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Clinical relevance of post-transplant pharmacodynamic analysis of cyclosporine in renal transplantation

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ABSTRACT

Although therapeutic drug monitoring based on blood concentration has been widely implemented in transplant recipients treated with immunosuppressive agents, clinical adverse events such as rejection, infection or drug-induced toxicity caused by inappropriate dosage cannot be completely controlled. Development of an effective assay for optimized immunosuppression would be desirable, which can potentially lead to personalized medicine in renal transplantation. Cyclosporine (CSA) pharmacodynamic analysis using carboxyfluorescein diacetate succinimidyl ester (CFSE)-based T cell proliferation assay was examined in 66 kidney transplant recipients before and after transplantation. Two parameters, the 50% inhibitory concentration (IC_{50}) and the percentage of T-cell proliferation values at the lower plateau (bottom), were compared with clinical events. A significant relation in CSA pharmacodynamic parameters was observed between pre- and post-transplantation. Analysis of the association between clinical outcomes and pharmacodynamic parameters in post-transplant samples demonstrated the following findings: (i) cytomegalovirus (CMV)/varicella zoster virus (VZV) reactivation and CSA-induced nephrotoxicity were significantly associated with high sensitivity to CSA (low bottom or low IC_{50}), (ii) acute T cell-mediated rejection (ATMR) was significantly related to low sensitivity to CSA (high bottom), and (iii) de novo human leukocyte antigen (HLA) antibody production was associated with lower bottom and IC_{50} values, although the elucidation of those mechanisms is still in progress. It was suggested that CSA pharmacodynamics applied at post-transplantation would be useful for optimizing immunosuppressive therapy.

1. Introduction

Recent advancement in more effective immunosuppressive drugs has brought about significant improvement of graft and patient survival after organ transplantation. Considering the fact that there is still room for improvement, particularly in long-term outcomes, it is essential to continue the effort to develop a novel monitoring method for the optimization of immunosuppressive therapy that will lead to personalized medicine.

In clinical renal transplantation, attention generally needs to be paid to acute rejection (T cell-mediated or antibody-mediated), drug-induced toxicity or infection during the early phase, and chronic rejection (antibody-mediated) during the late phase. As immunosuppressive agents generally have narrow therapeutic windows, treatment based on blood level monitoring has been implemented. However, such therapeutic drug monitoring cannot solve all problems [1]. Therefore, in addition to conventional blood level monitoring, the need for pharmacodynamics that shows the relationship between drug concentration and effectiveness has been recognized. Furthermore, since most patients are currently treated with multiple drug therapy, a comprehensive and accurate evaluation of an optimal level of immunosuppression would be required to prevent rejection or over-immunosuppression [2].

Various methods for detecting effective biomarkers in peripheral blood have been proposed as follows: enzyme-linked immunosorbent

Abbreviations: NFAT, nuclear factor of activated T cells; CFSE, carboxylfluorescein diacetate succinimidyl ester; CSA, cyclosporine; PRD, prednisolone; MPA, mycophenolic acid; MMF, mycophenolate mofetil; CNI, calcineurin inhibitor; CMV, cytomegalovirus; VZV, varicella zoster virus; ATMR, acute T cell-mediated rejection; HLA, human leukocyte antigen; AUC, area under the drug concentration–time curve; IC_{50}, 50% inhibitory concentration; eGFR, estimated glomerular filtration rate.

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spot assay, intracellular staining by flow cytometry, T cell proliferation and trans vivo delayed-type hypersensitivity response as antigen (donor)-specific immune monitoring, and analysis of cell surface marker or receptor repertoire, T cell function assay (soluble CD30, ATP, proliferating cell nuclear antigen mRNA), nuclear factor of activated T cells (NFAT)-regulated gene expression, and mRNA/miRNA profiling as non-antigen (donor)-specific immune monitoring [3–10]. Several approaches have been applied to clinical settings, and a few effective assays which could navigate suitable immunosuppressive therapy for each patient have been developed, but are not in widespread use at present.

We have developed a T-cell proliferation assay using flow cytometry with carboxylfluorescein diacetate succinimidyl ester (CFSE) labeling as a useful drug sensitivity test [11]. The results showed that there was considerable inter-individual variation in the inhibitory effects of cyclosporine (CSA), tacrolimus, and prednisolone (PRD). In contrast, only a small amount of inter-individual variation was observed with mycophenolic acid (MPA), the active metabolites of mycophenolate mofetil (MMF). Furthermore, the results revealed that patients with high sensitivity to CSA would be susceptible to viral reactivation, although that relation with acute rejection episodes has not been analyzed. In the previous study, pre-transplant evaluation was conducted just before initiation of immunosuppressive therapy, whereas in the current study, we attempted to expand such a monitoring assay to post-transplant evaluation using peripheral blood T cells withdrawn from immunosuppressive patients.

First, we compared individual T-cell sensitivity to CSA between patients before and after transplantation. Next, we estimated the relation between pharmacodynamic parameters and clinical outcomes such as cytomegalovirus (CMV)/varicella zoster virus (VZV) reactivation, acute T cell-mediated rejection (ATMR), CSA-induced nephrotoxicity, and de novo human leukocyte antigen (HLA) antibody production. The purpose of this study was to determine a valuable index for predicting clinical outcomes and to examine the feasibility of post-transplant monitoring.

