Cell Transplantation, Vol. 21, pp. 565–570, 2012

0963-6897/12 $90.00 + .00
DOI: http://dx.doi.org/10.3727/096368911X605493
E-ISSN 1555-3892
www.cognizantcommunication.com

Comparative Study of the Cellular Pharmacodynamics of Tacrolimus in Renal Transplant Recipients Treated With and Without Basiliximab

Kentaro Sugiyama,* Kazuya Isogai,† Satoshi Horisawa,† Akira Toyama,† Hiroshi Satoh,† Kazuhide Saito,‡ Yuki Nakagawa,‡ Masayuki Tasaki,‡ Kota Takahashi,‡ and Toshihiko Hirano*

*Department of Clinical Pharmacology, School of Pharmacy, Tokyo University of Pharmacy and Life Science, Tokyo, Japan
†Division of Pharmacy, Niigata University Medical and Dental Hospital, Niigata, Japan
‡Division of Urology, Graduate School of Medical and Dental Sciences, Niigata University, Niigata, Japan

Basiliximab is a recently developed immunosuppressive agent for the prevention of acute allograft rejection in renal transplant recipients. The combination use of basiliximab and a calcineurin inhibitor was suggested to be more effective in comparison to immunosuppressive therapy using calcineurin inhibitor without basiliximab. Cyclosporine has been generally administered with basiliximab for renal transplant recipients. However, in cases of tacrolimus-based immunosuppressive regimen, the clinical efficacy and safety of combined use of tacrolimus and basiliximab remains to be elucidated. This study evaluated the tacrolimus pharmacological efficacy using a lymphocyte immunosuppressant sensitivity test (LIST) with MTT assay procedures in 16 cases of renal transplant recipients treated by tacrolimus without basiliximab and in 13 cases treated by tacrolimus in combination with basiliximab. The rate of acute rejection episodes in the recipients treated with tacrolimus plus basiliximab was 1/13 (7.7%), whereas the rate in the recipients treated with tacrolimus without basiliximab was 6/16 (37.5%). The recipients were divided into two groups according to their peripheral blood mononuclear cell (PBMC) sensitivity to tacrolimus [i.e., including a tacrolimus high sensitivity group (IC_{50} <1.0 ng/ml) and a low sensitivity group (IC_{50} >1.0 ng/ml). In the recipients treated with tacrolimus without basiliximab, the rate of acute rejection episodes in the tacrolimus high sensitivity group was 1/10 (10.0%), which was significantly lower than the rate in the low sensitivity group of 5/6 (83.3%; $p = 0.008$). The incidence of cytomegalovirus infection was not significantly different between the tacrolimus high and the low sensitivity groups of the recipients treated with tacrolimus with and without basiliximab. Therefore, in the case of selected tacrolimus-based immunosuppressive therapy for renal transplant recipients, the tacrolimus pharmacological efficacy should be evaluated using LIST at a time just before the transplant procedure in order to accurately predict allograft rejection. The data also suggested that low tacrolimus sensitivity recipients should be treated with tacrolimus-based immunosuppressive therapy in combination with basiliximab.

Key words: Tacrolimus; Basiliximab; Renal transplantation; Lymphocyte immunosuppressant sensitivity test (LIST); Peripheral blood mononuclear cells (PBMCs)

INTRODUCTION

Basiliximab was recently developed for the prevention of acute rejection episodes in renal transplant recipients. Immunosuppressive therapy by basiliximab combined with calcineurin inhibitor is potentially effective in comparison to the usual regimen of immunosuppressive treatment without basiliximab. Cyclosporine has been frequently used with basiliximab for renal transplant recipients.

In contrast to cyclosporine-based therapy, tacrolimus-based immunosuppressive therapy has not been the standard selection, whether the therapy is carried out with or without basiliximab in renal transplantation. The pharmacological efficacy of tacrolimus is estimated by the lymphocyte immunosuppressant sensitivity test (LIST) to be superior to that of cyclosporine (3–6,8,12). Tacrolimus combined with basiliximab might result in over immunosuppression, in comparison with the therapy by tacrolimus without basiliximab in renal transplant recipients.

