Cardiovascular Drugs and Therapy 17 141–149 2003

CLINICAL PHARMACOLOGY AND DRUG STUDIES

Multicenter Prospective Investigation on Cardiovascular Adverse Effects of Tacrolimus in Kidney Transplantations

Yoshihiko Seino[1], *Masatsugu Hori*[2], *and Takao Sonoda*[3]

[1]*The First Department of Internal Medicine, Nippon Medical School, Tokyo;* [2]*The First Department of Internal Medicine, Osaka University, Osaka;* [3]*Osaka Prefecture General Hospital, Osaka*

Summary. **To clarify the incidence and pathophysiological mechanism of cardiovascular adverse effects of tacrolimus, the present prospective study performed scheduled cardiovascular examinations at 1, 2, 4, 8, 16, 20, and 24 weeks after starting the tacrolimus therapy in 68 consecutive kidney transplantation recipients enrolled from 26 institutes in Japan. Patients with previous coronary artery disease or congestive heart failure were excluded. The examinations included any subjective symptoms, changes in resting ECG, ambulatory Holter's dynamic ECG, two-dimensional echocardiography, and monitoring of serum drug concentrations and cardiac troponin T levels. Cardiac nuclear imaging and/or coronary angiography were performed in the case of suspicious coronary events. During the investigation, chest pain in 9 (13.2%) and palpitation in 6 patients (8.8%) were reported, both closely related to elevated blood drug concentrations (37.2 ± 18.7 ng/mL, mean ± SD). Cardiovascular examinations detected development of resting ECG abnormalities in 12 patients (17.6%), asymptomatic ST depression following increased heart rate in 11 (16.2%) and ventricular arrhythmias in 7 patients (10.3%) on Holter's dynamic ECG. Elevation of troponin T was detected in 3 patients (4.4%), which was also closely related to elevated drug concentrations and interpreted as myocardial damage associated with the therapy. Assessments by thallium(Tl)-201 myocardial scintigraphy and/or coronary angiography in patients with suspicious coronary events revealed only two patients (2.9%) were considered to be myocardial ischemia associated with coronary vasospasm or microcirculatory disturbance. Sequential evaluations on echocardiography revealed significant ($p < 0.05$) decrease in LV end-diastolic dimension (4, 8, 18 and 24 weeks) and LV end-systolic dimension (from 1 to 24 weeks), and significant ($p < 0.05$) increase in LV ejection fraction 1 to 4 weeks after the kidney transplantation. Thickening of LV wall (>2 mm compared with baseline) was detected in only one patient. The present prospective study detected totally 30.9% incidence of cardiovascular adverse events. Symptomatic events and troponin T elevation were closely related to elevated blood drug concentrations (>20 ng/ml). Coronary vasomotor dysfunction seemed to be related to these adverse events especially when the blood drug concentration was exceeding 20 ng/ml.**

Key Words. **tacrolimus, myocardial ischemia, angina pectoris, troponin T, kidney transplantation**

Introduction

Although tacrolimus is widely applied for the immunosuppressive therapy after organ transplantations, cardiovascular adverse events such as anginal pain, palpitation, electrocardiographic changes, or reversible myocardial hypertrophy were sometimes reported [1–3]. Atkison et al. [4] reported development of myocardial hypertrophy in pediatric patients undergoing immunosuppressive therapy using tacrolimus after transplantation of liver or small intestine, which was dose-dependent and reversible. Nakata et al. [5] also reported a similar drug-concentration dependent occurrence of myocardial hypertrophy in pediatric patients receiving the tacrolimus therapy after liver transplantation. Multicenter studies in United States [6] and Europe [7] compared the efficacy and safety of tacrolimus and cyclosporine in recipients of kidney transplantations. Both studies demonstrated that the incidence of anginal pain was higher in patients receiving tacrolimus therapy than in patients receiving cyclosporine therapy, while the incidence of arrhythmias and hypercholesterolemia were higher in patients receiving cyclosporine therapy.

On the other hand, it is well known that in recipients of kidney transplantations, left ventricular hypertrophy, ventricular arrhythmias, or coronary artery disease coexist frequently because of the underlying systemic diseases, neurohumoral derangements, coexisting anemia, and influences of long-term hemodialysis performed before the kidney transplantation. Thus, to better clarify the incidence and pathophysiological mechanism of cardiovascular adverse effects of tacrolimus, the present multicenter study prospectively investigated the incidence, clinical backgrounds,

Address for correspondence: Yoshihiko Seino, MD, The First Department of Medicine, Nippon Medical School, 1-1-5, Sendagi, Bunkyo-ku, Tokyo 113-8603, Japan. Tel.: ±81-3-3822-2131; Fax: ±81-3-5685-0987; E-mail: y-seino@nms.ac.jp

and mechanism of cardiovascular adverse effects of tacrolimus in patients with kidney transplantation.

Methods

The present study was conducted at 34 institutes in Japan, and the enrollment was started from June 1996 to May 1998. Before enrolment in the present study, each patient gave informed consent. Members of the committee for the present investigation are listed in an appendix to this report. The cardiac data and safety monitoring board which was consisted of 4 cardiologists (YS, MH, HH, NF) and one nephrologist (TS) was established to evaluate all the data provided from each institute for the present prospective study.