2. Materials and methods

2.1. Patients

Eighty-one peripheral blood samples from 66 kidney transplant recipients (age: 49.7 (mean) ± 12.5 (SD) years, male/female: 43/23) treated with CSA, mycophenolate mofetil (MMF) and PRD were collected and analyzed in this study. All transplantations were performed from August 2007 to January 2011 at Nagoya Daini Red Cross Hospital. This case–control study was analyzed as of August 31, 2013. The observation period after transplantation and the dialysis vintage of 66 recipients are 55.4 ± 11.4 months (range: 31–72), and 35.1 ± 61.1 months (0–305), respectively. This study was approved by the Institutional Ethics Committee of Nagoya University School of Medicine and by the Institutional Review Board of Nagoya Daini Red Cross Hospital in accordance with current standards for human research. Written informed consent was obtained from all the patients before inclusion in this study.

First, fifteen of the 66 recipients were examined for T-cell sensitivity to CSA both at pre-transplantation (before initiation of immunosuppressive therapy) and at post-transplantation (25.1 ± 16.5 months after transplantation: range: 6–48). Second, we compared the parameters of a post-transplant T-cell sensitivity test with clinical events such as CMV/VZV reactivation, ATMR, CSA-induced nephrotoxicity, and de novo HLA antibody production in all 66 recipients.

2.2. Immunosuppressive protocol

All the recipients received 20 mg of basiliximab on day 0 and day 4. Oral immunosuppressive agents consisted of CSA, MMF and PRD. The loading doses of CSA (8 mg/kg/day) were administered starting on day -2. During the first 3 months, the dosage was strictly adjusted to the target level of the area under the drug concentration–time curve from 0 to 4 h (AUC_{0-4}: 3500 ng·h/mL), which was reduced to 2000 ng·h/mL thereafter. MMF was given at a dosage of 3 g/day from day 1 and reduced to 2 g/day from day 14. The dosage was basically adjusted according to the trough level and adverse effects. PRD was tapered from 60 mg/day on day 0 to 10 mg/day on day 19, and maintained at a dose of 5.0–7.5 mg/day from 3 months after transplantation. Whole-blood levels of CSA were measured using the ARCHITECT assay (Abbott Japan, Chiba, Japan), and MPA were measured using the EMIT assay (Dade Behring, Cupertino, CA).

2.3. Elimination of immunosuppressive drugs in posttransplant peripheral blood samples

Post-transplant peripheral blood samples (7.5 mL) were collected from immunosuppressed recipients immediately before administering agents (12 h after the last administration). Mononuclear cells isolated by density gradient centrifugation over Histopaque-1077 (Sigma-Aldrich, St. Louis, MO) were washed twice and resuspended in culture medium, i.e., Roswell Park Memorial Institute (RPMI) medium (Gibco, New York, NY) containing 2% fetal bovine serum (Sanko, Tokyo, Japan) at a final concentration of 2×10^6 cells/mL. The cells were incubated overnight to eliminate immunosuppressive drugs in an atmosphere of 5% CO_2 at 37 °C (total culture medium volume was 2 mL in 24-well flat-bottom plates). After centrifugation, 2×10^5 cells were suspended in 200 μL of RPMI medium and 100 μL whole blood precipitation reagent (Abbott Japan) and CSA concentration was measured. The concentration of CSA after overnight incubation was proven to be undetectable, whereas the cells contained 60.0 ± 18.9 ng/mL of CSA before incubation, immediately after washing two times (data not shown).

2.4. CFSE based T-cell proliferation assay

The T-cell proliferation assay was performed as described previously [11]. Briefly, peripheral blood mononuclear cells, isolated from 20 mL of pretransplantation whole blood or 7.5 mL of posttransplantation whole blood were stained with CFSE (Invitrogen Co., Carlsbad, CA). Peripheral blood mononuclear cells (2×10^5 cells) were incubated in AIM V medium + 1% HEPES buffer (Gibco) containing CSA at final concentrations of 5, 10, 25, 50, 100, 200, 300 and 400 ng/mL in 96-well flat-bottom plates with phytohemagglutinin (Sigma-Aldrich) stimulation. After incubation for 3 days, the cells were stained with phycoerythrin-conjugated CD3 monoclonal antibodies (BD Pharmingen, San Diego, CA). Three-color flow cytometry was performed on a FACS Calibur dual-laser cytometer (Becton Dickinson, Mountain View, CA). Dead cells were excluded by light-scatter properties and propidium iodide staining. The percentage of inhibited CD3-positive cells was quantitatively analyzed.

2.5. Pharmacodynamic parameters

Concentration–effect curves were analyzed by the sigmoid maximum-effect model. Pharmacodynamic parameters were calculated using GraphPad Prism 4 (GraphPad Software, Inc., San Diego, CA) as described previously [11]. The percentage of T-cell proliferation was expressed as the relative value of maximum T-cell proliferation. “Top” and “bottom” are the T-cell proliferation percentage values at the upper and lower plateaus, respectively, and 50% inhibitory concentration (IC_{50}) is the drug concentration corresponding to the response halfway between the top and bottom values. Therefore, the value obtained by subtracting the bottom value from 100% is considered to express the maximum percentage of inhibition.