The therapeutic drug monitoring (TDM) of calcineurin inhibitors has been performed for dosing of the drug to optimize immunosuppressive efficacy. However, TDM of tacrolimus based on the blood concentration of

Received March 31, 2010; final acceptance July 18, 2011.
Address correspondence to Kentaro Sugiyama, Ph.D., Department of Clinical Pharmacology, School of Pharmacy, Tokyo University of Pharmacy and Life Science, 1432-1 Horinouchi, Hachioji City, Tokyo 192-0392, Japan. Tel: +81-42-676-5111; Fax: +81-42-676-5798; E-mail: sugiyama@toyaku.ac.jp.

the drug is not always an adequate index for prevention of rejection episodes in tacrolimus-based immunosuppressive treatment. A previous study reported that the individual variation in the pharmacological efficacy of prednisolone in the peripheral blood mononuclear cells (PBMCs) of chronic renal failure (CRF) patients is larger than that in the PBMCs of healthy subjects. In contrast, the effect of methylprednisolone against PBMC blastogenesis is almost the same in CRF patients and healthy subjects (2). Furthermore, both the LIST-estimated cyclosporine and tacrolimus pharmacological efficacies exhibit a wide variation in the PBMCs of renal transplant recipients (6,8,12). The individual pharmacological efficacies of calcineurin inhibitors in the PBMCs of cirrhosis patients also showed large deviations (3–5). Therefore, the accurate estimation of the clinical efficacy of immunosuppressive agents is necessary from the standpoint of not only pharmacokinetics, but also the pharmacodynamics of tacrolimus. The present study evaluated the individual tacrolimus potency with LIST to characterize the pharmacological background and determine whether tacrolimus-based therapy with or without basiliximab is preferable to prevent acute allograft rejection or infection in renal transplant recipients.

MATERIALS AND METHODS

Reagents

Tacrolimus was kindly provided by Astellas Co. (Tokyo, Japan). 3-(4,5-Dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was obtained from Sigma Chemical Co. (St. Louis, MO). Ficoll-Paque was obtained from Amersham Pharmacia Biotech (Buckinghamshire, UK). RPMI-1640 medium, fetal bovine serum, and Hank's balanced salt solution were obtained from Gibco Laboratories (Rockville, NY). Concanavalin A was obtained from Seikagaku Kogyo Co. (Tokyo, Japan). Dimethyl sulfoxide was purchased from Wako Chemical (Osaka, Japan). All other reagents were of the highest grade available.

Subjects

The pharmacological efficacy of tacrolimus was evaluated in 29 renal transplants. Sixteen (12 males and 4 females) out of these 29 recipients received immunosuppressive therapy by tacrolimus without basiliximab. The mean ± SD age of these 16 recipients was 37.4 ± 16.0 years. The mean ± SD for the human lymphocyte antigen (HLA)-AB and HLA-DR mismatch numbers were 2.1 ± 1.3 and 0.9 ± 0.7, respectively. The other 13 recipients (male 10 and female 3) received tacrolimus with basiliximab. The mean ± SD age of these 13 recipients treated with tacrolimus plus basiliximab was 38.5 ± 16.7 years. The mean ± SD for the HLA-AB and HLA-DR mismatch numbers in these 13 recipients was 2.2 ± 1.0 and 1.0 ± 0.6, respectively (Table 1).

All of the transplant recipients received renal allografts from living donors after blood sampling for analysis of their PBMC response to immunosuppressive agents in vitro. These renal transplant recipients underwent surgery from July 2002 to January 2008 at Niigata University Medical and Dental Hospital. The study was approved by the ethics review board of the Medical Faculty of Niigata University.

These patients were treated with maintenance immunosuppressive therapy after renal transplantation, which consisted of a combination of tacrolimus (Prograf cap., Astellas Co., Japan), either with 20 mg basiliximab at day 0 and day 4 or without basiliximab, plus methylprednisolone and mycophenolate mofetil (MMF; Celcept 250 mg cap., Chugai Co., Japan). The starting doses of these agents were 0.05 mg/kg/day intravenously or 0.2 mg/kg/day orally for tacrolimus, 125 mg/day for methylprednisolone, and 1000 or 2000 mg b.i.d. for MMF. To measure the response of PBMCs to the drugs in vitro, a drug sensitivity test (611), was carried out in renal transplant recipients just before the operation.