Patients

Sixty-eight patients (43 males and 25 females, mean 33.4 years old, range 16–58 years old) who were receiving living or cadaveric kidney transplants were enrolled from the 26 institutes. Patients with previous myocardial infarction, angina pectoris, documented coronary artery disease, or congestive heart failure were excluded from the present study. Of the 68 patients, 59 (86.8%) received tacrolimus until 24 weeks after transplantation according to the study protocol; however, tacrolimus was discontinued within 24 weeks (4–168 days after transplantation) in 9 patients (13.2%) because of the occurrence of rejection with an adverse event ($n = 1$), adverse events such as renal function disturbance, liver function disturbance, impaired glucose tolerance, and abnormal nervous findings ($n = 7$), or deterioration from complications($n = 1$).

Dosing schedule

The dosage and administration were pursuant to the approved dosage and administration for tacrolimus capsules and injections indicated for kidney transplantation from the Ministry of Health and Welfare, Japan, and the dosage was adjusted using, as indices, the clinical courses, blood tacrolimus concentrations and serum creatinine levels [1–3]. In principle, intravenous administration of tacrolimus was recommended as the loading therapy in the initial three days after kidney transplantation. In the present study, 57 of 68 patients (83.8%) were given intravenous administration of tacrolimus in the initial three days after kidney transplantation. As a result, the median loading dose given by intravenous administration was 0.100 mg/kg/day, and the median loading dose given by oral administration was 0.300 mg/kg/day two days before transplantation, then 0.291 mg/kg/day one week after transplantation.

On the basis of clinical studies conducted previously, the target blood concentration (trough level) during oral administration was set at 20 ng/mL or less until 10 days after transplantation, 15 ng/mL or less from 10 days to 3 months after transplantation, and 10 ng/mL or less from 3 months after transplantation.

Assessment of cardiovascular effects by cardiovascular examinations

Cardiovascular examinations concerning any subjective symptoms, resting electrocardiography, chest X-ray, ambulatory Holter's dynamic electrocardiography (DCG), two-dimensional echocardiography, and measurement of serum levels of cardiac troponin T, creatine kinase (CK), and CKMB were made before the initiation of tacrolimus therapy, and at 1, 2, 4, 8, 12, 16, 20 and 24 weeks after kidney transplantation. Bodyweight and cell blood counts including serum hemoglobin levels were measured at every visit to check the condition of circulating blood volume control.

Subjective symptoms such as chest pain, chest discomfort, or palpitation were monitored every day during hospitalization and every visit to the outpatient clinic after discharge. When a subjective symptom was noted, the duration, any triggering factor, medical treatment for the event, and the follow-up were examined precisely, and trough concentrations of the drug at the time of symptom onset were correlated with these events.

On the Holter's DCG analysis, occurrence of symptomatic or asymptomatic myocardial ischemia was assessed by ST segment deviation (ST depression or ST elevation >1.0 mm) or T wave inversion associated with increase in heart rate and its duration. Occurrence of arrhythmias was evaluated by the frequency and the severity according to Lawn's classification for ventricular arrhythmias. Cardiac function (left ventricular ejection fraction: LVEF, LV end-diastolic dimension: LVEDD, LV end-systolic dimension: LVESD) and LV wall thickness were measured by two-dimensional echocardiography according to the study protocol. Presence of LV hypertrophy was defined as the wall thickness equal or more than 13 mm. Electrocardiographic diagnosis of LV hypertrophy was not applied in the present study because of the limitation for the diagnostic accuracy in patients with end-stage renal disease. Occurrence of LV dysfunction was defined as decrease in LVEF >5% compared with the baseline, and the development of LV wall thickening was defined as an increase in wall thickness >2 mm compared with the baseline.

To detect whether any myocardial damage occurs following tacrolimus therapy, 5–10 mL of blood was collected according to the study protocol to measure serum levels of cardiac troponin T (Enzymun test, Boehringer Mannheim, Germany), CK and CKMB (immunoinhibition assay, Merk, USA), as we previously reported [8–10]. Recently, the Joint Committee of the European Society of Cardiology and American College of Cardiology published a redefinition of acute myocardial infarction, and recommended that troponin T should be measured as the first-choice because of

almost absolute myocardial specificity and prominent high sensitivity reflecting even microscopic minor myocardial damage [11]. Furthermore, we recently reported that elevation of troponin T identifies patients with ongoing myocardial damage and at increased risk for future cardiac events in chronic heart failure [10]. In the present study, we analyzed sequential changes in these cardiac markers to detect minor or ongoing myocardial damage following the tacrolimus therapy.

Additional examinations, including treadmill exercise ECG test, thallium(Tl)-201 myocardial scintigraphy and/or coronary angiography were performed in the case of suspicious coronary event to clarify the pathophysiological mechanism.

