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Japanese study of FK506 on kidney transplantation: 2. Follow-up study of FK506-treated patients

Japanese FK506 Study Group

Abstract. Thirty-seven primary renal transplant patients were enrolled in the early phase II study on kidney transplantation. All grafts survived during the follow-up period. However, 10 of the 37 patients were changed from FK506 to conventional drugs, and 3 were treated concomitantly with azathioprine (AZA) or mizoribine (MZR) in the 3-month period of observation. After 3 months posttransplantation, an additional 10 patients were treated continuously with AZA or MZR. In addition, 3 were converted from FK506 to conventional drugs. No additional conversion was observed after 4 months. Trough level monitoring was effective enough to regulate the FK506 dosage. Nephrotoxicity and hyperglycemia were associated with a high trough level of FK506 (whole blood, > 20 ng/ml).

Key words: FK506 – Renal transplantation – Clinical study – Follow-up study

Patients and methods

Exclusion criteria. The following patients were excluded: (1) under 16 years old, (2) liver dysfunction of more than 1.6 mg/dl of total bilirubin or twice or more higher glutamine-oxaloacetic (GOT) and glutamic-pyruvic (GPT) transaminase values than normal, (3) pulmonary dysfunction of less than 70 mm Hg of PaO_2, (4) cardiac problems with less than 60% of ejection fraction or abnormal ECG (6) peptic ulcer, (7) a history or present status of malignant carcinoma, (8) infectious disease including hepatitis B virus (HB) hepatitis, (9) drug hypersensitivity or allergy, (10) pregnant or expecting to become so, (11) a history of kidney transplantation, (12) a minor mismatch for ABO blood group, (13) positive T-cell crossmatch, (14) HLA identical or 2-haplotype mismatched living related donor, (15) did not consent to receive the FK506 treatment.

Monitoring of blood concentration of FK506. The trough level of FK506 in the plasma and whole blood as measured by the double sandwich enzyme-linked immunosorbent assay (ELISA) were monitored. The extraction of the blood for the measurement of the drug concentration involves a liquid phase method using dichloromethane [5]. This method is different from the column extraction method which is available in the United States of America. The detection limit was 0.5 ng/ml in whole blood and 0.05 ng/ml in plasma. The intra- and interassay coefficients of variation were both less than 20%.

Nephrotoxicity was diagnosed from mainly histology observations. Briefly, a foomy vacuolization of the proximal tubular cells, especially in the straight portion, was the main criteria [6].

Trial design and statistical analysis. An early phase II study on kidney transplantation was performed as a multicenter open trial. After the observation period of 3 months, 26 of the 37 patients were still being maintained on FK506 therapy.

The sign test was used in the analysis of dosage and blood levels. The Wilcoxon signed-rank test was used in the analysis of laboratory values.

Results

Characteristics of the study population

Table 1 lists the background characteristics of the patients. In all, 23 living related and 3 cadaveric cases were treated with FK506 for more than 3 months after transplantation.

Table 1. Characteristics of the study population

			n
Donor	Living related		23
	Cadaveric		3
HLA	A + B + DR mismatched		1.7 ± 1.0
Direct crossmatch		(−)	26
PRA	T	(−)	25
(*n* = 25)	B warm	(−)	24
		(+)	1
	B cold	(−)	23
		(+)	2

PRA, panel cell reactive antibody

Offprint requests to: S. Takahara, M.D., Department of Urology, Osaka University Medical School, 1-1-50 Fukushima, Fukushima-ku, Osaka 553, Japan

