distinct, their mechanisms of action are similar. Cyclosporine binds to the cytosolic protein Peptidyl prolyl cis-trans isomerase A (also known as cyclophilin), whereas tacrolimus binds to the Peptidyl-prolyl cis-trans isomerase FKBP12, and these complexes (cyclosporine–cyclophilin or tacrolimus–FKBP12) inhibit calcineurin, thereby blocking the dephosphorylation of nuclear factor of activated T cells (NFAT) and its nuclear translocation. These events prevent NFAT from exerting its transcriptional function, resulting in the inhibition of transcription of IL-2 and of other cytokines and ultimately leading to a reduced function of T-cells.[7]

Two multicenter, randomized, prospective trials conducted in the mid-1990s demonstrated decreased incidence of acute GvHD with the tacrolimus and methotrexate combination compared to cyclosporine and methotrexate, but overall survival was not significantly different. These findings led some centers to favour the tacrolimus and methotrexate combination. Nonetheless, a recent

survey estimated a much higher proportion of centers using cyclosporine over tacrolimus-based regimens.[7]

2. Research Topics

CNIs have played an important role in GvHD prophylaxis and have used for a long time. However there are some unmet needs in selection of CNIs and dosing in GvHD prophylaxis.

Although there have been much studies which compared the efficacy and safety between tacrolimus and cyclosporine, all of studies were conducted in adult HSCT patients. In this situation, the majority of the pediatric centers prefered cyclosporine in combination with methotrexate for GvHD prophylaxis. Thus a comparison study is needed to establish the efficacy and safety of tacrolimus in pediatric HSCT patients. The methods and results are presented in SECTION I.

Second, adequate concentrations of CNIs are important to prevent GvHD and adverse drug reactions. To maintain concentrations in target range, therapeutic drug monitoring is carried out. Nevertheless, large inter- and intra-variability in pharmacokinetics interrupt maintaining adequate concentrations of CNIs. Thus a

population pharmacokinetic (PopPK) studies of CNIs are needed and the methods and results are presented in SECTION II.

3. Overall Results

In SECTION I, a total of 50 pediatric HSCT patients were included. The cumulative incidence of grade II to IV acute GvHD was not significantly different between tacrolimus and cyclosporine groups (75.4% vs. 66.7%, p = 0.910). The cumulative incidence grade III to IV acute GvHD was also not significantly different between tacrolimus and cyclosporine groups (15.4% vs. 21.6%, p = 0.627) Furthermore relapse free survival and non-relapse mortality at 100 days post-transplantation was not significantly different between two groups (100% vs. 91.3%, p = 0.961 and 0% vs. 8.7%, p = 0.576). All of adverse drug reactions were reported in less than 10% of patients.

In SECTION II, PopPK models of tacrolimus in pediatric HSCT patients and cyclosporine in adult HSCT patients were developed. PopPK model of tacrolimus included BSA and azole antifungals use as covariates. The final model was:

$CL\ (L/h) = 6.74 \times (BSA/1.102)^{0.552} \times 0.4$ (if azole antifungals use)

$V\ (L) = 1160 \times (BSA/1.102)^{0.503}$

PopPK model of cyclosporine included weight as covariates. The final model was:

$CL = THETA(1) \times (weight/70)^{0.419} \times EXP(ETA(1))$

Both models were well validated by bootstrap and VPC.

4. Overall Conclusions

In these studies, the efficacy and safety of tacrolimus and cyclosporine were compared in pediatric HSCT patients. In conclusion the efficacy of tacrolimus was not superior to cyclosporine in pediatric patients and both CNIs had a low incidence of adverse drug reactions.

In addition, tacrolimus in pediatric HSCT patients and cyclosporine in adult HSCT patients were developed. Using the models, adequate dosing of tacrolimus and cyclosporine might be possible.

Finally these studies might give insight to the optimization strategies of CNIs in GvHD prophylaxis.

Keywords: Calcineurin inhibitors, Tacrolimus, Cyclosporine, Effectiveness and safety, Population pharmacokinetics, Graft-versus-host disease, Hematopoietic stem cell transplantation

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Contents

Tables

Figures

SECTION I:

Comparison of Effectiveness and Safety of Calcineurin Inhibitors in Pediatric HSCT Patients

1. Introduction

GvHD is a severe complication of allogeneic HSCT. Several immunosuppressive strategies were developed to prevent acute GvHD. Among these, calcineurin inhibitor (CNI)-based regimens are the most commonly used.[8] CNIs, cyclosporine and tacrolimus, prevent T-cell activation by inhibiting calcineurin. After introduction of CNI-based regimen, GvHD incidence was notably reduced and survival after HSCT was improved. The combination of CNIs with methotrexate has shown superior efficacy over single agent use and the combination therapy has become the most widely used GVHD prophylaxis regimen.[8] Other combination regimen which includes mycophenolate mofetil and CNIs also has shown a synergistic effect for GvHD prophylaxis.[9] There have been some researches which compared the efficacy of cyclosporine and tacrolimus. Of the two CNIs, tacrolimus-based regimens were associated with a statistically significant reduction in acute GvHD (RR 0.62, 95% CI 0.52 to 0.75) and in severe acute GvHD (RR 0.67, 95% CI 0.47 to 0.95) compared to cyclosporine-based regimens. However, tacrolimus-based regimens were associated with a significantly higher rate of renal failure (RR 1.20, 95% CI 1.03 to 1.39)[10]

Children are at less risk for acute GvHD than adults. However, acute GvHD risk is still high when using unrelated donor sources.[11] Acute GvHD prophylaxis regimen is not well established in pediatric HSCT patients yet. The European Group for Blood and Marrow Transplantation (EBMT) Working Party Paediatric Diseases (EBMT-WP PD) and the International BFM Study Group - Subcommittee Bone Marrow Transplantation (IBFM-SG) evaluated current local standards in the prevention of acute GvHD. Several conferences with their members assessed practices which are mainly applied or under investigation in pediatric patients. As a result, the majority of the pediatric centers prefered cyclosporine in combination with methotrexate for GvHD prophylaxis.[12] Because the result conflicted to that of the meta-analysis, a prospective study is needed to compare the efficacy and safety of tacrolimus with cyclosporine in pediatric HSCT patients.

The aim of this study was to compare the efficacy and safety of tacrolimus with cyclosporine in pediatric allogeneic HSCT patients.

2. Methods

Study design and patients

A multicentre prospective study was conducted to compare the efficacy and safety of tacrolimus with cyclosporine. This study was approved by the institutional review board and followed the recommendations of the Declaration of Helsinki.

Pediatric patients who need GvHD prophylaxis after allogeneic HSCT were included. Eligibility criteria were age less than 18 years old, total bilirubin less than 1.5 times of upper limit of normal (ULN), alanine aminotransferase and aspartate aminotransferase less than 2.5 times of ULN, serum creatinine less than 1.5 times of ULN, and alkaline phosphatase less than 2.5 times ULN. Patients with human immunodeficiency virus, severe or uncontrolled comorbidity, and previous history of HSCT or solid organ transplantation were excluded.

GvHD prevention regimen

Patients received either tacrolimus or cyclosporine in combination with methotrexate. Tacrolimus was administered intravenously at 0.03 mg/kg/day from day −1 and changed to oral from around day 20 at a 4 times of the last intravenous dosage. Cyclosporine was administered intravenously at 3 mg/kg/day from day −1 and

changed to oral from around day 20 at a 2 to 3 times of the last intravenous dosage. Mehtotrexate was given intravenously at 15 mg/m^2 on day 1 and at 10 mg/m^2 on days 3, 6 and 11 after HSCT. Tacrolimus dosing was adjusted to maintain concentrations at 10 to 20 ng/mL, while cyclosporine dosing was adjusted to maintain whole blood trough concentrations at 200 to 300 ng/mL. Drugs for prophylaxis of infection or veno-occlusive disease were permitted and used according to the institutional regimen.

Outcomes

Patient's demographic information including sex, age, weight, height, original disease, cells source, conditioning regimens were collected. All patients were followed up until 100 days post-transplantation. During the period, signs and symptoms of acute GvHD, adverse drug reactions, and infections were monitored.

The primary efficacy outcome was the incidence of grade II to IV acute GvHD which was a clinically important acute GvHD. The grade of acute GvHD was scored according to the stage of liver, skin, and gastrointestinal tract involvement.[13]

The secondary efficacy outcomes were the incidence of grade III to IV acute GvHD (severe acute GvHD), relapse free survival at day 100 post-transplantation, and non-relapse mortality at day 100 post-transplantation.

The safety outcomes were adverse drug reactions and infections. Adverse drug reactions were evaluated about the relevance with tacrolimus or cyclosporine according to Naranjo algorithm.[14] Only "definitely", "probably" or "possibly" related adverse drug reactions were analyzed.

Statistical analysis

To calculate the sample size, it was assumed that approximately 20% of patients in tacrolimus group would experience grade II to IV acute GvHD while 55% of patients in cyclosporine group would experience grade II to IV acute GvHD. A sample size of 24 evaluable subjects per study group allowed detection of an absolute difference of 35% (one-sided alpha = 0.05, power = 0.80). Thus the expected sample size was 26 subjects per study group, adjusting 10% drop-out rate.

Statistical analysis was conducted by SPSS version 21. A Kaplan-meier survival analysis and a log-rank test were used to assess significance of differences in cumulative incidence of grade II to IV acute GvHD, grade III to IV acute GvHD, relapse free survival, non-relapse mortality between two groups.

3. Results

Patients

Twenty six patients in tacrolimus group and twenty four patients in cyclosporine group were included. Two patients in tacrolimus group were dropped-out because of engraftment failure and non-compliance. Patient and transplantation characteristics of the study population are summarized in Table 1. There were no differences in recipient age, sex, cells source, HLA matching, and conditioning regimen. Especially more than 90% of patients in both group received hematopoietic stem cells derived from peripheral blood stem cells.

Acute GvHD

Sixteen patients in cyclosporine group and nineteen patients in tacrolimus group. The cumulative incidence of grade II to IV acute GvHD was not significantly different between tacrolimus and cyclosporine groups (75.4% vs. 66.7%, p = 0.910). The cumulative incidence grade III to IV acute GvHD was also not significantly different between tacrolimus and cyclosporine groups (15.4% vs. 21.6%, p = 0.627) (Figure 1). In Cox proportional hazard model, type of CNIs (p = 0.235), recipient age (p = 0.398), conditioning regimen (p = 0.051), original disease (p = 0.078), sex (p = 0.157), degree of HLA matching (p = 0.48), and number of methotrexate administration (p = 0.069) were not affected the incidence of grade II to IV acute GvHD. However degree of HLA matching significantly affected the incidence of grade III to IV acute GvHD (p = 0.013).

Relapse free survival and non-relapse mortality

Relapse free survival at 100 days post-transplantation was not significantly different between tacrolimus and cyclosporine groups (100% vs. 91.3%, p = 0.961). (Figure 2) Two patients in cyclosporine group relapsed after HSCT. Both patients were acute

lymphoblastic leukemic patients and one patient relapsed on day 51 while the other relapsed on day 85.

Non-relapse mortality was also not significantly different between tacrolimus and cyclosporine groups (0% vs. 8.7%, p = 0.576). (Figure 2) Two patients in cyclosporine group died.