The cardiac data and safety monitoring board

The members of cardiac data and safety monitoring board (five members; YS, MH, TS, HH, and NF) regularly assessed all data of the cardiovascular examinations, including the original resting ECG, ambulatory Holter's DCG, and original records of two-dimensional echocardiography. The board members independently analyzed the data to detect any possible adverse effects or abnormal findings in these cardiovascular examinations.

Further precise assessment of the events detected by this primary screening were made again by the treating physician based on the clinical course, laboratory data, and additional examinations such as treadmill exercise test, Tl-201 myocardial scintigraphy or coronary angiography. Final judgment on the cardiovascular adverse events was made on the basis of these precise assessments by the cardiac data and safety monitoring board.

Results

Patient backgrounds

The patient backgrounds are shown in Table 1. The average duration of dialysis was 36.2 months (range, 1–218 months), and 14 patients (20.6%) received dialysis for 5 years or more. Hypertension was a complication for 36 patients (52.9%). Most (92.6%) of the patients received kidney from living donors; only 5 patients underwent cadaveric kidney transplantation. The mean donor age was 54.5 years (range, 20–76 years), with 23 patients (33.8%) receiving kidneys from donors older than 60 years. The ABO blood types were concordant in 53 patients (77.9%), discordant in 13 patients (19.1%), and incompatible in 2 patients.

The immunosuppressive therapy performed immediately after transplantation was a triple therapy with tacrolimus, a steroid and azathioprine or mizoribine in 57.4% of the cases, a quadruple therapy, in which antilymphocyte globulin was added to the triple therapy in 27.9% of the cases, and a dual therapy with tacrolimus and a steroid in 8.8% of the cases. In the two patients of ABO incompatible (19 year-old woman and 32 year-old man, both from AB to A transplantation), a triple therapy with tacrolimus, methyl-predonisolone and azathioprine was applied. In the case of 32 year-old man, antilymphocyte globulin was added to the triplet therapy in the initial 2 weeks of postoperative period. Both patients had successful transplantation.

Cardiovascular examinations before starting the tacrolimus therapy revealed ST or T wave abnormalities on resting ECG (13.6%, 9/66), asymptomatic ST depression following increased heart rate (8.3%, 5/60), arrhythmias (8.3%, 5/60) on ambulatory Holter's DCG, and the presence of LV hypertrophy (28.8%, 17/59) on two-dimensional echocardiography (Table 1).

Table 1. *Patients background-demographic and clinical criteria*

Recipient age	33.4 ± 9.8 years (mean ± SD, range: 16–58 years, male 43, female 25),
Duration of HD	36.2 ± 45.3 months (Range: 1–218 months)
Complication	
No complication	20 (29.4%)
Hypertension	36 (52.9%)
Anemia	13 (19.1%)
Hyperuricemia	6 (8.8%)
Impaired glucose torelance	5 (7.4%)
Others	20 (29.4%)
Cardiothoracic ratio (%)	45.8 ± 4.2
ST or T wave abnormalities in ECG	9/66 (13.6%)
Asymptomatic ST depression following HR increase	5/60 (8.3%)
Arrhythmias (sinus arrhythmia, PVC, or PAC)	5/60 (8.3%)
LV hypertrophy on 2D-echocardiography	17/59 (28.8%)
LVEF (%)	66.2 ± 10.1
LVEDD (mm)	51.9 ± 10.7
LVESD (mm)	32.4 ± 6.1
Type of transplantation	
Living kidney	63 (92.6%)
Cadaveric kidney	5 (7.4%)
Donor age	54.5 ± 10.6 years old (Range: 20–76 years)

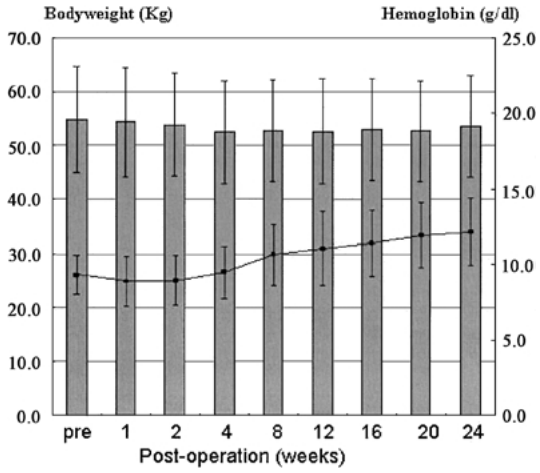

Fig. 1. *Changes in bodyweight and hemoglobin concentrations following kidney transplantation and tacrolimus therapy.*

Sequential changes in bodyweight, hemoglobin concentrations and blood drug concentrations

Sequential changes in bodyweight and hemoglobin concentrations are shown in Figure 1. Overfilling following the operation was not observed in the present study patients. The mean blood drug concentration was 16 ng/mL at one week after kidney transplantation, 17.5 ng/mL at 2 weeks, 12 ng/mL at 12 weeks, 11 ng/mL at 16 weeks, and 9.45 ng/mL at 20 weeks after the transplantation (Fig. 2).