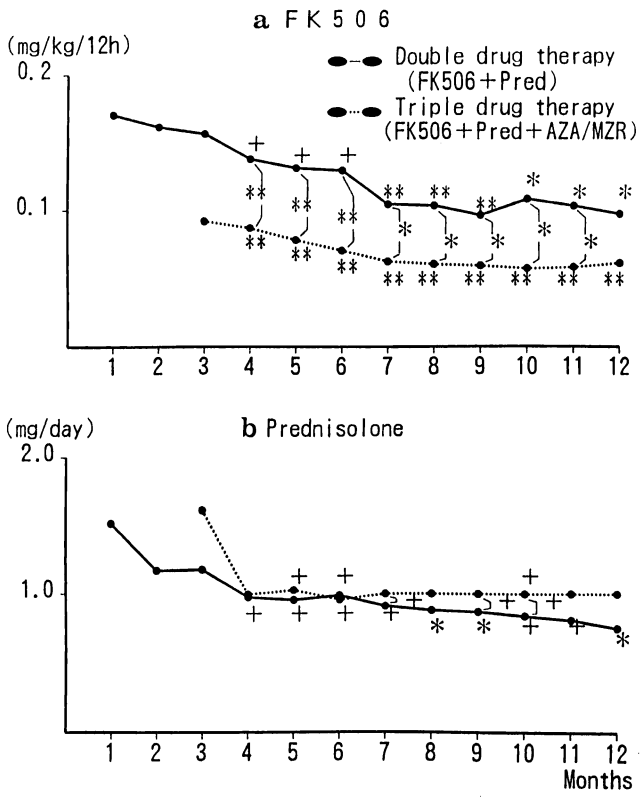

Fig. 1. Mean oral dosage of FK506 and prednisolone (Pred)

Mean HLA-A, B, DR mismatches was 1.7 ± 1.0.

All direct crossmatches and panel cell reactive antibody (PRA) titers against T cells were negative, although the PRA against B warm cells was positive in 1 patient and against B cold cells, positive in 2 patients.

Dosage of FK506 and prednisolone

The mean oral dosage of FK506 in the double drug therapy group (FK506 and prednisolone) was 0.16 ± 0.04 mg/kg b.i.d. at 3 months and thereafter was 0.13 ± 0.05 mg/kg at 6 months, 0.10 ± 0.05 mg/kg at 9 months, and 0.10 ± 0.05 mg/kg at 12 months (Fig. 1a).

The mean oral dosage of FK506 in the triple drug therapy group (FK506, prednisolone, and azathioprine or mizoribine, AZA/MZR) was 0.09 ± 0.04 mg/kg b.i.d. at 3 months, 0.07 ± 0.03 mg/kg at 6 months, 0.06 ± 0.02 mg/kg at 9 months, and 0.06 ± 0.01 mg/kg at 12 months.

In the double drug therapy group, the dosage of FK506 was decreased over 7 months. A statistically significant difference in the dosage was observed between 3 and 7–12 months ($P < 0.01$ or $P < 0.05$, respectively). A statistically significant difference in the dosage was observed between 3 and 4–12 months ($P < 0.01$) in the triple drug therapy group.

The mean oral dosage of prednisolone in the double drug therapy group was 11.8 ± 4.4 mg/day at 3 months, 9.9 ± 1.4 mg/day at 6 months, 8.7 ± 2.2 mg/day at 9 months, and 7.5 ± 3.6 mg/day at 12 months (Fig. 1b).

The mean oral dosage of prednisolone in the triple drug therapy group was 16.1 ± 8.6 mg/day at 3 months, 9.6 ± 1.5 mg/day at 6 months, 10.0 ± 0.0 ng/day at 9 months, and 10.0 ± 0.0 mg/day at 12 months.

FK506 trough levels

The FK506 trough level in the whole blood was 15.4 ± 8.1 ng/ml at 3 months, 13.1 ± 6.5 ng/ml at 6 months, 11.4 ± 5.9 ng/ml at 9 months, and 9.3 ± 1.6 ng/ml at 12 months in the double drug therapy group Fig. 2a). The trough level at 9 months was statistically significantly lower than that at 3 months ($P < 0.05$).

The FK506 trough level in the plasma was 0.35 ± 0.16 ng/ml at 3 months, 0.26 ± 0.16 ng/ml at 6 months, 0.18 ± 0.11 ng/ml at 9 months, and 0.12 ± 0.15 ng/ml at 12 months in the double drug therapy group (Fig. 2b). The trough levels were not stable during the follow-up period.