Adverse drug reactions and infections

Nephrotoxicity (7.7%), hypertension (3.8%), and thrombotic microangiopathy (3.8%) were reported in tacrolimus group. Otherwise skin rash (8.3%), hypertension (4.2%), urticarial (4.2%), and diarrhea (4.2%) were reported in cyclosporine group. All of adverse drug reactions were reported in less than 10% of patients. (Table 2)

Cytomegalovirus was the most frequently reported pathogens in both tacrolimus and cyclosporine groups (26.9% vs. 41.7%). Other pathogens were *Enterococcus* (3.8%), mycoplasma (3.8%), BK virus (3.8%), respiratory syncytial virus (3.8%), and Epstein-Barr virus (7.7%) in tacrolimus group. Otherwise *Enterococcus* (12.5%), *Klebsiella pneumoniae* (4.2%), *Escherichia coli* (4.2%), BK virus

(4.2%), Ebstein-Barr virus (4.2%), and fungi (8.3%) in cyclosporine group. (Table 2)

4. Discussion

This study was the first pediatric exclusive study. Although children have less risk of acute GvHD, none of study was conducted in pediatric population in our knowledge.

The efficacy of tacrolimus was not superior to cyclosporine in pediatric patients. Previous meta-analysis which compared tacrolimus/methotrexate regimen and cyclosporine/methotrexate regimen included three trials. There was a significant reduction of grade II to IV acute GvHD with tacrolimus/methotrexate (RR = 0.62, 95% CI = 0.52-075). Similarly, there was a significant reduction in grade III to IV acute GvHD with tacrolimus/methotrexate (RR = 0.67, 95% CI = 0.47-0.95). However there was no difference in all-cause mortality between tacrolimus/methotrexate and cyclosporine/methotrexate groups (RR = 1.10, 95% CI = 0.93-1.3). The included studies were published during 1998~2001. After the publication of the meta-analysis, several articles were published which conflicted with the result of the meta-analysis.

Most recently, a retrospective nationwide survey was published and a comparison was conducted stratified by stem cell source. Incidence of acute GvHD after matched related donor was significantly lower in tacrolimus group (21.6% vs. 31.6%, p = 0.006). In subgroup analysis, stem cells derived from peripheral blood induced a lower GvHD incidence in tacrolimus group (21.9% vs. 33.8%, p = 0.04) while stem cells from bone marrow did not (21.4% vs. 29.4%, p = 0.08). Incidence of acute GvHD was not significantly different between tacrolimus and cyclosporine groups after unrelated bone marrow stem cell transplantation (38.3% vs. 42.6%, p = 0.06).[15] Because the study was conducted in Japan, there was no stem cells derived from unrelated peripheral blood. Another international retrospective study suggested insignificant results. All of transplantation from sibling bone marrow, sibling peripheral blood, unrelated bone marrow, and unrelated peripheral blood did not shown significant differences of acute GvHD in tacrolimus and cyclosporine groups (p = 0.12, 0.83, 0.98, and 0.47, respectively).[16] Grade III to IV acute GvHD, overall survival, and non-relapse mortality were not significantly different in those two studies. The insignificant results in grade II to IV acute GvHD,

grade III to IV acute GvHD, relapse free survival, and non-relapse mortality were in accordance with those studies.

In our study the incidence of adverse drug reactions was low. Previously tacrolimus in pediatric HSCT patients caused hypomagnesemia (58%), nephrotoxicity (12%), tremor (12%), hyperglycemia (4%), and hypertension (8%) in a retrospective study.[17] In another study, hypomagnesemia (98%), hypertension (49%), nephrotoxicity (34%), tremor (32%), hyperglycemia (7%), and tremor (5%) were reported.[18] Application of Naranjo algorithm might be the reason of the lower incidence of adverse drug reactions in this prospective study. Concurrent use of medications such as conditioning regimens, infection prophylactic regimens could induce several adverse drug reactions. Also the complications of an infusion with hematopoietic stem cells of cryopreserved marrow or peripheral blood include cardiac alterations, dyspnea, nausea, vomiting, allergic reactions, hypotension, hypertension, tremors, fever, chest pain, and feeling of constriction in the larynx, abdominal cramps, and exhalation of a characteristic odor for 24 to 36 hours.[19]

There were several limitations in this study. First sample size of the study did not fulfilled the number planned. Second, protocols

except for immunosuppressive regimen were different among study centers.

The strength of this study was that study population was specific. A low birth rate increased cases of unrelated donor transplantation. Furthermore stem cells derived from donor's peripheral blood reduced the inconvenience of donor compared to bone marrow. Thus study in unrelated peripheral blood stem cell transplantation might be more valuable.

In this study, we compared the efficacy and safety of tacrolimus and cyclosporine in pediatric HSCT patients. In conclusion the efficacy of tacrolimus was not superior to cyclosporine in pediatric patients and both CNIs had a low incidence of adverse drug reactions.

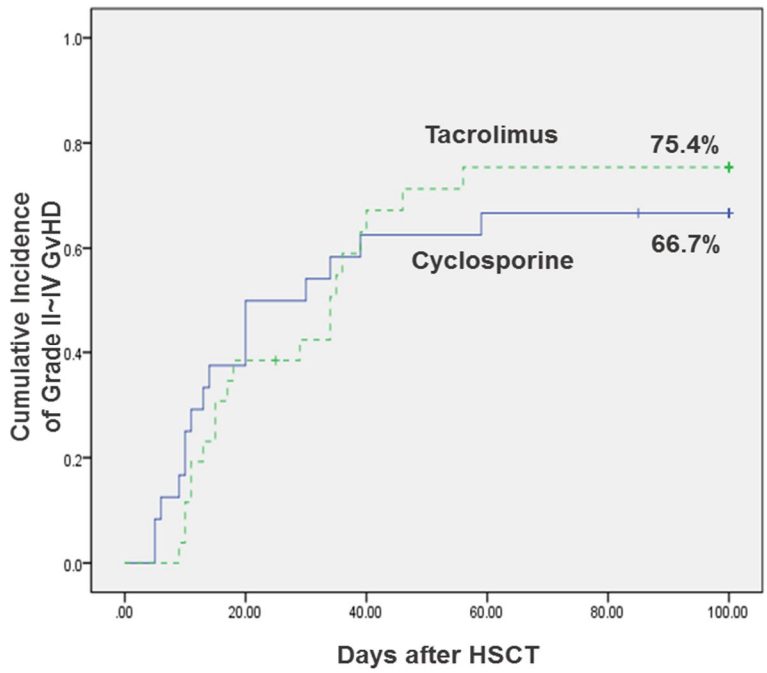
Table I-1. Patient demographics

Characteristics	Tacrolimus (N = 26)	Cyclosporine (N = 24)	*p*-value
Age, median (range)	10 (1-17)	9 (1-18)	0.365
Male, n(%)	17 (65.4)	13 (54.2)	1.0
Cell source, n(%)			1.0
Peripheral blood stem cells	24 (92.3)	23 (95.8)	
Bone marrow	2 (7.7)	1 (4.2)	
HLA matching, n(%)f			1.0
Full matched	19 (73)	17 (71)	
Conditioning regimens, n(%)			1.0
High intensity	24 (92.3)	23 (95.8)	

Table I-2. The adverse drug reactions (ADRs) and infections

Type	Tacrolimus (N = 26)	Cyclosporine (N = 24)
ADRs	0	2 (8.3%)
Skin rash	2 (7.7%)	0
Nephrotoxicity	1 (3.8%)	1 (4.2%)
Urticaria	0	1 (4.2%)
Diarrhea	0	1 (4.2%)
Thrombotic microangiography	1 (3.8%)	0
Infections		
Cytomegalovirus	7 (26.9%)	10 (41.7%)
Enterococcus	1 (3.8%)	3 (12.5%)
Klebsiella	0	1 (4.2%)
Mycoplasma	0	1 (4.2%)
BK virus	1 (3.8%)	0
Respiratory syncytial virus	1 (3.8%)	1 (4.2%)
Epstein-Barr virus	2 (7.7%)	1 (4.2%)
Fungi	0	2 (8.3%)

Figure I-1. Grade II to IV (upper) or grade III to IV (lower) acute GvHD



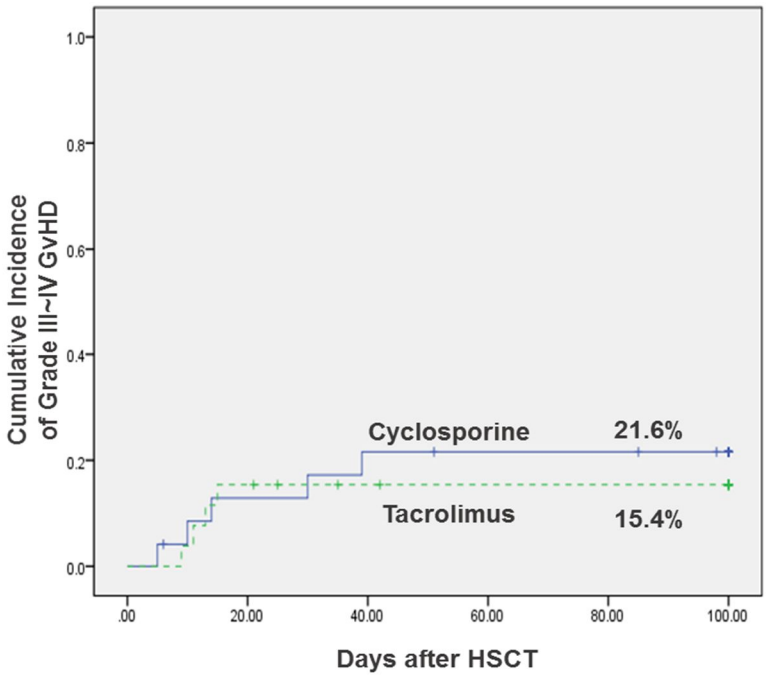
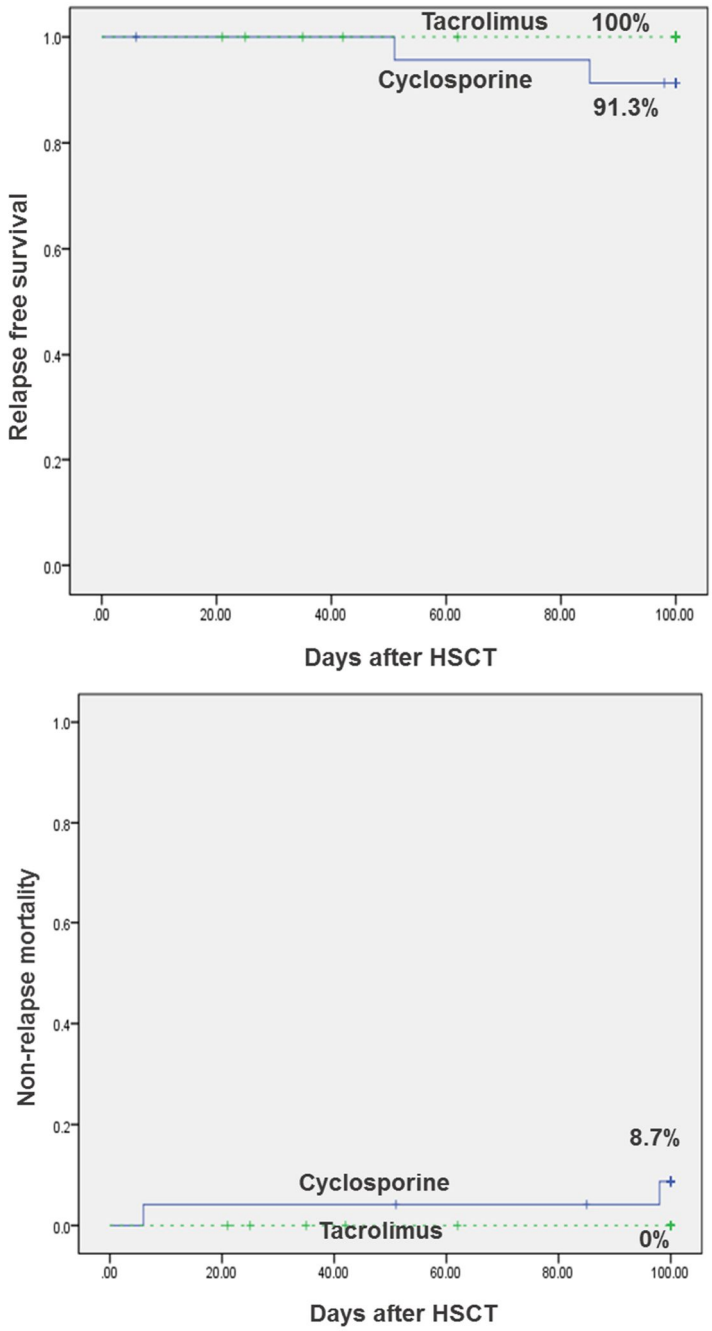