Table 2. *Blood drug concentrations at the onset of subjective symptoms*

Symptom	Number	Drug concentration (administration route at onset)
Chest pain or discomfort	4	21.0 ng/mL (oral) 31.2 ng/mL (oral) 54.0 ng/mL (iv + oral) 60.0 ng/mL (iv)
Palpitation	1	20.0 ng/ml (oral)
Cardiac failure	1	63.0 ng/ml (iv)

Subjective symptoms and relationship to drug concentrations

Subjective cardiovascular symptoms were reported in 13 patients (19.1%) as 17 events: chest pain or chest discomfort in 9 patients (13.2%), palpitation in 6 patients (8.8%), tachycardia in one patient, and heart failure in one patient. These symptoms appeared within one week after transplantation in 7 out of 13 patients (53.8%), and within 4 weeks in 9 (62.9%), suggesting that subjective cardiovascular symptoms appeared early phase after the treatments (Fig. 3).

The blood drug concentration at the time of cardiac symptom development was determined in 6 of the 13 patients. The median value was 42.6 ng/mL and the minimum was 20 ng/mL (Table 2). These symptoms were eliminated or ameliorated by standard treatment in 12 out of 13 patients (92.3%, 16 events); 6 patients (46.2%) required no special treatment, and 5 patients (38.5%) received an application of nitroglycerin tape,

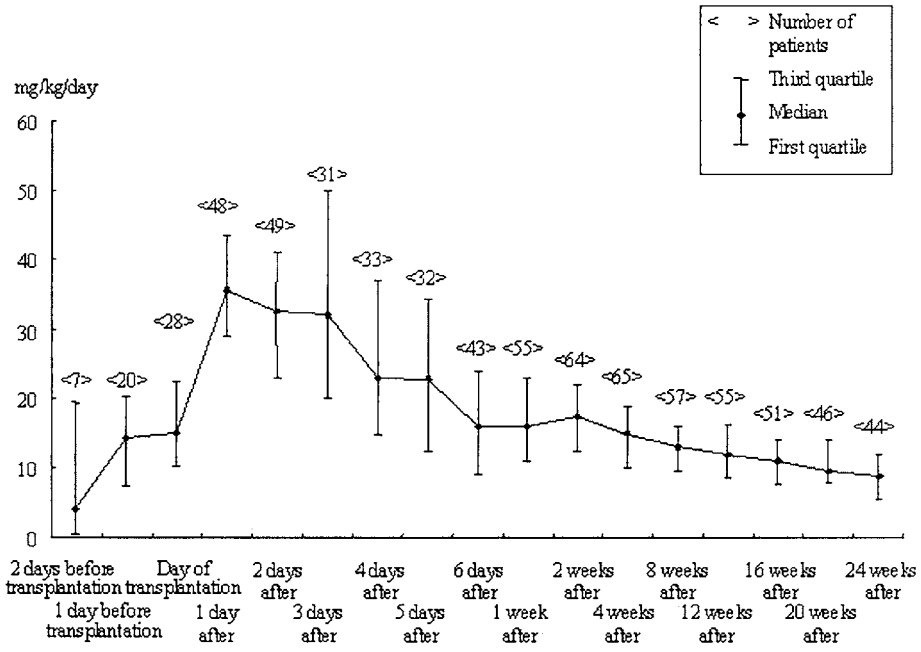

Fig. 2. *Changes in blood drug concentration following intravenous and succeeding oral administration of tacrolimus. Each bar represents median, third quartile (upper part) and first quartile (lower part).*

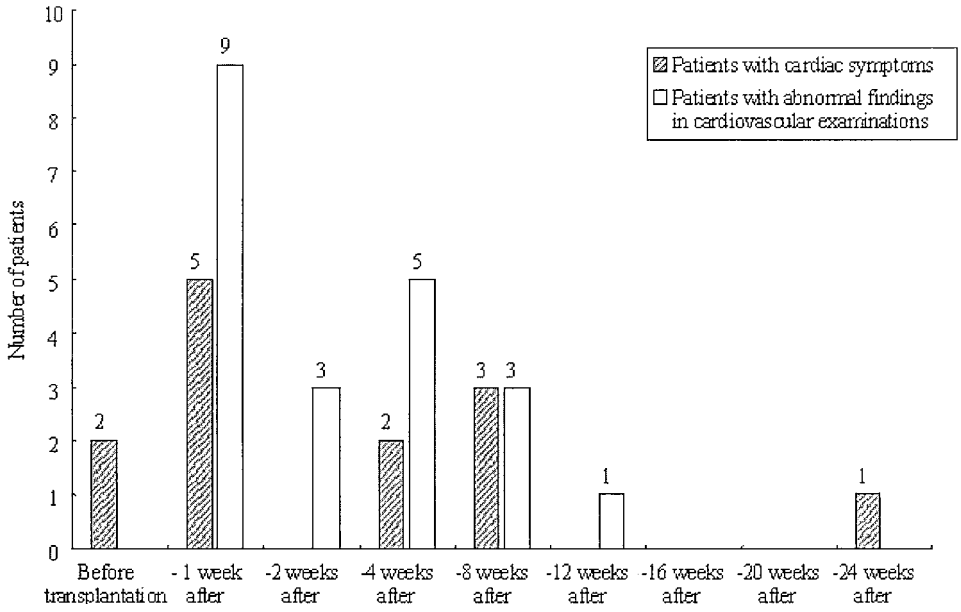

Fig. 3. *Time-course of the development of cardiovascular symptoms and abnormalities in cardiovascular examinations. Oblique line bar: patients number with cardiovascular symptoms. Open bar: patients number with detected abnormalities in cardiovascular examinations.*