Graft survival and immunosuppressive protocol

All 26 grafts survived during the follow-up period (4–14 months after transplantation, average 332 ± 83 days).

At 3 months after transplantation, 23 of the 26 patients (88%) were treated only with FK506 and prednisolone. AZA or MZR was administered to 3 patients in addition to FK506 and prednisolone (Fig. 3).

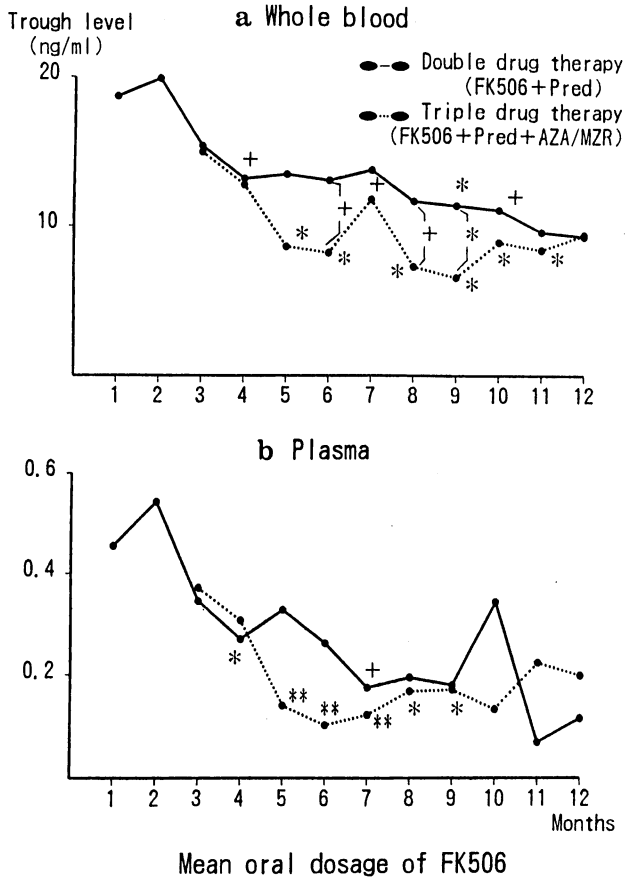

Fig. 2. Mean FK506 trough levels. AZA/MZR, azathioprine or mizoribine

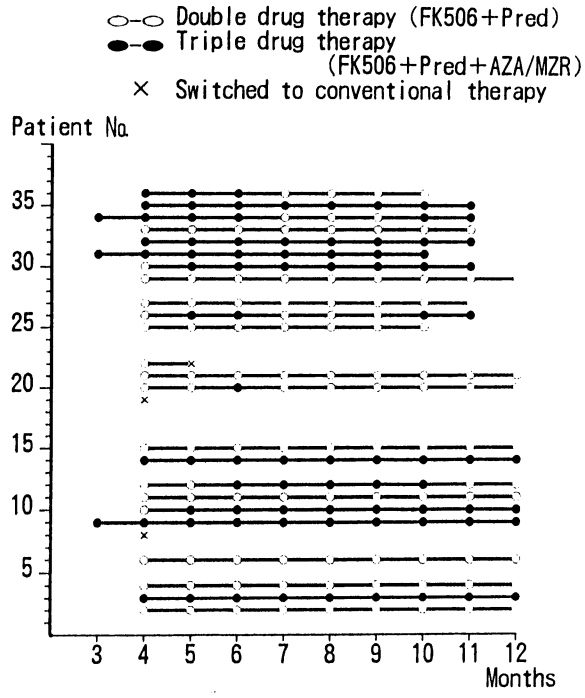

Fig. 3. Immunosuppressant therapy in the follow-up period

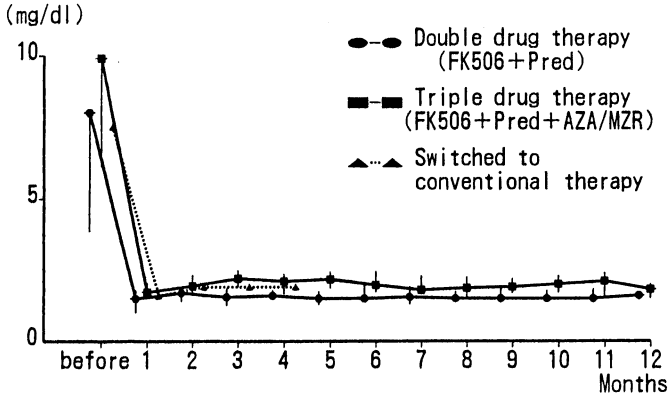

Fig. 4. Median serum creatinine levels

Between 4 and 6 months, 10 other patients were transferred to the triple drug regimen group (rejection 3 cases, nephrotoxicity 6 cases, both 1 case) from the double drug group.

FK506 was stopped, and the immunosuppressive protocol was changed to the protocols without FK506 in the other 3 patients of the double drug therapy group (rejection 1 case, nephrotoxicity 2 cases). No further drop-out case from FK506 therapy was observed thereafter. Between 7 and 9 months, 14 of the 23 recipients were treated with FK506 and prednisolone. The triple drug regimens of FK506/AZA/prednisolone or FK506/-MZR/prednisolone were administered to the other 9 recipients.

More than 10 months after transplantation, 12 of the 23 recipients were treated with FK506 and prednisolone. The triple drug regimens were administered to the other 11 recipients.

Rejection

Rejection was observed in 3 cases (4 episodes) in this follow-up period. Three cases were treated with bolus administration of methylprednisolone and/or by adding AZA or MRZ. In only 1 case was FK506 stopped, and CyA and antilymphocyte immunoglobulin (ALG) were administered. All grafts recovered from their rejection episodes and kept to the previous serum creatinine levels.