Figure I-2. Relapse free survival (upper) and non-relapse mortality (lower)



SECTION II: Model-based approach to individualized dosing

SECTION II-1: PopPK Study of Tacrolimus in Pediatric HSCT Patients

1. Introduction

Tacrolimus, a calcineurin inhibitor, is used for prevent a graft-versus-host disease (GvHD) in hematopoietic stem cell transplantation (HSCT). To prevent GvHD, maintaining adequate concentrations of tacrolimus is necessary in HSCT patients. Furthermore oversuppression can lead to serious nephrotoxicity, neurotoxicity, infections, and increased risk of further malignancies. However a narrow therapeutic range and large inter- and intra-individual pharmacokinetic variability are challenging in spite of routine therapeutic drug monitoring (TDM).

Factors suggested to affect the pharmacokinetics of tacrolimus include the population studied, hepatic function, post-transplantation days, patient age, race, hematocrit and albumin concentrations, food administration, corticosteroid dosage, diarrhea, and cytochrome P450 (CYP) isoenzyme and P-glycoprotein expression.[20]

Population pharmacokinetic analysis are adding to the understanding of the pharmacokinetics of tacrolimus. The population pharmacokinetic (PopPK) models could explain those covariate effects to pharmacokinetic parameters and suggest personalized doses. Previously many studies developed tacrolimus PopPK models in various transplantation types. Most were conducted in solid organ transplantation patients. However, the gastro-intestinal integrity of HSCT patients is physiologically different from that of solid organ transplantation patients because of barrier destroy secondary to conditioning chemotherapy, GvHD, and infections.[21] Furthermore, concurrent medications like itraconazole, a known CYP3A4 inhibitor, are usually administered to prevent infection, and can alter tacrolimus pharmacokinetics.

Furthermore pediatrics have different pharmacokinetic properties compared to adults. First absorption can be different because of the differences in gastric pH and stomach emptying time that have been observed in the pediatric population. A lower plasma protein concentrations and a higher body water composition can change drug distribution. Metabolic processes are often immature at birth, which can lead to a reduced clearance and a prolonged half-life for those drugs for which metabolism is a significant mechanism for

elimination. The expression of CYP3A4, 2C9, and 2C19 occurs during the first weeks of life. Renal excretion is also reduced in neonates due to immature glomerular filtration, tubular secretion, and reabsorption.[22]

Nevertheless, there are few PopPK studies of tacrolimus in HSCT patients. In 2001, first PopPK study was reported which included 122 HSCT patients aged 13~60 years.[23] And another PopPK study was conducted in 68 Chinese HSCT patients.[24] However there is only one study that exclusively conducted in pediatric HSCT patients. The study identified that the clearance (CL) of tacrolimus was influenced by serum creatinine and bioavailability (F) of tacrolimus was affected by post-transplantation days. According to the model, the authors concluded that current intravenous dose recommendations of 0.03 mg/kg/day may produce potentially toxic drug concentrations in this patient population, whereas current oral conversion of 4 times the adjusted intravenous dose may lead to subtherapeutic concentrations. Although the study suggested new initial dosage recommendations and a conversion factor of 6, there was a limitation that the results were deducted from 22 patients.[25]

Thus the aim of this study was to develop a PopPK model of tacrolimus in a large population of pediatric HSCT patients and

improve tacrolimus dosage regimen through the model.

2. Methods

Patients and data

Eligibility criteria were pediatric patients at age less than 18 years old, who were going to receive allo-HSCT at a tertiary care academic hospital (Seoul, Korea) between September 2006 and February 2015 and receive tacrolimus as immunosuppressant therapy. Our study was approved by the institutional review board and followed the recommendations of the Declaration of Helsinki.

Patients received tacrolimus and methotrexate for GVHD prophylaxis. Tacrolimus was administered by 24-hour continuous infusion at an initial dose of 0.03 mg/kg/day and converted to oral administration as soon as possible. The initial daily dose of oral CsA was equal to four times the last infusion dose and CsA was given in two divided doses 12 hours apart. The dose was adjusted according to trough therapeutic drug monitoring (10-20 ng/mL).

The following data were collected retrospectively from electronic medical records: age, sex, weight, height, body surface area, post-

transplantation days, white blood cells, hemoglobin, hematocrit, platelets, total bilirubin, alkaline phosphatase, alanine transaminase, aspartase transaminase, serum creatinine, glomerular filtration rate, albumin, cholesterol, gastrointestinal GvHD, concomitant steroid dose, co-administered CYP interacting drugs (azole antifungals, proton-pump inhibitors, calcium channel blockers, and phenytoin).

Dependent variable was tacrolimus trough concentrations which were analysed by liquid chromatography-tandem mass spectrometry for therapeutic drug monitoring.

Population pharmacokinetic modeling

A PopPK model was developed using the nonlinear mixed-effects modeling software program NONMEM (version. 7.3; ICON Development Solutions, Hanover, MD, USA). Parameters were estimated by the first-order conditional estimation using the interaction (FOCE-I) method. The concentration-time data after both intravenous and oral routes of administration were fitted simultaneously.

Various compartmental distribution models and absorption models were compared during base model building step. The bioavailability (F1) was estimated using logit-transform model to keep F1

between 0 and 1 for oral administration data. Inter-individual pharmacokinetic parameter variability was tested using the additive, proportional, and exponential models. Inter-occasional variability was incorporated to each week during first month and each month after that. A combined model was used for residual error.

Potential covariates were tested by forward selection and backward elimination to determine if these potential covariates affected the CsA pharmacokinetic parameters. The influence of covariates on the pharmacokinetic parameters was tested by linear, exponential, and power function models. Continuous covariates were centralized by median values and some were also analyzed as categorical covariates with normal or abnormal values. A significant level of $P < 0.05$ (ΔOBFV (objective function value) > 3.84) was used in forward selection and $P < 0.01$ (ΔOBFV (objective function value) > 6.63) was used in backward elimination process. The modeling process was controlled by statistical criteria and visual inspection.

The final model were validated using bootstrap and prediction corrected visual predictive check (pcVPC). One thousand samples for bootstrap and pcVPC were simulated by the software program PsN (Perl speaks NONMEM version. 4.2.0) and R software version. 3.1.0. The parameters estimated from the bootstrap were compared

with the parameter estimates obtained from the original data set.

3. Results

Population pharmacokinetic modeling

The characteristics of the 100 patients are presented in Table 1. A total of 2,632 therapeutic drug monitoring data were collected. Intravenous and oral tacrolimus data were successfully modeled simultaneously. A one-compartment model with fixed absorption rate constant was the best fit to the data. The estimated parameters were population mean value of clearance (CL), volume of distribution (V), log-transformed bioavailability (FLGT) and absorption rate constant for transit compartment (k_a). For inter-individual variability (IIV) and inter-occasional variability (IOV) the exponential model for CL and V yielded the smallest OBFV and the best visual fitting.

During stepwise covariate model building process, body surface area (BSA) and azole antifungals use were incorporated into CL and BSA was incorporated into V.

$CL\ (L/h) = 6.74 \times (BSA/1.102)^{0.552} \times 0.4$ (if azole antifungals use)

$V\ (L) = 1160 \times (BSA/1.102)^{0.503}$

Because weight, height, and age showed co-linearity with BSA, those covariates were excluded in the process. Although the effects of other demographic, clinical and variables on the pharmacokinetic parameters (CL, V, FLGT) of tacrolimus were evaluated during a stepwise process, significant associations were not found.

The final estimate of typical CL, V and k_a was 6.74 L/h, 1,160 L, and 4.48 h^{-1}, respectively. The typical value of logit-transformed bioavailability of oral tacrolimus was estimated as −0.529 thus the value of F1 was 0.371. The final estimated pharmacokinetic parameters, IIV, and residual errors are presented in Table 2.

Model evaluation

The diagnostic plots of the final model which show the relationship between the observed concentrations (DV) and population model-predicted concentration (PRED) or individual model-predicted concentration (IPRED) indicated a good fit between the model and the data (Figure 1). The plots of conditional weighted residuals (CWRES) vs. PRED and the CWRES vs. time were symmetrically distributed and were mostly within 3 units of the null ordinate, indicating a good fit of the model to the data (Figure 1).

Most of the 1,000 bootstraps ran successfully. The 2.5th percentile and 97.5th percentile results are described in Table 2. The estimates of each value calculated by NONMEM were near the center of the bootstrap runs. The pcVPC showed the median and 95th percentile of the simulated data captures the median and 95th percentile of the observed PK data while the simulated 5th percentile value was less than the observed data (Figure 2).

4. Discussion

This study was the first large PopPK study of tacrolimus in pediatric HSCT patients. The base model of tacrolimus was one-compartment model with fixed k_a, because concentration data were collected retrospectively and most of them were trough concentration. The IIV and IOV were incorporated into CL and V with exponential model. The IOV incorporation into the model reduced statistically significant spurious period effects and it is the strength of this study.

In the final model, BSA affected both CL and V of tacrolimus. Over the years, many studies revealed that BSA was related with CL and V of drugs.[26] In pediatric oncology, the typical range of BSA is 0.4–

2 m^2. In such a population, for almost any drug, there is a correlation between CL and BSA. It is obvious that for children a scale parameter such as BSA or weight for dose calculation is absolutely necessary.[27]

Azoles antifungal agents also significantly affected CL of tacrolimus. The use of azoles reduced CL by 60%. Tacrolimus is a well-known drug that is metabolized by CYP3A4 and azoles antifungal agents inhibit the enzyme activity. Especially most patients received itraconazole which is a potent inhibitor of CYP3A4.[28]

The estimated value of bioavailability was as high as 37.1%, which was higher than common value of 25%. In normal gut, enteral metabolism of tacrolimus by gastrointestinal CYP3A isoenzymes and excretion to gut lumen by P-glycoprotein transporter is extensive. Especially P-glycoprotein lowers intracellular concentration of tacrolimus by pumping absorbed drug back out into the gut lumen. However conditioning chemotherapy, gastrointestinal infections because of low immune activity, and gastrointestinal GvHD damage to CYP3A isoenzyme and P-glycoprotein transporter. The lower gastrointestinal intensity in HSCT patients explains higher bioavailability.