Isolation of PBMCs

After informed consent was obtained, venous blood was taken and heparinized just before the immunosuppressive agents were administered for renal transplant recipients on a day in which no hemodialysis was performed. The isolation and culturing of PBMCs were carried out according to a method described previously (2,3,6,12). In brief, 5 ml of heparinized blood was loaded onto 4 ml of Ficoll-Paque and centrifuged at $900 \times g$ for 20 min at room temperature. The buffy coat was taken and rinsed three times with Hank's balanced salt solution. PBMCs, including lymphocytes, were suspended in RPMI-1640 medium containing 10% fetal bovine serum to a cell density of 1×10^6 cells/ml.

PBMC Culture and Evaluation of Drug Potency

Aliquots (200 μl) of the cell suspension prepared as described above were placed into each well of microplates with 96 flat-bottomed wells. Saline containing concanavalin A was added to each well at a final mitogen concentration of 5.0 μg/ml. Subsequently, an ethanol solution containing tacrolimus was added to give a final drug concentration of 0.0001, 0.001, 0.01, 0.1, 1, 10, 100, or 1,000 ng/ml. The same volume of each vehicle solution was added to control wells. The plates were then incubated for 4 days in an atmosphere of 5% CO_2 at 37°C.

MTT Assay

After 4 days of culture, 10 μl of 5 mg/ml MTT solution dissolved in saline was added to each well and then the cultures were reincubated under 5% CO_2 at 37°C for 4–5 h (1,6,11). The plates were centrifuged at $375 \times g$

Table 1. Characteristics of Renal Transplant Recipients

Immunosuppressant Treatment Group	Mean Age ± SD	Male	Female	Mean HLA-AB (Match Number ± SD)	Mean HLA-DR (Match Number ± SD)
Tacrolimus without basiliximab group ($n = 16$)	37.4 ± 16.0	12	4	2.1 ± 1.3	0.9 ± 0.7
Tacrolimus with basiliximab group ($n = 13$)	38.5 ± 16.7	10	3	2.2 ± 1.0	1.0 ± 0.6

HLA, human leukocyte antigen.

for 5 min to precipitate cells and formazan produced by growing cells. The aliquots of the supernatant were removed from each well, and dimethyl sulfoxide was added followed by shaking of the plate on a microshaker for 10 min to dissolve the formazan crystals. The absorbance was read with a microplate reader at 550 nm. Dose–response curves were plotted, and the concentrations of drugs giving IC_{50}s were calculated.

Statistical Analysis

The renal transplant recipients were divided into two subgroups according to their PBMC sensitivity to tacrolimus estimated in vitro by LIST as described above. The tacrolimus group IC_{50} <1.0 ng/ml showed a high sensitivity to tacrolimus, while the tacrolimus >1.0 ng/ml group demonstrated a low sensitivity. The difference in the incidence of acute rejection episode (within 3 months) or reinfection of cytomegalovirus (CMV) antigenemia$^+$ (within 3 months) episode between any two recipient subgroups was estimated by the Fisher's exact probability test. These data analyses were performed using SPSS 11.0J statistical software package (SPSS Japan Inc.) and EXCEL 2003 (Microsoft).

RESULTS

Tacrolimus pharmacological efficacy on mitogen-induced blastogenesis of PBMCs in vitro was evaluated in 29 renal transplant recipients by LIST with an MTT assay procedure. The typical dose–response curves for tacrolimus against the blastogenesis of PBMCs obtained from one renal transplant recipient are shown in Figure 1. The mean ± SD and the median of the tacrolimus IC_{50} values against PBMCs of the recipients under tacrolimus-based immunosuppressive treatment without basiliximab were 202.4 ± 397.7 and 0.77 ng/ml, respectively. The IC_{50} values ranged from 0.01 to 1000 ng/ml (Table 2).

The mean ± SD and the median of tacrolimus IC_{50} values against PBMCs of the recipients under tacrolimus-based immunosuppressive treatment with basiliximab were 77.2 ± 277.3 and 0.32 ng/ml, respectively. The IC_{50}s ranged from 0.00017 to 1000 ng/ml (Table 2).