Serum creatinine

The median serum creatinine level in the double drug therapy group was 1.6 mg/dl at 3 months, 1.5 mg/dl at 6 months, 1.5 mg/dl at 9 months and 1.6 mg/dl at 12 months (Fig. 4). The median serum creatinine level in the triple drug therapy group was 2.2 mg/dl at 3 months, 2.0 mg/dl at 6 months, 1.9 mg/dl at 9 months, and 1.8 mg/dl at 12 months.

A statistically significant difference was observed between the two groups at 3 months ($P < 0.01$) and 6 months ($P < 0.05$).

Adverse events

No life-threatening adverse events were observed.

Insulin-dependent hyperglycemia occurred in 7 of the 26 cases (27%) within 3 months after transplantation (Fig. 5a). However, insulin was required in only 5 of the 26 cases (19%) between 4 and 6 months, and 2 of 23 (9%) between 7 and 9 and 10 and 12 months.

Antihyperuricemic drugs were used in 3 of the 26 cases (12%) within 3 months, 5 of 26 (19%) between 4 and 6 months, 6 of 23 (26%) between 7 and 9 and 10 and 12 months (Fig. 5b).

Antihyperkalemic drugs were administered to 8 of the 26 cases (13%) within 3 months, 2 of 26 (8%) between 4 and 6 months, and 1 of 23 (4%) between 7 and 9 months. No patient needed these drugs after 10 months (Fig. 5c).

Antihypertension drugs were administered to 15 of the 26 cases (58%) within 3 months, 8 of 26 (31%) between 4 and 6 months, 8 of 23 cases (35%) between 7 and 12 months (Fig. 5d). Therefore, no "de novo" hypertension was observed during the follow-up period.

No hepatotoxic episode was observed during the follow-up period.

In one case, mild chest pain was noted on day 154. Tachycardia was noted in another case on day 121. Nitroglycerin was administered temporarily, and the symptom disappeared.