Several limitations existed in this study. First because of the nature of retrospective study, most of concentration data were the trough concentration. Thus the estimation of absorption phase was constrained and literature value of absorption rate constant was used. Second information about factors affecting the pharmacokinetics of tacrolimus such as diarrhea, genetic polymorphisms were not evaluated. Pharmacokinetics of tacrolimus may be influenced by diarrhea because of changes in gastrointestinal transit time. And genetic differences in CYP3A and P-glycoprotein expression also affect pharmacokinetic properties of tacrolimus.[20]

This study showed that BSA significantly influenced the CL and V of tacrolimus in the model. Thus BSA-based dosage regimen of intravenous and oral tacrolimus would be appropriate in pediatric HSCT patients. Furthermore dosage adjustments are necessary when azoles antifungal agents initiate or discontinue. High bioavailability suggests that the conventional oral conversion ratio may result in high tacrolimus concentration.

Table II-1. Patient demographics (N = 100)

Characteristics	N = 100
Male, n (%)	53 (53.0)
Age, median (range), year	10 (0.58-17.99)
Body weight, median (range), kg	33.7 (7.65-72.4)
Height, median (range), cm	142.2 (66.1-181.9)
Disease type, n (%)	
Leukemia	65 (65.0)
Aplastic anemia	20 (20.0)
Others	15 (15.0)

Table II-2. Final parameter estimates for tacrolimus

Parameter	Estimate (RSE %)	95% CI	Bootstrap Median (2.5^{th}, 97.5^{th})
CL (L/h)	6.74 (10)	5.42, 8.06	6.74 (5.52-8.05)
V (L)	1160 (6)	1024, 1296	1163 (1035-1301)
FLGT	-0.53 (42)	-0.96, -0.09	-0.53 (-0.94, -0.1)
k_a (h^{-1})	4.48 fix		
Impact of azoles on CL	0.4 (16)	0.27, 0.53	0.39 (0.29, 0.54)
Impact of BSA on CL	0.55 (13)	0.41, 0.69	0.55 (0.39, 0.69)
Impact of BSA on V	0.50 (15)	0.35, 0.65	0.50 (0.35, 0.64)
IIV of CL (CV%)	30.2 (9)	24.9, 35.5	29.5 (24.5, 35.0)
IIV of V (CV%)	31.9 (18)	20.6, 43.2	31.3 (20.3, 43.4)
IOV of CL, V (CV%)	30.1 (7)	26.0, 34.2	30.0 (26.5, 34.4)
Residual error			
Proportional	0.32 (3)	0.30, 0.34	0.32 (0.30, 0.34)

(%)			
Additive (ng/mL)	0.60 (16)	0.41, 0.79	0.58 (0.36, 0.76)

CI, confidence interval; CL, clearance; CV, coefficient of variation; FLGT, log-transformed bioavailability; IIV, inter-individual variability; IOV, inter-occasional variability; k_a, absorption rate constant; RSE, relative standard error; V, volume of distribution

Figure II-1. Goodness-of-fit plots of the model

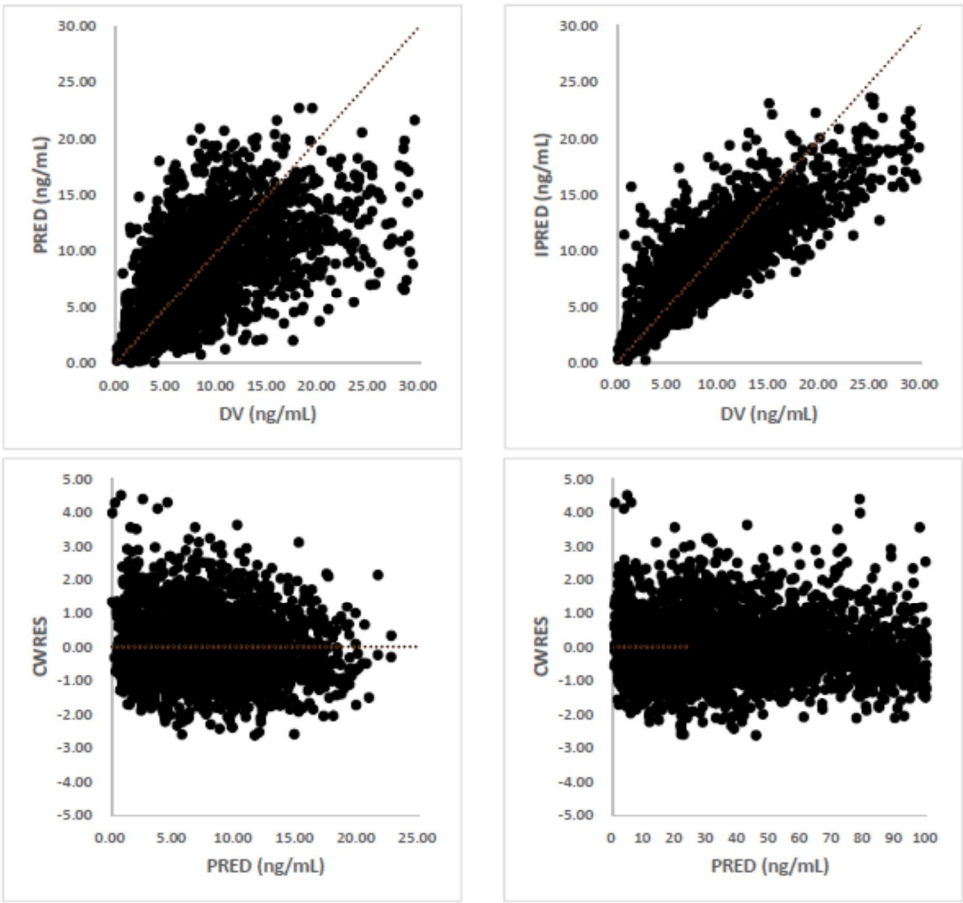
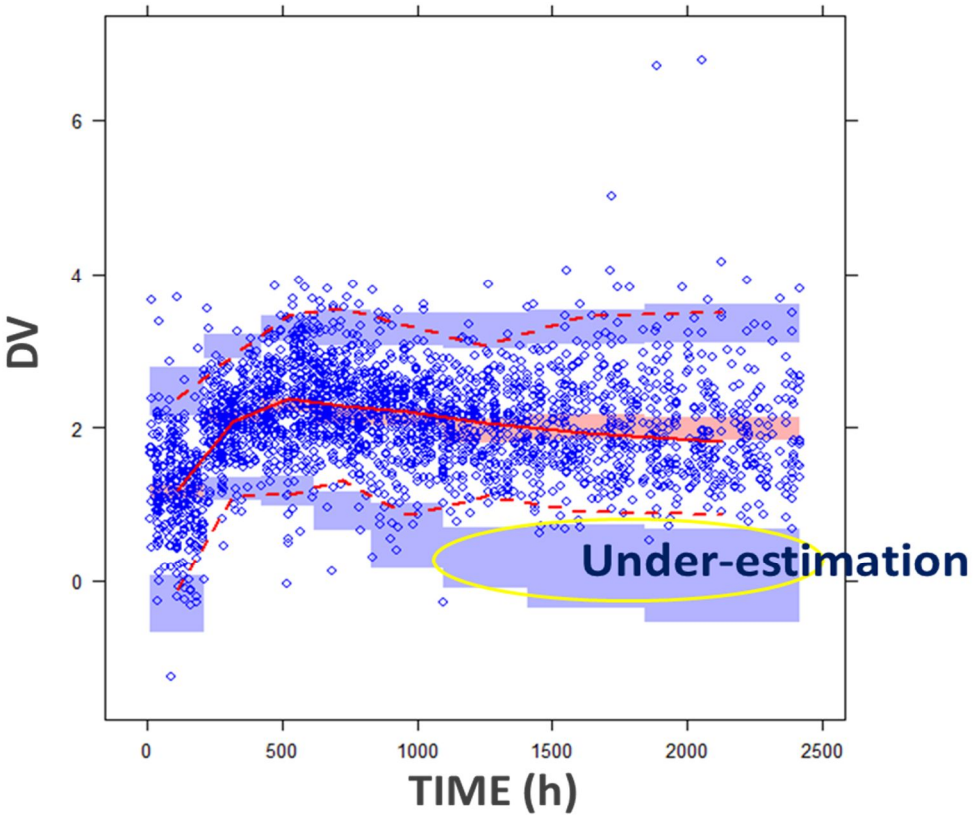


Figure II−2. Visual predictive check of the final model



SECTION II-2: PopPK Study of Cyclosporine in Adult HSCT Patients

1. Introduction

Cyclosporine (CsA) is a commonly used immunosuppressant for prevention of graft-versus-host disease (GVHD) in allogeneic hematopoietic stem cell transplants (allo-HSCT).[29] CsA has a narrow therapeutic range and large inter- and intra-individual pharmacokinetic variability.[30] Many studies have examined the role of genetic polymorphisms in CsA pharmacokinetic variations. *CYP3A5* *3 polymorphism, a single nucleotide polymorphism (SNP) in the gene encoding the enzyme CYP3A5, is the most studied and influential polymorphism.[31] Because vitamin D receptors (VDR) control the expressions of several CYP genes, *VDR* genetic polymorphisms could also affect CsA pharmacokinetics.[32] Other important genetic polymorphisms are variations in *ABCB1*, which encodes the drug transporter P-glycoprotein (P-gp). P-gp has a role in preventing oral absorption and promoting clearance into bile.[33] In *ABCB1*, the most influential SNPs are 3435C>T,

2677G>T/A, and 1236C>T.[34] Furthermore, several studies have also evaluated the effects of other genetic polymorphisms in *CYP2C19*, *CYP2C8*, *ABCC2*, and *TGFB1* on CsA pharmacokinetics.[35,36]

Because of individual variability, it is challenging to maintain CsA levels adequate enough to suppress the immune system while avoiding side effects. Population pharmacokinetic (PopPK) modeling appears to be the best method to predict individual CsA pharmacokinetic parameters.[37] At present, PopPK models for CsA identified in our PubMed and Embase searches have been established mostly in solid organ transplants. However, the intestinal integrity of allo-HSCT patients is physiologically different from that of solid organ transplant patients because of mucositis secondary to conditioning regimen, GVHD, and infections.[21] Furthermore, concurrent medications like fluconazole, a known CYP3A4 and CYP2C19 inhibitor, are usually administered to prevent infection, and can alter CsA pharmacokinetics. Nevertheless, PopPK studies of CsA in allo-HSCT are scarce and only one PopPK study has evaluated genetic polymorphisms as covariates in allo-HSCT.

Therefore, the goal of our study was to build a PopPK model of CsA in allo-HSCT in consideration of *CYP3A5*, *CYP2C19, VDR, ABCB1,* and *ABCC2* genetic polymorphisms and demographic and clinical data.