The incidence of acute rejection episodes in the recipients administered tacrolimus without basiliximab immunosuppressive treatment was 6/16 (37.5%). The incidence of acute rejection episodes in the tacrolimus high sensitivity group treated with tacrolimus without basiliximab was 1/10 (10.0%), whereas the incidence of rejection episodes in the low tacrolimus sensitivity recipients was 5/6 (83.3%). The difference of the incidence of rejection episodes was statistically significant according to Fisher's exact probability test between the tacrolimus high and the low sensitivity recipients ($p = 0.008$) (Table 3). Calcineurin inhibitor conversion was not carried out in cases where the recipients were treated without basiliximab. The incidence of CMV antigenemia$^+$ reinfection in the recipients under tacrolimus without basiliximab therapy was 5/16 (31.3%). The incidence of CMV antigenemia$^+$ reinfection episodes in the tacrolimus high sensitivity group treated with tacrolimus without basiliximab was 5/10 (50.0%). The incidence in the low sensitivity group was 0/6 (0%), although the difference in the incidence of CMV antigenemia$^+$ reinfection was not significantly different. In cases where the recipients were treated by tacrolimus without basiliximab, the rate of CMV antigenemia$^+$ reinfection episodes in the tacrolimus high sensitivity group was higher than that of the low sensitivity group, although the rate was not statistically significant (Table 3).

Acute rejection episodes occurred in 1/13 cases treated by tacrolimus with basiliximab (7.7%). In recipients treated with tacrolimus plus basiliximab, acute rejection episodes in the tacrolimus high sensitivity recipients occurred in 1/12 cases (8.3%), while acute rejection episodes in the tacrolimus low sensitivity recipient occurred in 0/1 cases (0%). Furthermore, in the tacrolimus high sensitivity group of the recipients treated with tacrolimus plus basiliximab, CMV antigenemia$^+$ reinfection were detected in 5/12 (41.7%). In the tacrolimus low sensitivity recipients, CMV antigenemia$^+$ was detected in 1/1 (100%) (Table 3). The incidence of acute rejection episodes in the tacrolimus high sensitivity group of the recipients treated with tacrolimus plus basiliximab was lower than that in the low sensitivity group, although the difference was not statistically significant.

DISCUSSION

Cyclosporine-based immunosuppressive treatment combined with basiliximab has been generally administered for prevention of allograft rejection in renal transplant recipients. In contrast, the possible superiority of tacrolimus-based immunosuppressive therapy without basiliximab not been thoroughly evaluated in Japanese

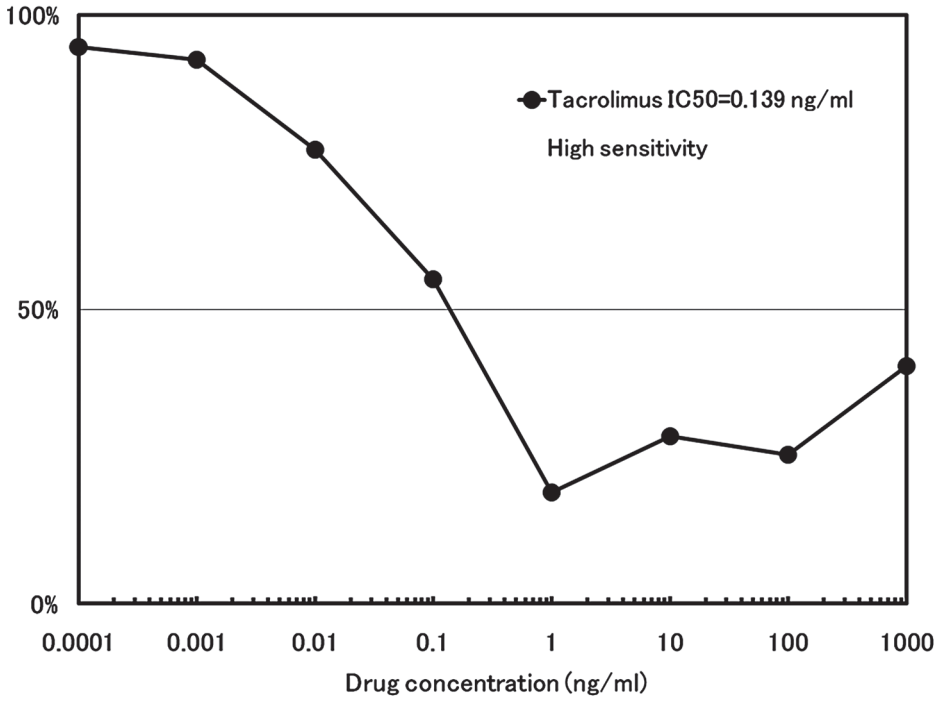

Figure 1. Typical dose–response curve for tacrolimus against the blastogenesis of peripheral blood mononuclear cells (PBMCs) obtained from one renal transplant recipient.

renal transplant recipients. Therefore, the tacrolimus pharmacological efficacy was evaluated by LIST to estimate whether combined use of basiliximab with tacrolimus has an apparent clinical efficacy with regard to the prevention of rejection and safety.