Infections

Cytomegalovirus pneumonia was observed in 2 cases at 89 and 158 days posttransplant. Siagonanthitis was identified in another patient at 84 days posttransplant. All patients recovered after the administration of an antimicrobial agent or ganciclovir. In only 1 case was septicemia ob-

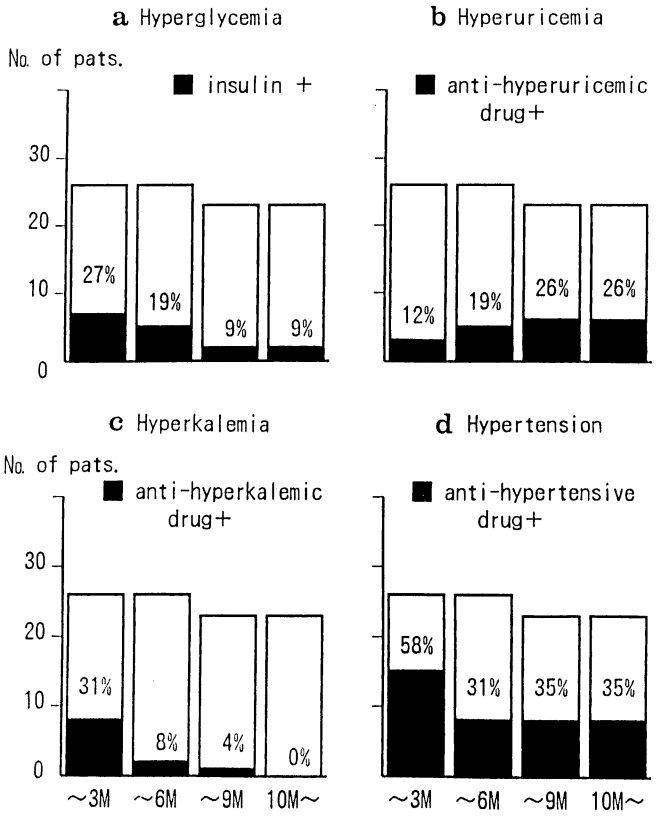

Fig. 5. Incidence of adverse events: **a** diabetes mellitus, **b** hyperuricemia, **c** hyperkalemia, **d** hypertension; *M*, months

served on day 314 posttransplant. This patient also recovered after the administration of antibiotics.

Discussion

This early phase II study produced several data on the efficacy, safety, and optimal therapeutic dose and blood level of FK 506 in patients undergoing kidney transplantation [4]. In particular, a higher reproducibility was indicated for the blood level monitoring by whole blood samples than for that by plasma samples. This new monitoring method was achieved by extracting with dichloromethane [5]. Our recent blood level monitoring of FK 506 was only performed with whole blood samples. The therapeutic trough level in whole blood is suggested to be 10–15 ng/ml at 3–9 months and 10 ng/ml at 12 months in the double drug therapy group.

Although 3 patients were switched to another regimen without FK 506 at 3–5 months, all patients and grafts had survived. Almost all rejection and nephrotoxic episodes were reversible. No additional case of switching was identified thereafter.

In addition, the median serum creatinine level low remained low during the follow-up period in both double and triple drug therapy groups. Therefore, the trough level monitoring in whole blood was sufficient to monitor good graft function for a long period.

We also succeeded in reducing the dosage of prednisolone; in more than half of the cases, it was less than 10 mg/day at 4 months posttransplant. We could not find any major adverse events. Although infections were observed in 4 patients, recovery followed the administration of an antimicrobial agent or ganciclovir.

No hepatotoxicity occurred for more than 10 months, and the incidence of hypertension was very low. These merits have not been previously reported in CyA clinical studies [1–3].

Although hyperglycemia and hyperkalemia were observed in several cases, all these events were reversible.

In conclusion, this multicenter study of FK 506 therapy showed that a good graft function could be maintained for more than 10 months via monitoring the FK 506 trough level in whole blood.

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