2. Methods

Patients and data

Eligibility criteria were patients at age 18 years or older, who were going to receive allo-HSCT at a tertiary care academic hospital (Seoul, Korea) between November 2009 and March 2011 and receive CsA (Neoral; Novartis Pharma, Basel, Switzerland) as immunosuppressant therapy. Exclusion criteria included current significant disease that could affect the study: severe psychiatric comorbidity; addiction to drugs or alcohol; and pregnancy or breast-feeding. All participants gave written informed consent. Our study was approved by the institutional review board and followed the recommendations of the Declaration of Helsinki.

Patients received CsA and methotrexate for GVHD prophylaxis. CsA was administered by 24-hour continuous infusion and converted to oral administration as soon as possible. The initial

daily dose of oral CsA was equal to two to three times the last infusion dose and CsA was given in two divided doses 12 hours apart at least one hour after meals. The dose was adjusted according to trough therapeutic drug monitoring (150–400 ng/mL). The following data were collected from electronic medical records: age, sex, weight, height, red blood cells, hemoglobin, hematocrit, platelets, total bilirubin, alkaline phosphatase, alanine transaminase, aspartase transaminase, serum creatinine, glomerular filtration rate, albumin, cholesterol, concomitant steroid dose, co-administered CYP interacting drugs.

CsA blood concentration assay

Whole blood samples (1 mL) were transferred into Vacutainer tubes with sodium heparin (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) at one time point during continuous infusion, prior to dosing and 1, 2, 3, 4, and 5 h after dosing when the oral dose reached steady state. Steady state was assumed to be reached in 4–5 half-lives after regular dosing is started (median half-life 4.43 h).[38] All samples were stored at −70° C until analysis. CsA concentrations in whole blood were analyzed by liquid

chromatography coupled with tandem mass spectrometry (LC–MS/MS). LC was performed using a Waters 2795 Alliance HT LC system (Waters, Milford, CT, USA) with a C18 column (5 μm, 2.1 mmφ × 150 mm). The Waters Quatro Premier XE system (Waters, Milford, CT, USA) was used to measure analytes as positive ions by MS/MS. The lower limit of quantification was 100 ng/mL. Between 100–5000 ng/mL, the accuracy was 98.5%–103.3% and the precision coefficient of variation was 0.8%–2.4%.

Genotyping

Whole blood samples were collected for genotyping before stem cell transplantation. The genotype of the *CYP3A5* *1/*3 polymorphism was screened using a TaqMan fluorogenic 5′ nuclease assay (Life Technologies, Grand Island, NY, USA). The 5 μL polymerase chain reaction (PCR) contained 10 ng of genomic DNA and 2.5 μL of TaqMan Universal PCR Master Mix with 0.13 μL of 40X Assay Mix (Assay ID C_26201809_30). The thermal cycling conditions were as follows: initial incubation at 50° C for 2 min and denaturation at 95° C for 10 min, followed by 45 cycles of annealing at 95° C for 15 s and extension at 60° C for 1 min. All PCRs were performed in

a Dual 384-Well GeneAmp PCR System 9700 (Life Technologies), and the endpoint fluorescent readings were performed on an ABI PRISM 7900 HT Sequence Detection System (Life Technologies). Polymorphism genotyping for the *CYP2C19* *1/*2/*3 variant, *ABCB1* 3435C>T, 1236C>T, and 2677G>T/A, *ABCC2* -24C>T and 1249G>A, and *VDR BsmI* and *ApaI* was analyzed by a SNaPshot assay (ABI PRISM SNaPshot Multiplex Kit, Life Technologies). The SNaPshot assay was performed according to the manufacturer's instructions. Analysis was carried out using GeneMapper software (version 4.0; Life Technologies). Duplicate samples and negative controls were included to ensure accuracy in genotyping.

Population pharmacokinetic modeling

A PopPK model was developed using the nonlinear mixed-effects modeling software program NONMEM (version. 7.2; ICON Development Solutions, Hanover, MD, USA). Parameters were estimated by the first-order conditional estimation using the interaction (FOCE-I) method. Because the concentration-time data after both intravenous and oral routes of administration were fitted simultaneously, subroutine ADVAN13 of the NONMEM program

was selected to describe the pharmacokinetics of CsA expressed by differential equation.

One- vs. two-compartmental distribution models and lagged time vs. transit compartment absorption models were compared during base model building step. The bioavailability (F1) was fixed to 1 for intravenous data or estimated using logit-transform model to keep F1 between 0 and 1 for oral administration data. Inter-individual pharmacokinetic parameter variability was tested using the additive, proportional, and exponential models. A combined model was used for residual error.

Age, sex, weight, height, red blood cells, hemoglobin, hematocrit, platelets, total bilirubin, alkaline phosphatase, alanine transaminase, aspartate transaminase, serum creatinine, glomerular filtration rate, albumin, cholesterol, concomitant steroid dose, co-administered CYP interacting drugs, and genetic polymorphisms were tested by forward and backward selection to determine if these potential covariates affected the CsA pharmacokinetic parameters. The influence of covariates on the pharmacokinetic parameters was tested by linear, exponential, and power function models. Continuous covariates were centralized and some were also analyzed as categorical covariates with normal or abnormal values.

Genetic covariates including *CYP3A5* *1/*3, *CYP2C19* *1/*2/*3, *ABCB1* 3435C>T, 1236C>T, 2677G>T/A, *ABCC2* −24C>T, 1249G>A, and *VDR BsmI* and *ApaI* polymorphisms, and diplotype combinations of the three *ABCB1* variants were analyzed as categorical covariates. When the number of patients with homozygous mutant was less than five, the dominant model (wild type vs. heterozygous + homozygous mutant) was used to evaluate mutation effect. Further combination of *CYP3A5, CYP2C19* genotype, and use of CYP interacting drugs was also analyzed. A significant level of $P < 0.05$ (ΔOBFV (objective function value) > 3.84) was used in forward and backward selection process. The modeling process was controlled by statistical criteria and visual inspection.

The stability and performance of the developed final model were evaluated using bootstrap and prediction corrected visual predictive check (pcVPC). One thousand samples for bootstrap and pcVPC were simulated by the software program PsN (Perl speaks NONMEM version. 4.2.0) and R software version. 3.1.0. The parameters estimated from the bootstrap were compared with the parameter estimates obtained from the original data set.

3. Results

Demographics

The characteristics of the 34 patients are presented in Table 1. A total of 104 blood samples were collected at a median of 2.5 days (range: 1.5–3.5 days) after intravenous CsA was started and when converted to oral formulation. All samples were collected at more than 4–5 half-lives of CsA. The median (range) dose of intravenous CsA was 230 mg/day (145–400 mg/day) and that of oral CsA was 300 mg/day (150–700 mg/day), respectively. Nine individuals (26.5%) were taking steroids at the time of sampling. Prednisolone, methylprednisolone, and dexamethasone were used, and the prednisolone converted dose ranged from 5 to 62.5 mg. Fluconazole, a CYP isoenzyme inhibitor, was administered orally to 19 patients for infection prophylaxis at the sampling time for intravenous CsA. Voriconazole was administered orally to two patients to treat invasive aspergillosis at the sampling time for oral CsA. Among them, one patient used both fluconazole and voriconazole, but the drugs were not administered at the same time. Phenobarbital, which induces CYP isoenzymes, was co-

administered to two patients. All genotype frequencies of *CYP3A5*, *CYP2C19, ABCB1, ABCC2,* and *VDR* were in Hardy-Weinberg equilibrium. Allelic frequencies of these genes and Hardy-Weinberg equilibrium data are presented in Table 2.

Population pharmacokinetic modeling

Intravenous and oral CsA data were successfully modeled simultaneously. A one-compartment model with 2 transit compartments for delayed oral absorption and central elimination provided the best fit to the data (Figure 1). The estimated parameters were population mean value of clearance (CL), volume of distribution (V), log-transformed bioavailability (FLGT) and rate constant for transit compartment (k_{tr}). For inter-individual variability (IIV), the exponential model for CL and V yielded the smallest OBFV and the best visual fitting.

Demographic, clinical, and genetic polymorphisms data were tested during stepwise covariate model building process. However only actual body weight was incorporated into CL as power function model with the exponent value of 0.419.

$$CL = THETA(1) \times (weight/70)^{0.419} \times EXP(ETA(1))$$

Although the effects of other demographic, clinical and genetic

variables on the pharmacokinetic parameters (CL, V, FLGT, k_{tr}) of CsA were evaluated during a stepwise process, significant associations were not found. Fluconazole also did not show significant effects either individually or compositely with voriconazole.

The final estimate of typical CL, V and k_{tr} was 21.2 L/h, 430 L, and 2.87 h^{-1}, respectively. The typical value of logit-transformed bioavailability of oral CsA was estimated as 1.49 thus the value of F1 was 0.81. The final estimated pharmacokinetic parameters, IIV, and residual errors are presented in Table 3.

Model evaluation

The diagnostic plots of the final model which show the relationship between the observed concentrations (DV) and population model-predicted concentration (PRED) or individual model-predicted concentration (IPRED) indicated a good fit between the model and the data (Figure 2). The plots of conditional weighted residuals (CWRES) vs. PRED and the CWRES vs. time were symmetrically distributed and were mostly within 3 units of the null ordinate, indicating a good fit of the model to the data (Figure 2).

Most of the 1,000 bootstraps ran successfully (82.1%). The 2.5th percentile and 97.5th percentile results are described in Table 2. The estimates of each value calculated by NONMEM were near the center of the bootstrap runs. The pcVPC showed the median and 95th percentile of the simulated data captures the median and 95th percentile of the observed PK data while the simulated 5th percentile value was less than the observed data (Figure 3).

4. Discussion

This study was implemented prospectively to establish a PopPK model of CsA in allo-HSCT patients. Although genetic polymorphisms may influence CsA pharmacokinetics, only one study by Xue *et al.* has evaluated genetic polymorphisms as covariates in allo-HSCT patients.[39] Our model is a simpler model with fewer proportional residual errors than the previous study. Furthermore, we could estimate an absorption rate as k_{tr} because most of our data were collected by dense sampling while the previous study used a fixed absorption rate. Analysis of CsA concentration measured by LC-MS/MS in our study is another strength because the immunoassay used in the previous study could overestimate CsA concentrations due to cross-reactivity.