dose-reduction in 3 patients (23.1%) or withdrawal of tacrolimus in one patient (4.3%). The duration until the recovery was within 1 week in 9 patients (75.0%) and within 2 weeks in 11 patients (91.7%). In the case with withdrawal of tacrolimus, occurrence of chest pain was reported on the 95th day of postoperative period, and development of exercise-induced ST depression was documented on the ambulatory Holter's DCG. Although elevation of cardiac troponin T was not detected in this case, tacrolimus was changed to cyclosporine, and the symptom was recovered soon after. However, further investigation such as cardiac nuclear imaging or coronary angiography was not performed in this case.

Sequential changes in the echocardiographic measurements

Table 3 shows sequential changes in the cardiothoracic ratio and echocardiographic measurements regularly evaluated according to the study protocol. The cardiothoracic ratio slightly increased ($p < 0.05$) one week after treatment. LVEDD decreased significantly ($p < 0.05$) at 4, 8, 16, and 24 weeks after the treatment, and LVESD decreased significantly ($p < 0.05$) from 1 to 24 weeks after the treatment, and resulted in a significant increase ($p < 0.05$) in LVEF from 1 to 4 weeks after transplantation. Echocardiographic evaluations detected a decrease in LVEF (>5% from baseline) in only one patient, and thickening of LV wall (>2 mm from baseline) in another patient.

Development of abnormal findings in cardiovascular examinations

The cardiac data and safety monitoring board analyzed all the scheduled examinations to compare with each pre-treatment record, and detected newly development of resting ECG abnormalities in 12 patients (17.6%), asymptomatic ST depression following increased heart rate in 11 patients (16.2%) and arrhythmias in 7 patients (10.3%) on ambulatory Holter's DCG analysis. However, assessment with Tl-201 myocardial scintigraphy ($n = 4$) or coronary arteriography ($n = 2$) performed in the case of suspicious coronary events

Table 3. *Changes in cardiothoracic ratio and echocardiographic measurements following kidney transplantation and tacrolimus therapy*

	Pre	1 week	2 week	4 week	8 week	12 week	16 week	24 week
CTR (%)	45.8 ± 4.2	47.2 ± 5.3*	46.2 ± 4.7	45.0 ± 3.7	45.6 ± 3.9	45.6 ± 4.2	45.7 ± 6.3	45.8 ± 6.3
LVEDD (mm)	51.9 ± 10.7	51.8 ± 11.2	50.4 ± 10.2	49.3 ± 9.5*	46.6 ± 5.6*	49.3 ± 12.5	46.3 ± 6.2*	49.3 ± 15.8*
LVESD (mm)	32.4 ± 6.1	30.6 ± 6.2*	28.9 ± 5.0*	28.8 ± 5.0*	28.6 ± 5.6*	29.0 ± 5.4*	27.6 ± 4.7*	28.6 ± 4.2*
LVEF (%)	66.2 ± 10.0	71.0 ± 9.7*	72.7 ± 7.7*	71.4 ± 8.5*	65.4 ± 18.6	68.3 ± 9.9	71.3 ± 5.5	70.5 ± 8.2

Data were expressed as mean ± SD, *: $p < 0.05$ compared with pre-treatment value.

revealed no significant coronary stenotic findings. Only two patients (2.9%) were considered to have myocardial ischemia because of another symptomatic episode of anginal pain and the typical efficacy of nitroglycerin for the relief of the episode.

Troponin T elevation and relationship to blood drug concentrations

Elevation of troponin T suggesting minor or ongoing myocardial damage was found in 3 patients (4.4%) during the study period. None of three patients had subjective symptoms. In the first case, persistent elevation of troponin T (0.22, 0.11, and 0.27 ng/ml) associated with appearance of inverted T wave on resting ECG, and increase in premature ventricular contractions on Holter's DCG were detected at 1, 12 and 16 weeks after the operation, which was interpreted as myocardial damage associated with the therapy because the trough drug concentrations were elevated (>20 ng/ml) during the corresponding 8 to 12 weeks postoperative period. In the second case, transient but marked elevation of troponin T (3.29 ng/ml) associated with marked elevation of CK (1,945), transient ST elevation in leads II and aVF, and appearance of pericardial effusion was detected. This was interpreted as marked myocardial damage. In the third case, elevation of troponin T (0.56 ng/ml) was detected one week after the operation. Neither ECG nor Holter's DCG showed significant changes, however sequential evaluation of echocardiography revealed enlargement of left atrium with appearance of mild mitral regurgitation and lowering in LVEF (43%). In this case, elevated trough drug concentration (>40 ng/ml) was detected during this period, and the troponin T elevation was interpreted as myocardial damage associated with the therapy.