The in vitro response of PBMCs to the suppressive effects of immunosuppressive drugs correlates with the clinical efficacy of the drugs used in treating renal transplantation (2,5,12). Although the number of cases examined was not sufficient, the data of a previous study raised the possibility that the response of PBMCs to the effects of immunosuppressive drugs correlated with the clinical efficacy of tacrolimus in liver transplantation (5). In addition, the current study showed that large individual variations existed in the IC_{50} values for calcineurin inhibitors in renal transplant recipients. According to these observations, the dose of tacrolimus should be changed according to the individual sensitivity of PBMCs to the drugs used in treating recipients in not only liver but also in renal transplantations. Moreover, immunosuppressive drugs including calcineurin inhibitors are generally expensive, and the individualized dose management of these drugs based on the drug sensitivity of PBMCs is also encouraging from this point of view.

In the present study, the rate of acute rejection episodes in the patient group under tacrolimus with basiliximab immunosuppressive therapy was lower than the group under tacrolimus without basiliximab therapy. Furthermore, the rate of acute rejection episodes in the tacrolimus high sensitivity recipients was significantly lower than the tacrolimus low sensitivity recipients ($p = 0.008$). In contrast, the recipients under tacrolimus with

Table 2. Comparison of the Pharmacological Efficacy of Tacrolimus Against Lymphocyte Blastogenesis Between Recipients Treated by Tacrolimus With and Without Basiliximab Immunosuppressant Treatment

	Tacrolimus IC_{50} (ng/ml)	
Group	Mean ± SD	Median (Range)
Tacrolimus without basiliximab group ($n = 16$)	202.4 ± 397.7	0.77 (0.01–1000.0)
Tacrolimus with basiliximab group ($n = 13$)	77.2 ± 277.3	0.32 (0.00017–1000.0)

Table 3. Comparison of the Incidence of Acute Rejection Episodes and Cytomegalovirus (CMV) Antigenemia Reinfection Between the High and Low Tacrolimus Sensitivity Groups Treated by Tacrolimus With and Without Basiliximab

Immunosuppressive Therapy	Acute Rejection	Tacrolimus Sensitivity	Rate of Acute Rejection
Tacrolimus-based immunosuppressive therapy without basiliximab ($n = 16$)	6/16 (37.5%)	high	1/10 (10.0%)*
		low	5/6 (83.3%)*
Tacrolimus-based immunosuppressive therapy with basiliximab ($n = 13$)	1/13 (7.7%)	high	1/12 (8.3%)†
		low	0/1 (0%)†

Immunosuppressive Therapy	CMV	Tacrolimus Sensitivity	Rate of CMV
Tacrolimus-based immunosuppressive therapy without basiliximab ($n = 16$)	5/16 (31.3%)	high	5/10 (50%)†
		low	0/6 (0%)†
Tacrolimus-based immunosuppressive trerapy with basiliximab with basiliximab ($n = 13$)	6/13 (46.2%)	high	5/12 (41.7%)†
		low	1/1 (100%)†

Statistical significance assessed by Fisher' exact probability test ($p < 0.05$).
*$p < 0.008$, between high and low sensitivity.
†N.S., between high and low sensitivity.

basiliximab therapy showed a high rate of CMV reinfection in comparison to the recipients under the therapy with tacrolimus without basiliximab, although the difference was not statistically significant. Therefore, renal transplant recipients under tacrolimus-based immunosuppressive therapy should be evaluated for their tacrolimus pharmacological efficacy by LIST at a time just before the operation. If the tacrolimus pharmacological efficacy is as high as that estimated by LIST, such recipients should therefore be treated with tacrolimus without basiliximab. It has been suggested that the risk of CMV antigenemia$^+$ reinfection increases in transplant recipients under tacrolimus therapy combined with basiliximab. Conversely, the tacrolimus low sensitivity recipients should be treated with tacrolimus combined with basiliximab.

The results of this study indicate that the pharmacological efficacy of tacrolimus as estimated by LIST is useful for the prevention of acute rejection. The results also suggest that LIST will contribute to the pharmacoeconomics of tacrolimus-based immunosuppressive treatment for renal transplant recipients.

ACKNOWLEDGMENT: The authors declare no conflicts of interest.