A one-compartment model with 2 transit absorption compartments fitted better than a multi-compartment model because of insufficient blood sampling during the terminal elimination phase. A transit compartment model might be preferable over a lagged absorption model because numerical instability could arise from the discontinuous feature of the lagged absorption model.[40] Irtan *et al.* and Saint-Marcoux *et al.* developed transit compartment models for CsA in heart, lung, and kidney transplant patients. The mean transit time (= k_{tr} / (number of transit compartment + 1)) in our study was 0.96 h^{-1}, which is similar to those of previous studies (1.17 h^{-1} and 0.88 h^{-1}, respectively).[41,42] The CL value in our study ranged from 17.8 L/h to 22.7 L/h when weight increased from 46.3 kg to 82.75 kg and the value is similar to the previously determined CL for CsA in allo-HSCT patients (CL = 21.9, 28.2, and 22.3 L/h) and cardiopulmonary, liver, and kidney transplant patients (CL = 22.1, 23.1, and 23.7 L/h, respectively).[37,43-47]

The bioavailability of the microemulsion formulation of CsA (Neoral) was variable and usually estimated to be about 40%.[48] Thus a ratio of 2 to 1 was commonly used when patients were switch from intravenous to oral CsA.[21] However, the estimated bioavailability value in our study was relatively high as 81% and similarly high

bioavailability values were also reported in previous CsA PopPK studies in allo-HSCT patients (71% in two studies and 75.4% in another study).[39,44,49] When we assumed that oral mucositis after the conditioning regimen represents gastrointestinal (GI) inflammation, all patients in our study had GI inflammation (only four with diarrhea) at the time of blood collection. These patients might have high bioavailability because of GI inflammation as suggested in previous research.[50] CsA maximum concentration was 65% (P=0.19) and area under the curve of a concentration-time graph was 71% (P=0.02) higher in those who had GI inflammation.[50] There are four factors that might increase CsA absorption during inflammatory state. The first one is capillary permeability, which is generally increased by inflammation.[23] Second, inflammation may reduce the P-gp mediated multidrug resistance efflux function in the intestinal wall epithelium, resulting in reduced pumping of CsA from the enterocyte into the intestinal lumen.[23] Third, CYP3A function and intra cellular metabolism of CsA may be decreased within the enterocyte because of inflammation, allowing for increased drug absorption.[50] Finally, decreased gut motility due to inflammation may result in prolonged CsA transit through the intestine, leading to increased absorption.[50] Although

diarrhea could decrease CsA absorption because of shortened gut transit time, the small portion of diarrhea patients did not show significant effects on CsA pharmacokinetics in our study. Thus re-evaluating the conversion ratio of oral CsA might be necessary in allo-HSCT protocols.

The relationship between weight and CsA pharmacokinetics is still controversial.[37,43,44,49] Jacobson *et al.* found that weight had a significant effect in the CL of CsA in allo-HSCT patients.[9] Other studies performed in solid organ transplantation patients also found an association between weight and the CL of CsA.[51-53] However Zhou *et al.* did not find that weight had a significant effect on the CL of CsA.[54] This may be because the weight of most patients' in the study was mostly in the range of 50-60 kg.[54] With a weight range of 46.3-82.75 kg, we found a significant relationship between weight and CL of CsA. The exponent value of 0.419 which reflects the effect of weight on CL of CsA was similar to a previous exponent value found in Chinese kidney transplant patients.[51]

None of the genotype covariates were found to be significant. There have been conflicting findings about *CYP3A5* and *ABCB1* genetic effects on CsA pharmacokinetics although several studies have been conducted.[51,52,54-56] Other candidate covariates also were not

considered to be significant in our study, in accordance with previous prospective PopPK studies in allo-HSCT. Wilhelm *et al.* and Eljebari *et al.* evaluated candidate covariates such as weight, body surface area, enzyme inducers and inhibitors, age, sex, serum creatinine, bilirubin, liver enzymes, and post-transplantation day, but did not find any significant covariate in the PopPK model of CsA.[43,49]

The current study has some limitations. First, blood sampling time during oral CsA administration would miss a major part of CsA distribution and elimination phases and also potentially the enterohepatic recycling profile. Second, patients of the same ethnicity and from a single center might cause selection bias in our study. Further external validation is needed to apply our model to other settings. Third, more caution is needed for interpreting the diagnostics because eta-shrinkage was more than 20-30%; that means inter-individual variability data were less informative. Finally, a small number of patients might reduce the chance of finding the genotype-related covariates, which is reflected in remaining random error of the final model. However the prospective study design had reduced bias and accuracy of the data was the strength of this study. Because of the PopPK study design and one

compartment model, 34 patients were enough to perform a reasonable PopPK of CsA. Bréant *et al.* noted that for a one compartment model with V and CL parameters, 15 to 20 patients with 2 blood levels may be enough to perform a reasonable PopPK analysis.[57]

Our study showed that weight significantly influenced the CL of CsA in the model. Thus weight-based dosage regimen of intravenous and oral CsA would be appropriate in allo-HSCT patients. However, high bioavailability suggests that the conventional oral conversion ratio may result in high CsA concentration. Genetic polymorphisms did not affect CsA pharmacokinetics in allo-HSCT patients. Further large prospective studies are needed to validate the results of this study.

Table II-3. Patient demographics (N = 34)

Male, n (%)	19 (55.9)
Age, median (range), year	36 (18-62)
Body weight, mean ± SD, kg	61.0 ± 10.6
Height, mean± SD, cm	165.4 ± 9.7
Donor type, n (%)	
Sibling donor	16 (47.1)
Unrelated donor	17 (50.0)
Haploidentical donor	1 (2.9)
Disease type, n (%)	
AML	8 (23.6)
ALL	10 (29.4)
ABL	2 (5.9)
MDS	3 (8.8)
AA	6 (17.6)
Others (IMF, NK-cell leukemia, PNH, lymphoma)	5 (14.7)
Conditioning regimen, n (%)	
Busulfan, cyclophosphamide	13 (38.2)
Busulfan, fludarabine	16 (47.1)
Fludarabine, cyclophosphamide	5 (14.7)

Laboratory test values, mean ± SD	
Red blood cells, $\times 10^6/\mu L$	2.9 ± 0.4
Hemoglobin, g/dL	9.2 ± 1.3
Hematocrit, %	27.2 ± 3.9
Platelets, $\times 10^3/\mu L$	100.5 ± 76.8
Total bilirubin, mg/dL	0.8 ± 0.3
Alkaline phosphatase, IU/L	81.9 ± 50.8
Alanine transaminase, IU/L	77.8 ± 84.2
Aspartate transaminase, IU/L	47.2 ± 54.2
Serum creatinine, mg/dL	0.95 ± 0.6
Glomerular filtration rate, mL/min/1.73 m^2	98.7 ± 40.6
Albumin, g/dL	3.6 ± 0.4
Cholesterol, mg/dL	160.4 ± 39.5

AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; ABL, acute biphenotypic leukemia; MDS, myelodysplastic syndrome; AA, aplastic anemia; IMF, idiopathic myelofibrosis; PNH, paroxysmal nocturnal hematuria

Table II−4. Patient’s genotype frequencies

Gene	Variants	N (%)	P-value
CYP3A5	*1/*1	23 (67.7)	0.10
	*1/*3	8 (23.5)	
	*3/*3	3 (8.8)	
CYP2C19	*1/*1	18 (52.9)	0.26 (*2)
	*1/*2	11 (32.4)	0.12 (*3)
	*1/*3	4 (11.8)	
	*3/*3	1 (2.9)	
ABCB1 1236C>T	CC	2 (5.8)	0.44
	CT	16 (47.1)	
	TT	16 (47.1)	
ABCB1 3435C>T	CC	13 (38.2)	0.98
	CT	16 (47.1)	
	TT	5 (14.7)	
ABCB1 2677G>T/A	GG	4 (11.8)	0.09
	GT	15 (44.1)	
	GA	8 (23.5)	
	TT	5 (14.7)	
	AA	1 (2.95)	
	AT	1 (2.95)	
ABCC2	CC	17 (50.0)	0.54
-24C>T	CT	13 (38.2)	

	TT	4 (11.8)	
ABCC2	GG	30 (88.2)	0.72
1249G>A	GA	4 (11.8)	
VDR ApaI G>T	GG	18 (53.0)	0.77
	GT	13 (38.2)	
	TT	3 (8.8)	
VDR BsmI G>A	GG	29 (85.3)	0.64
	GA	5 (14.7)	

CYP, cytochrome P450

Table II-5. Final parameter estimates for cyclosporine

Parameter	Estimate (RSE %)	95% CI	Bootstrap Median (2.5th-97.5th)[a]
CL (L/h)	21.2 (18)	13.6-28.8	21.6 (9.7-29.8)
V (L)	430 (33)	146-714	446 (224-803)
FLGT	1.49 (80)	-0.89-3.87	1.93 (0.36-11.8)
k_{tr} (h^{-1})	2.87 (31)	1.09-4.65	2.92 (1.43-4.36)
Impact of weight on CL	0.419 (42)	0.067-0.771	0.449 (0.02-1.44)
IIV of CL (CV%)	40.2 (33)	13.7-66.7	39.5 (4.6, 67.1)
IIV of V (CV%)	55.1 (29)	23.1-87.1	56.8 (21.1, 103.8)
Residual error			
Proportional (%)	13.2 (41)	2.4-24.0	15.0 (1.2-40.0)
Additive (ng/mL)	35.8 (77)	-19-91	42.6 (1.5-149.2)

CI, confidence interval; CL, clearance; CV, coefficient of variation; FLGT, log-transformed bioavailability; IIV, inter-individual

variability; k_{tr}, rate constant for transit compartment; RSE, relative standard error; V, volume of distribution