Development of abnormal findings in cardiovascular examinations and those backgrounds

Final judgments of abnormal findings in cardiovascular examinations by the cardiac data and safety monitoring board are listed in Table 4. These abnormal findings were noted in 21 patients (30.9%) as 33 events. Most of the abnormalities were mild: developments of electrocardiographic abnormalities were found in 12 patients (17.6%), arrhythmia in 7 patients (10.3%), myocardial damage in 4 patients (5.9%), silent myocardial ischemia in 2 patients (2.9%), and cardiac dysfunction and LV wall thickening in 1 patient each. These abnormalities were detected within 1 week in 9 out of 21 patients (42.9%), within 2 weeks in 12 out of 21 patients (57.1%) and within 4 weeks in 17 patients (81.0%), suggesting that these events appeared relatively early phase of the treatment, the same as in the case of occurrence of subjective symptoms (Fig. 3).

These adverse events were not related to changes in bodyweight nor hemoglobin concentrations, and the causative possibility of overfilling following the operation was ruled out in the present study; median (the first and third quartile) hemoglobin concentration (g/dl) was 8.6 (6.0, 11.1) at one week, 8.8 (6.0, 11.6) at 2 week, and 9.9 (7.0, 12.7) at 4 week for patients presenting these adverse events compared with 9.1 (7.8, 10.2), 8.9 (7.8, 10.1), and 9.3 (8.3, 10.7) for those without it, respectively. When the relationship with use of anti-lymphocyte globulin was analyzed, incidence of development of cardiovascular subjective symptoms was 9.5% (2/21) for patients given anti-lymphocyte globulin versus 23.4% (11/47) for those without it, and incidence of abnormal findings in cardiovascular examinations was 33.3% (7/21) versus 29.7% (14/47), respectively. Thus the use of anti-lymphocyte globulin (n = 21 patients) was not related to development of these adverse events. The blood drug concentrations at the time of detecting these cardiovascular abnormal findings were determined in 19 out of the 21 patients. Blood drug concentrations of 20 ng/mL or more were seen in 7 patients (36.8%). Development of abnormal findings in cardiovascular examinations was not necessarily confirmed to be dose-dependent for the all cases.

Table 4. *Development of abnormal findings in cardiovascular examinations*

Abnormalities	Number of patients (%)
Resting ECG	
Tall T wave	5 (7.4%)
ST elevation	4 (5.9%)
T wave inversion	2 (2.9%)
ST depression	1 (1.5%)
Pulmonary P-wave	1 (1.5%)
PR shortening	1 (1.5%)
Echocardiogram	
Decrease in LVEF (>5% from baseline)	1 (1.5%)
LV wall thickening (>2 mm from baseline)	1 (1.5%)
Pericardial effusion	4 (5.9%)
Holter's DCG	
Silent myocardial ischemia	2 (2.9%)
Worsening of VPC	2 (2.9%)
AV-block	2 (2.9%)
Non-sustained VT	1 (1.5%)
Sinus arrhythmia	2 (2.9%)
Biochemical markers of myocardial injury	
Troponin T elevation	3 (4.4%)
Other finding of myocardial damage	1 (1.5%)

Discussion

Cardiovascular adverse events and relationship to elevated drug concentration

Tacrolimus suppresses T-cell activation by binding to FK binding protein (FKBP). The drug-FKBP complex stably associates with calcineurin and inhibits the phosphatase activity of this Ca-dependent enzyme, and as a consequence inhibits the nuclear translocation of nuclear factor of activated T-cells, and suppresses

induction of cytokine gene expression [12]. In myocytes, FKBP couples with ryanodine receptors, which control intracellular calcium ion incorporation, and tacrolimus enhances calcium ion release from sarcoplasmic reticulum by affecting the receptors [13].

Previous clinical trials of tacrolimus in Japanese recipients of kidney transplants reported that adverse cardiovascular events such as chest pain, palpitation, ECG abnormalities, or decrease in LVEF developed in 15 to 28% of the patients [1–3]. The incidence of cardiovascular adverse events detected in the present prospective investigation was slightly higher compared with the previous reports. The most frequent adverse event was chest pain (19.8% in early phase II study, 12.9% in late phase II study, 13.3% in phase III study, and 13.2% in the present study) [1–3]. The present prospective study further demonstrated that such symptomatic events closely correlated with elevated drug concentrations (37.2 ± 18.7 ng/mL) in the relatively early phase after transplantation. Development of troponin T elevation which is suggestive of minor or ongoing myocardial damage also closely related to elevated levels of blood drug concentrations (>20 ng/ml).