REFERENCES

1. Hirano, T.; Abe, K.; Gotoh, M.; Oka, K. Citrus flavone tangeretin inhibits leukaemic HL-60 cell growth partially through induction of apoptosis with less cytotoxicity on normal lymphocytes. Br. J. Cancer 72:1380–1388; 1995.
2. Hirano, T.; Oka, K.; Takeuchi, H.; Sakurai, E.; Matsuno, N.; Tamaki, T.: Kozaki, M. Clinical significance of glucocorticoid pharmacodynamics assessed by antilymphocyte action in kidney transplantation. Marked difference between prednisolone and methylprednisolone. Transplantation 57: 1341–1348; 1994.
3. Kihara, Y.; Matsuno, N.; Mijiti, A.; Nagao, T.; Takeuchi, T.; Unezaki, S.; Hirano, T. Comparative study of the cellular pharmacodynamics of calcineurin inhibitors between patients with chronic renal failure awaiting renal transplantation and cirrhosis patients awaiting liver transplantation. Cell Transplant. 18:639–646; 2009.
4. Mijiti, A.; Matsuno, N.; Iwahori, T.; Takeuchi, H.; Nagao, T.; Oka, K.; Hirano, T. Increased sensitivities of peripheral blood mononuclear cells to immunosuppressive drugs in cirrhosis patients awaiting liver transplantation. Cell Transplant. 15:885–891; 2006.
5. Mijiti, A.; Matsuno, N.; Takeuchi, H.; Unezaki, S.; Nagao, T.; Hirano, T. Clinical significance of cellular pharmacodynamics of tacrolimus in living-donor liver transplantation. Cell Transplant. 18:657–664; 2009
6. Sugiyama, K.; Arakawa, K.; Satoh, H.; Saito, K.; Takahashi, K.; Saito, N.; Hirano, T. Correlation between pharmacological efficacy of cyclosporine A and tacrolimus, evaluated by lymphocyte immunosuppressant-sensitivity test (LIST) with MTT assay procedure in renal transplant recipients. J. Immunoassay Immunochem. 27:195–205; 2006.
7. Sugiyama, K.; Isogai, K.; Toyama, A.; Satoh, H.; Saito, K.; Nakagawa, Y.; Tasaki, M.; Takahashi, K.; Saito, N.; Hirano, T. Cyclosporine pharmacological efficacy estimated by lymphocyte immunosuppressant sensitivity test (LIST) before and after renal transplantation. J. Clin. Pharm. Ther. 34:539–545; 2009.
8. Sugiyama, K.; Isogai, K.; Toyama, A.; Satoh, H.; Saito, K.; Nakagawa, Y.; Tasaki, M.; Takahashi, K.; Saito, N.; Hirano, T. Pharmacodynamic parameters of immunosuppressive drugs are not correlated with age, duration of dialysis, percentage of lymphocytes or lymphocyte stimulation index in renal transplant recipients. Biol. Pharm. Bull. 31:2146–2149; 2008.
9. Sugiyama, K.; Kawada, T.; Sato, H.; Hirano, T. Comparison of suppressive potency between prednisolone and pre-

dnisolone sodium succinate against mitogen-induced blastogenesis of human peripheral blood mononuclear cells in-vitro. J. Pharm. Pharmacol. 53:727–733; 2001.
10. Sugiyama, K.; Satoh, H.; Hirano, T. Comparison of suppressive potency between azathioprine and 6-mercaptopurine against mitogen-induced blastogenesis of human peripheral blood mononuclear cells in-vitro. J. Pharm. Pharmacol. 55:393–398; 2003.
11. Sugiyama, K.; Satoh, H.; Saito, K.; Takahashi, K.; Saito, N.; Hirano, T. Immunosuppressive efficacy of mycophenolate mofetil when compared with azathioprine and mizoribine against peripheral lymphocytes from renal transplant recipients. Transpl. Int. 18:590–595; 2005.
12. Takeuchi, H.; Hirano, T.; Oka, K.; Mizumoto, K.; Akashi, T.; Sakurai, E.; Degawa, T.; Uchiyama, M.; Kozaki, K.; Matsuno, N.; Nagao, T.; Kozaki, M. Lymphocyte sensitivity to cyclosporine and tacrolimus in chronic renal failure patients and clinical significance in renal transplantation. Transplant. Proc. 30:36–39; 1998.