[a] percentile

Figure II-3. Compartmental model of the final model

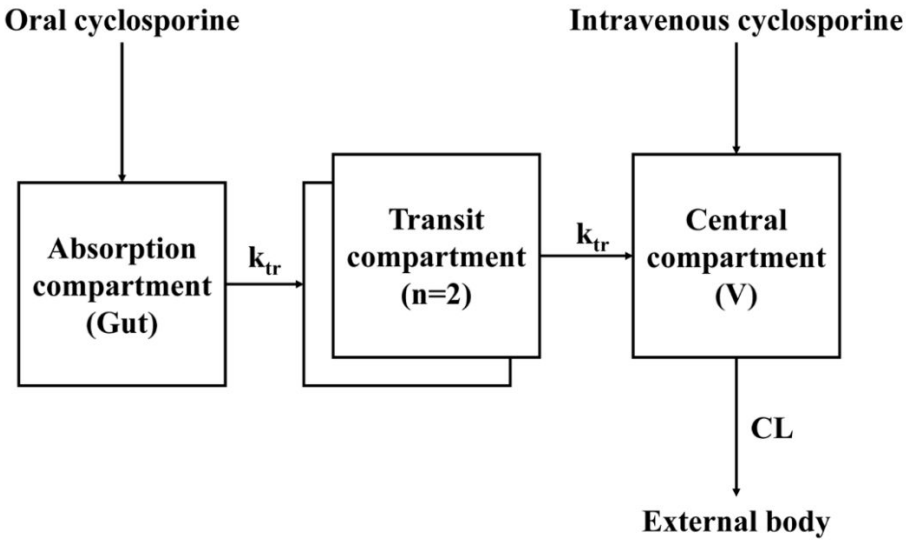


Figure II-4. Goodness-of-fit plots of the model

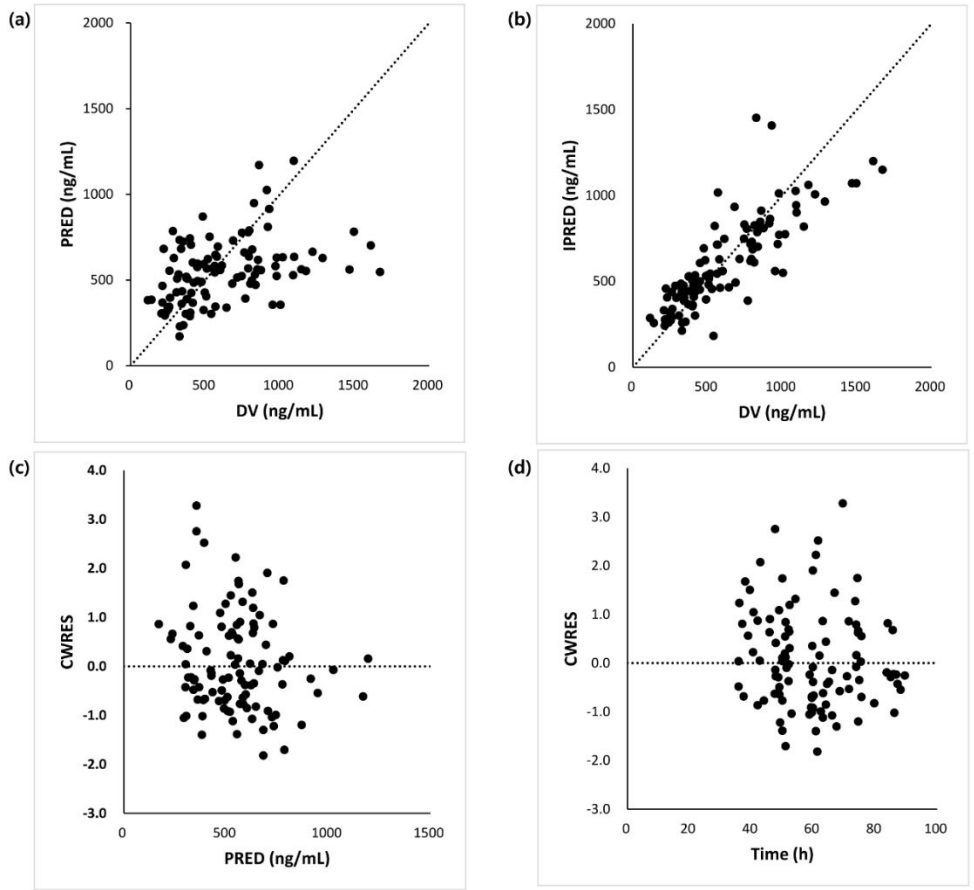
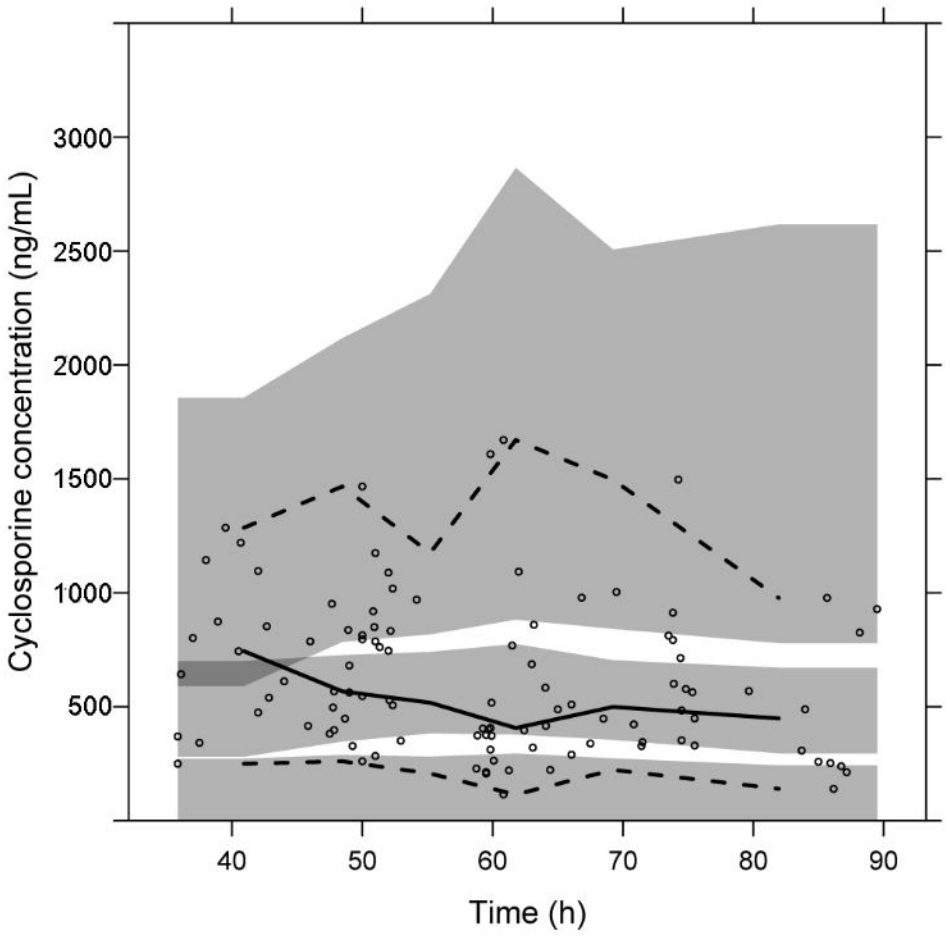


Figure II-5. Visual predictive check of the final model



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요약

이식편대숙주질환 예방에서의 칼시뉴린 억제제 효과, 안전성 및 집단약동학 연구

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1. 소아 조혈모세포이식 환자에서의 타크로리무스(tacrolimus)와 사이클로스포린(cyclosporin)의 효과, 안전성 비교 임상 연구

혈액종양질환에서 조혈모세포이식은 질병 완치를 위한 유일한 방법으로 급성림프구성백혈병을 포함한 소아 혈액종양질환 환자에서 빈번히 사용되고 있다. 그러나 조혈모세포이식으로 인해 발생하는 이식편대숙주질환(graft versus host disease), 그 중에서도 특히 이식 후 100일 이내에 발생하는 II~IV 등급의 급성 이식편대숙주질환은 총 생존기간과 이식 관련 사망률에 밀접한 연관이 있는 합병증이다.

칼시뉴린억제제(calcineurin inhibitor)에 속하는 타크로리무스(tacrolimus)와 사이클로스포린(cyclosporin)은 조혈모세포이식 후 이식편대숙주질환 예방 목적으로 일반적으로 사용되는 면역조절제이다. 현재 소아 조혈모세포이식 환자에 대해서는 'EBMT(European Group for Blood and Marrow Transplantation)'에서 사이클로스포린/메토트렉세이트(methotrexate) 요법을 이식편대숙주질환 예방의 일차적 요법으로 추천하고 있다.

그러나 타크로리무스 기반의 면역조절요법에 대한 연구가 증가함에 따라, 최근에 수행된 메타 분석 연구에서는 타크로리무스 병용요법이 사이클로스포린 병용요법보다 이식편대숙주질환 예방에 효과적임이 보고하고 있다. 메타 분석에 포함된 기존에 수행된 두 약제간의 비교 임상 연구는 주로 성인을 대상으로 수행되었으며, 소아 조혈모세포이식 환자에서도 타크로리무스가 사이클로스포린에 비해 이식편대숙주질환 예방에 더 효과적이고 안전할 것인지에 대해서는 확립되어 있지 않다. 따라서 본 연구는 소아 조혈모세포이식 환자에서 타크로리무스와 사이클로스포린의 안전성과 유효성을 비교하기 위하여 수행되었다.

본 연구는 전향적 다기관 활성대조군 비교 연구로 2011년부터 2013년까지 3개 병원에서 수행되었다. 연구 참여 대상자는 비혈연간 동종 조혈모세포 이식 후 발생하는 이식편대숙주질환 예방을 필요로 하는 악성혈액질환 환자로 이식 시 연령이 만 18세 이하인 남아 또는 여아, 혈액학적 질환으로 비혈연간 동종 조혈모세포 이식을 받을 자로

선정하였다. 피험자 수는 35%의 이식편대숙주질환 발생률 차이를 검정하기 위해 탈락률을 고려하여 각 군당 26명씩 산출하였다.

본 연구의 1차 유효성 평가변수는 피부, 간, 위장관의 II~IV 등급의 급성 이식편대숙주질환의 발생률로 Keystone criteria에 따라 평가하였고, 안전성 평가변수로는 이상반응의 발생 빈도와 감염의 발생 빈도를 평가하였다. 통계 분석을 위해 SPSS version 19를 사용하였으며, modified intention-to-treat (ITT) 분석을 통해 $P < 0.05$ 일 때 통계적으로 유의한 것으로 하였다.

본 연구에 모집된 환자 중 이식 후 생착이 일어나지 않아 이식편대숙주질환이 발생하지 않는 환자를 제외한 modified ITT 집단(타크로리무스군 18명, 사이클로스포린군 21명)에 대하여 분석을 시행하였다. 대상 임상시험 대상자의 인구학적 특성(나이, 성별, 키, 체중, 진단명, 조혈모세포 기원, HLA 적합도)는 두 집단에서 통계적으로 유의한 차이가 없었다.

1차 유효성 평가변수인 Ⅱ~Ⅳ 등급의 급성 이식편대숙주질환은 χ^2과 Fisher's exact test에 따라 타크로리무스군의 83.3%(n=13), 사이클로스포린군의 66.7%(n=14)에서 발생하였으나 유의한 차이를 보이지는 않았다 (p=0.29). 중도탈락 된 환자를 고려한 Ⅱ~Ⅳ 등급의 이식편대숙주질환 발생률을 Kaplan-Meier test로 분석하였을 때 타크로리무스군의 83.3%, 사이클로스포린군의 74.8%에서 Ⅱ~Ⅳ 등급의 이식편대숙주질환이 발생하였으며, 역시 유의한 차이는 보이지

않았다 (p=0.994). III~Ⅳ 등급의 중증 급성 이식편대숙주질환은 χ^2과 Fisher's exact test에 따라 타크로리무스군의 27.8%(n=5), 사이클로스포린군의 19.0%(n=4)에서 발생하였으나 유의한 차이를 보이지는 않았다 (p=0.71).

타크로리무스나 사이클로스포린으로 인해 발생하는 이상반응을 Naranjo scale로 평가하여 "probable" 또는 "definite"인 것만 포함하였다. 타크로리무스군에서는 "probable" 크레아티닌 상승이 2명, 오심이 1명에서 발생하였고, 사이클로스포린군에서는 "probable" 피부 발진이 1명에서 발생하였다. 타크로리무스군의 1명에서는 이식편대숙주질환 치료 목적으로 시롤리무스(sirolimus)가 병용되었고, 이로 인하여 혈전미세혈관병증(thrombotic microangiopathy)이 발생하여 약물이 중단되었다.

감염으로는 거대세포바이러스(cytomegalovirus)에 의한 항원혈증(antigenemia) 또는 감염이 타크로리무스군 61.1%(n=11), 사이클로스포린군의 19.0%(n=4)으로 가장 많은 빈도로 보고되었다.

결론적으로 임상시험 결과, 타크로리무스는 기존 사이클로스포린 예방요법에 비하여 우월성은 입증하지 못하였으나 비슷한 효과를 보였으므로, 소아 환자에서 타크로리무스의 사용을 인정할 수 있다.

2. 소아 조혈모세포이식 환자에서의 타크로리무스 집단 약동학 연구

타크로리무스의 농도는 이식편대숙주질환의 예방에 중요한 인자이다. 그러나 타크로리무스는 개체간 및 개체내 약물농도의 차이가 큰 약물로, TDM을 통하여 목표치로 최대한 조절하려하였으나 실제로 목표 농도를 유지하지 못하는 환자와 경구로 전환 후 약물농도가 높아 일시 중단하는 환자가 많았다. 타크로리무스의 유효성을 보장하기 위하여, 약물의 농도는 목표치료 유지하는 것이 중요하다.