On Holter's DCG analysis, asymptomatic ST depression following increased heart rate was detected in 16.2% of the patients, which suggested occurrence of silent myocardial ischemia associated with the therapy. However, precise analysis including treadmill exercise test, Tl-201 myocardial scintigraphy or coronary angiography revealed no significant coronary artery stenotic findings. Furthermore, no significant relationship was found between the ST depression and hemoglobin concentrations or bodyweight changes following the operation, thus the causative possibility of overfilling following the operation was ruled out. Possibility of the influence of anti-lymphocyte globulin therapy was also ruled out. Although elevated levels of drug concentration exceeding scheduled levels was considered as the cause of abnormal findings in cardiovascular examinations, it was not necessarily confirmed in all the cases because of lack of symptoms and time-lag of the blood sampling. The reason for we could not evidence a clear relationship between occurrence of the abnormalities in cardiovascular examinations and the drug concentration might be related to difficulties in the assessment based on the trough drug concentrations to correlate such events in clinical situations. However, appearance and amelioration of the events, which were concentrated in relatively early phase after staring the treatment as presented in Figures 2 and 3 suggests a dose-related phenomenon as same as in the case of occurrence of symptomatic events.

Possible mechanism for cardiovascular adverse events

Coronary vasospasm or microcirculatory disturbance (microvascular angina) was suspected as the cause of the silent myocardial ischemia and other symptomatic events in the present investigation. However in the precise analysis, only two patients (2.9%) were considered to have experienced silent myocardial ischemia because of another symptomatic episode accompanied by transient ST elevation and the efficacy of nitroglycerin. Proposed mechanisms of vasoconstriction were considered to be similar as the mechanism of coronary vasomotor dysfunction precipitated by another immunosuppressive agent cyclosporine: that is, increased intracellular Ca concentrations in vascular smooth muscle cells, inhibition of nitric oxide synthase (NOS), activation of endothelin gene expression, and activation of renin-angiotensin-aldosterone system [14]. Weis et al. [15] reported comparison of the effects of different immunosuppressive regimens on coronary vasomotor function in patients undergoing cardiac transplantation. They found that prevalence of epicardial endothelial dysfunction, assessed by induction of coronary vasospasm, and microvascular dysfunction, assessed by limitation in the flow velocity increase to intracoronary injection of adenosine, were both significantly larger in patients treated with tacrolimus than in those treated with cyclosporine. They further demonstrated that the observed coronary vasomotor dysfunction was associated with increased myocardial inducible-NOS gene expression, decreased endothelial-NOS gene expression, and enhanced cardiac release of interleukin-6. Their report strongly suggests the crucial role of these neurohumoral factors in the occurrence of symptomatic or asymptomatic myocardial ischemic events in tacrolimus therapy after kidney transplantation.

Rare incidence of hypertrophic change following tacrolimus therapy

Two reports demonstrated development of myocardial hypertrophy in pediatric patients treated with tacrolimus after transplantation of liver or small intestine; this hypertrophic change was dose-dependent (drug concentration >15 ng/mL) and reversible [5,6]. However, no such a report was found in adult patients treated with tacrolimus, and the present prospective investigation did not detect cardiac hypertrophic changes except one border-line case. In this case, drug concentrations of tacrolimus were controlled under the target dose during the study, and some influence associated with recovery from postoperative volume-loading state was considered as the cause rather than the adverse effects of the drug.

The contribution of calcineurin activation or inhibition to the development of cardiac hypertrophy is still debated. Molkentin et al. [16] reported that calcineurin plays a pivotal role in the development of cardiac hypertrophy through activation of the nuclear factor of activated T-cell, a member of transcription factor, in the transgenic mice. The calcineurin inhibitors, tacrolimus and cyclosporine, prevented cardiac hypertrophy of activated calcineurin transgenic mice. Shimoyama et al.

[17] also reported that calcineurin plays a critical role in pressure overload-induced cardiac hypertrophy produced by constriction of the abdominal aorta in the rats. Tacrolimus inhibited the activation of calcineurin and prevented cardiac hypertrophy in their study. They further demonstrated calcineurin is involved in the development of cardiac hypertrophy in Dahl salt-sensitive hypertension rats and that tacrolimus could induce regression of cardiac hypertrophy [18].

In contrast, Minamino et al. [19] investigated the effects of tacrolimus on NOS activity and on p70S6K activity, which plays an important role in cardiac hypertrophy by regulating protein synthesis in rabbit hearts. They found an inverse correlation between reduced NOS activity and elevated p70S6K activity after chronic treatment with high-dose tacrolimus, which might contribute to the cardiac hypertrophy in some previously reported pediatric patients [4,5].

Clinical implication of the present study

The present prospective study confirmed that careful monitoring of blood drug concentration should be essential to prevent cardiovascular adverse events, such as occurrence of chest pain, palpitation, symptomatic or asymptomatic myocardial ischemia or development of ongoing myocardial damage. Occurrence of symptomatic adverse events and troponin T elevation were closely related to elevated drug concentrations. Appropriate dose adjustment should be employed to minimize adverse cardiovascular effects especially when the drug concentration exceeds 20 ng/ml after the transplantation.

Appendix

First Department of Internal Medicine, Nippon Medical School: Yoshihiko Seino, MD, Hiroshi Honma, MD, Nagaharu Fukuma, MD
First Department of Internal Medicine, School of Medicine, Osaka University: Masatsugu Hori, MD
Osaka Prefecture General Hospital: Takao Sonoda, MD
Department of Surgery, Institute of Clinical Medicine, University of Tsukuba: Katashi Fukao, MD
Second Department of Surgery, School of Medicine, Chiba University: Takenori Ochiai, MD
Third Department of Surgery, Kidney Center, Tokyo Women's Medical University: Tatsuro Kawai, MD
Department of Urology, School of Medicine, Kitasato University: Tadao Endo, MD
Department of Urology, School of Medicine, Niigata University: Kota Takahashi, MD
Department of Urology, Shakaihoken Chukyo Hospital, Department of Urology: Shin-ichi Oshima, MD (present affiliation: Nagoya University)
Second Department of Surgery, School of Medicine, Nagoya University: Itsuo Yokoyama, MD
Department of Transplantation Surgery, Nagoya Second Red Cross Hospital: Kazuharu Uchida, MD
Second Department of Surgery, Kyoto Prefectural University of Medicine: Tadaki Yasumura, MD (present affiliation: Yasumura Hospital)
Department of Urology, School of Medicine, Osaka University: Shirou Takahara, MD
Kidney Center, Tokyo Women's Medical University: Kazuo Ota, MD (present affiliation: Ota Medical Institute)
Second Department of Surgery, School of Medicine, Nagoya University: Hiroshi Takagi, MD (present affiliation: JR Tokai General Hospital).

References

1. FK506 Kidney Transplant Study Group: Early phase II study of FK506 (tacrolimus) for kidney transplantation. *Jpn J Transplant* 1994;29:294–313.
2. FK506 Kidney Transplant Study Group: Late phase II study of FK506 (tacrolimus) for kidney transplantation. *Jpn J Transplant* 1994;29:614–631.
3. FK506 Kidney Transplant Study Group: Phase III comparative study of FK506 (tacrolimus) for kidney transplantation. *Jpn J Transplant* 1994;29:650–681.
4. Atkison P, Joubert G, Barron A, et al. Hypertrophic cardiomyopathy associated with tacrolimus in pediatric transplant patients. *Lancet* 1995;345:894–896.
5. Nakata Y, Yoshibayashi M, Yonemura T, et al. Tacrolimus and myocardial hypertrophy. *Transplantation* 2000;69:1960–1962.
6. Pirsch JD, Miller J, deierhoi MH, et al. A comparison of tacrolimus (FK506) and cyclosporine for immunosuppression after cadaveric renal transplantation. *Transplantation* 1997;63:977–983.
7. A Report of The European Tacrolimus Multicenter Renal Study Group: Multicenter randomized trial comparing tacrolimus (FK506) and cyclosporine in the prevention of renal allograft rejection. *Transplantation* 1997;64:436–443.
8. Seino Y, Tomita Y, Takano T, Hayakawa H. Early identification of cardiac events with serum troponin T in patients with unstable angina. *Lancet* 1993;342:1236–1237.
9. Seino Y, Tomita Y, Hoshino K, et al. Pathophysiological analysis of serum troponin T release kinetics in evolving ischemic myocardial injury. *Jpn Circ J* 1996;60:265–276.
10. Setsuta K, Seino Y, Ogawa T, et al. Use of cytosolic and myofibril markers in the detection of ongoing myocardial damage in patients with chronic heart failure. *Am J Med* 2002;113:717–722.
11. The Joint European Society of Cardiology/American College of Cardiology Committee: Myocardial infarction redefined—A consensus document of The Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction. *J Am Coll Cardiol* 2000;36:959–969.
12. McCaffrey PG, et al. NF-ATp, a T lymphocyte DNA-binding protein that is a target for calcineurin and immunosuppressive drugs. *J Biol Chem* 1993;268:3747–3752.
13. Brillantes AB, Ondrias K, Scott A, et al. Stabilization of calcium release channel (ryanodine receptor) function by FK506-binding protein. *Cell* 1994;77:513–523.
14. Ventura HO, Malik FS, Mehta MR, et al. Mechanism of hypertension in cardiac transplantation and the role of cyclosporine. *Curr Opin Cardiol* 1997;12:375–381.

15. Weis M, Wildhirt SM, Schulze C, et al. Coronary vasomotor dysfunction in the cardiac allograft: Impact of different immunosuppressive regimens. *J Cardiovasc Pharm* 2000;36:776–784.
16. Molkentin JD, Lu JR, Antos CL, et al. A calcineurin-dependent transcriptional pathway for cardiac hypertrophy. *Cell* 1998;93:215–228.
17. Shimoyama M, Hayashi D, Takimoto E, et al. Calcineurin plays a critical role in pressure overloaded-induced cardiac hypertrophy. *Circulation* 1999;100:2449–2454.
18. Shimoyama M, Hayashi D, Zou Y, et al. Calcineurin inhibitor attenuates the development and induces the regression of cardiac hypertrophy in rats with salt-sensitive hypertension. *Circulation* 2000;102:1996–2004.
19. Minamino T, Kitakaze M, Ueda Y, et al. Chronic treatment with FK506 increases p70S6 kinase activity associated with reduced nitric oxide synthase activity in rabbit heart. *Cardiovasc Drugs Ther* 2000;14:329–336.