조혈모세포이식 환자는 전처치로 투여된 항암제, 이식편대숙주질환, 감염 등으로 발생하는 위장관 점막염 및 장벽의 손상, 그리고 CYP 억제제인 항진균제의 병용으로 약물의 흡수 및 대사가 고형장기이식 환자와는 다르다. 게다가 소아는 성인과 장기 기능 및 대사 효소의 발달 차이로 약동학적으로 차이가 발생할 수 있다. 그럼에도 불구하고 기존의 연구에서 소아 환자를 대상으로 진행된 집단 약동학 연구는 1건 밖에 없었으며 22명의 소수의 환자만을 포함하였다. 게다가 해당 연구에서는 약물의 상호작용을 고려하지 않았다. 따라서 본 연구에서는 소아 조혈모세포이식 환자에서 타크로리무스의 집단 약동학 모델을 구축하고자 한다.

본 연구는 후향적으로 2006년 9월부터 2015년 2월까지 서울대학교병원에서 조혈모세포이식을 받고 이식편대숙주질환 예방 목적으로 타크로리무스를 투여받은 18세 미만의 소아 환자를 포함하였다.

타크로리무스는 0.03 mg/kg/day 초기용량으로 이식 하루 전부터 8

mL/h 속도로 지속 주입하며, 이식 21일 후부터 경구로 전환하였다. 경구 용량은 마지막 지속 주입 용량의 약 4배로 1일 2회 분할하여 투여하였다. 타크로리무스의 용량은 TDM을 통하여 혈중농도인 10-20 ng/ml에 도달하도록 용량을 조절하였다. 집단 약동학 모델의 종속변수인 타크로리무스의 농도는 서울대학교병원에서 liquid chromatography-tandem mass spectrometry (LC-MS/MS)로 측정한 TDM 분석 결과를 사용하였다.

공변량 평가를 위하여 환자의 나이, 성별, 체중, 키, 체표면적, 이식 후 기간, 백혈구, 헤모글로빈, 적혈구용적, 혈소판, 혈중요소질소, 크레아티닌, 총 빌리루빈, 알칼리인산분해효소, 알라닌 아미노전이효소, 아스파라진산 아미노전이효소, 위장관 이식편대숙주질환여부, 그리고 병용되는 azole계 항진균제, 프로톤펌프억제제, 칼슘채널차단제, 페니토인(phenytoin), 스테로이드 정보를 수집하였다.

집단 약동학 모델은 NONMEM version 7.3을 통해 구축하였으며, 1-컴파트먼트 모델과 2-컴파트먼트 모델을 비교하고, 다양한 흡수 모델(단순 흡수, 지연 시간 적용 모델, 트랜싯 컴파트먼트 모델)을 비교하였다. 총 100일간의 추적관찰 기간에 대하여 이식 후 1개월간은 매주, 이후 매달로 기간을 나누어 inter-occasional variability (IOV)를 적용하였다. 개체 간 차이는 exponential 모델로 적용하였다.

수집한 정보는 전향적 선택과 후향적 제거 방법으로 평가하여 타크로리무스의 약동학에 통계적으로 유의한 영향을 주는 공변량을

선택하였으며, 기준은 전향적 선택에서 $p < 0.05$, 즉 objective function value (OFV) 3.84, 후향적 제거에서 $p < 0.01$, 즉 OFV 6.63을 기준으로 하였다. 최종적으로 선택된 모델의 내적 타당성은 bootstrap과 visual predictive check를 통해 평가하였다.

기본 모델로 1-컴파트먼트 모델이 사용되었다. 대부분의 채혈이 경구 복용 중 채혈직전에 시행되었기 때문에 trough 농도였으며, 흡수 모델은 단순 모델로 흡수속도상수가 문헌 값인 4.48 h^{-1}로 고정되었다.

전향적 선택의 과정에서 azole계 항진균제의 사용여부를 clearance (CL)에 공변량으로 포함하였을 때 OFV를 가장 크게 감소시켰으며 (-134.5), 이후 체표면적이 CL와 volume of distribution (V)에 공변량으로 포함되었다. 후향적 제거에서 제거된 공변량은 없었다. 따라서 최종 모델은 다음과 같이 구축되었다.

$CL = 6.74 \times (\text{체표면적}/1.102)^{0.552} \times 0.4$(azole계 항진균제를 사용하는 경우)

$V = 1160 \times (\text{체표면적}/1.102)^{0.503}$

최종 모델의 bootstrap 결과는 실제 값을 잘 추정함을 보였으며, visual predictive check 결과도 40일 이후의 5 percentile 값이 낮게 추정되는 경향이 있기는 하였지만, median 값이나 95 percentile 값 등은 잘 추정되어 좋은 모델로 평가되었다.

결론적으로 본 연구를 통하여 소아 조혈모세포이식 환자에서 적절한 약물농도 조절을 위한 타크로리무스의 집단 약동학 모델이 개발되었다.

추정 생체이용률이 37%로 기존에 일반적으로 사용되는 25%보다 높아 경구 전환 시 높은 약물 농도의 원인이 될 것으로 생각된다. 또한 azole계 항진균제의 사용이 CL를 40%까지 감소시켜 약물용량 선정에 반영되어야 하며, 환자의 체표면적을 고려해야 한다.

3. 성인 조혈모세포이식 환자에서의 사이클로스포린 집단 약동학 연구

이식편대숙주질환 예방을 위해 사용되는 사이클로스포린은 좁은 치료역을 가지며, 약동학적으로 개체 간, 개체 내 차이가 큰 약물이다. 특히 많은 연구들에서 보고하였듯이 사이클로스포린의 약동학에는 약물대사에 관여하는 *CYP3A5*3* 변이나 약물 수송체인 P-glycoprotein의 발현에 관여하는 *ABCB1* 유전자의 다형성이 관여할 수 있다.

이러한 개체 간 차이로 인해 사이클로스포린 농도를 부작용은 최소화하며 이식편대숙주질환을 예방할 수 있는 면역억제작용을 나타내도록 조절하기란 어렵다. 집단 약동학 연구는 개체 간 차이를 수치화하고 모델을 구축함으로써 개인 맞춤 용량을 설정하는데 뛰어난 방법이다. 최근까지도 사이클로스포린에 대한 집단 약동학 연구는 고형장기이식 환자를 대상으로 많이 수행되었다. 그러나 조혈모세포이식 환자는 전처치로 투여된 항암제, 이식편대숙주질환, 감염 등으로 약물의 흡수와 CYP 억제제인 항진균제의 병용으로 약물의 대사가

고형장기이식 환자와는 다르다. 따라서 본 연구에서는 조혈모세포이식 환자에서 유전적 다형성을 고려한 사이클로스포린의 집단 약동학 모델을 구축하고자 하였다.

본 연구는 2009년 11월부터 2011년 3월까지 서울대학교병원에서 조혈모세포이식을 받고 사이클로스포린을 투여받은 18세 이상의 성인 환자를 대상으로 하였다. 사이클로스포린은 이식 1~2일전부터 3 mg/kg 용량을 24시간 지속 주입하였고, 이식 21일 후부터 전일 투여량의 2~3배를 1일 2회 분할투여한 후 복용하였다. 사이클로스포린의 용량은 TDM을 통해 150~400 ng/mL로 조절되었다. 사이클로스포린 농도 분석을 위한 혈액 검체 1 mL를 지속 주입 중 한 시점과 경구 복용 기간 중 복용 직전, 복용 1, 2, 3, 4, 5 시간 후에 수집하였다. 사이클로스포린의 농도는 LC-MS/MS로 측정하였다.

집단 약동학 모델 구축을 위하여 환자의 나이, 성별, 체중, 키, 적혈구, 헤모글로빈, 적혈구용적, 혈소판, 총 빌리루빈, 알칼리인산분해효소, 알라닌 아미노전이효소, 아스파라진산 아미노전이효소, 혈청 크레아티닌, 사구체여과율, 알부민, 콜레스테롤 수치, 병용하는 스테로이드 용량과 CYP 상호작용 약물들을 수집하였다. 유전자는 조혈모세포이식 전에 수집한 혈액 검체를 사용하였으며, TaqMan 또는 SNaPshot assay를 통해 분석되었다.

집단 약동학 모델 구축은 비선형 혼합효과 모델링 프로그램인 NONMEM version 7.2를 통해 수행되었다. 1 또는 2-컴파트먼트

모델과 다양한 흡수모델이 기본 모델로 평가되었으며, 생체이용률은 0~1 사이의 값을 가지도록 로짓 변환(logit transformation) 하였다. 개체 간 약동학 파라미터의 차이는 additive, proportional, exponential 모델로 비교되었다. 수집한 정보를 전향적과 후향적 방법으로 평가하여 사이클로스포린의 약동학에 유의한 공변량을 평가하였다. 전향적과 후향적 선택의 기준은 $p < 0.05$인 OFV의 변화량 3.84로 하였다.

구축된 최종 모델의 평가는 bootstrap과 visual predictive check 기법을 통해 수행하였으며 PsN (Perl speaks NONMEM version. 4.2.0)과 R software 프로그램을 사용하였다.

본 연구에 포함된 환자는 총 34명으로 분석된 유전형은 모두 Hardy-Weinberg equilibrium을 만족하였다. CYP 상호작용 약물로는 플루코나졸(fluconazole)이 19명의 환자에서 지속 주입 기간 동안 사용되었고, 보리코나졸(voriconazole)이 2명의 환자에서 경구복용 기간 동안 사용되었다.

기본 모델로 2개의 트랜싯 컴파트먼트(transit compartment)를 통해 지연흡수를 설명하는 1-컴파트먼트 모델이 구축되었으며, clearance (CL), volume of distribution (V), log-transformed bioavailability (FLGT)와 rate constant for transit compartment (ktr)의 약동학 파라미터가 추정되었다. 개체 간 다양성은 CL와 V에 exponential 모델로 포함되었다. 최종 모델에는 체중이 CL에 power function 모델로 포함되었으며 지수값은 0.419이었다.

$CL = 21.2 \times (weight/70)^{0.419}$

다른 임상적, 유전적 지표는 사이클로스포린의 집단 약동학 모델에 포함되지 않았다. 최종 모델에서 도출된 CL, V, ktr의 추정값은 각각 21.2 L/h, 430 L, 2.87 h^{-1}이며, 생체이용률 추정치는 81%이었다. 최종 모델의 bootstrap 결과와 visual predictive check도 예측치의 2.5 percentile 값과 97.5 percentile 값이 실제 측정값을 포함하여 모델의 내적 타당성도 검증되었다.

결과적으로 본 연구를 통하여 성인 조혈모세포이식 환자에서 사이클로스포린의 집단 약동학 모델을 구축하였으며, CL에 체중이 공변량으로 영향을 줌을 확인하였다. 평가한 유전형은 사이클로스포린의 약동학에 영향을 주지 않았으며 본 연구에서 구축한 모델을 바탕으로 조혈모세포이식 환자에서 적절한 이식편대숙주질환 예방요법을 시행할 수 있을 것이다.

주요어: 칼시뉴린억제제, 타크로리무스, 사이클로스포린, 효과 및 안전성, 집단약동학, 이식편대숙주질환, 조혈모세포이식

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