

#870

Microvascular Changes in Renal Allografts Associated with FK506 (Tacrolimus) Therapy

P.S. Randhawa, M.D., A.C. Tsamandas, M.D., M. Magnone, M. Jordan, M.D., R. Shapiro, M.D., T.E. Starzl, M.D., Ph.D., and A.J. Demetris, M.D.

FK506 (Tacrolimus) recently has been shown to be an effective immunosuppressant after renal transplantation. It is associated with less hypertension, hypercholesterolemia and steroid use compared with cyclosporine. We report 10 patients on FK506 who showed fibrin thrombi within the glomerular capillaries and/or arterioles at renal allograft biopsy. These biopsies were generally performed to assess increasing serum creatinine levels; laboratory evidence of hemolytic uremic syndrome was present in one instance. Plasma or whole blood FK506 levels were elevated in eight of 10 cases. Reduction of immunosuppression led to clinical improvement or biopsy-proven resolution of thrombi in all cases. These observations suggest that FK506 may occasionally produce microvascular changes in the renal allograft. The estimated incidence of this occurrence (1%) is comparable with that reported with cyclosporine (3%).

Key words: FK506—Tacrolimus—Kidney—Allografts—Thrombi—Toxicity.

Am J Surg Pathol 20(3): 306-312, 1996.

FK506 (Tacrolimus, Fujisawa USA, Inc.) is a new immunosuppressive agent that recently has been approved by the U.S. Food and Drug Administration for use in liver transplant recipients (3,26). The results in kidney transplantation also have been encouraging. Patient and graft survival rates are as good as or better than those possible with cyclosporine (CS), often at lower maintenance doses of steroids, with lower cholesterol levels and a reduced incidence of hypertension (21). Nephrotoxicity, comparable in degree to that seen with CS, is a

significant side effect of FK506 but is readily managed by adjustments in drug dosage (7,8,21). We and others have shown that renal allograft biopsies performed during acute episodes of FK506 toxicity most commonly show tubular or myocyte vacuolization (4,7,19). In patients maintained long-term on FK506, arteriolar hyalinosis and interstitial fibrosis similar to that reported with CS is described (19). There are isolated case reports suggesting that FK506 also can injure the renal allograft microvasculature (6,8,19,20). However, detailed clinical information was not provided in these studies, making a reasonable evaluation of this possibility difficult. The present work focuses on 10 patients in whom histopathological changes within the microvasculature correlated with high blood FK506 levels and elevated serum creatinine. Reduction in the dosage of FK506 led to clinical or histologic improvement in allograft function.

MATERIALS AND METHODS

Patients were retrieved from a coded database of renal transplant recipients maintained by The Pittsburgh Transplant Institute over a 4-year period from July 1990 through June 1994. Accrual of cases into the study was based on the presence of fibrin thrombi within glomerular capillaries or afferent/interlobular arterioles with one to three layers of smooth muscle in the media. Cases with tubulitis and/or active inflammation of the arterial intima were excluded to rule out immunologic microvascular injury. Biopsies samples from kidneys known to have thrombosis of medium to large sized vessels, or presence of discrete infarcts also were excluded because the microvascular pathology in such cases can be explained by ischemia alone.

All patients were maintained on FK506 and pred-

From the Divisions of Transplantation Pathology (P.S.R., A.C.T., A.J.D.) and Transplantation Surgery (M.M., M.J., R.S.), Departments of Pathology and Surgery, and the Pittsburgh Transplantation Institute (T.E.S., A.J.D.), University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, U.S.A.

Address correspondence to Parmjeet S. Randhawa, M.D., Division of Transplant Pathology, Presbyterian University Hospital, Pittsburgh, PA 15213, U.S.A.

nisone as previously described (21). Pertinent clinical information was obtained by discussion with the attending physicians and review of the medical records. Particular attention was directed to serial changes in the FK506 dose, blood levels, and serum creatinine levels. Plasma FK506 levels of 0.5–2.0 ng/ml, whole blood FK506 levels of 10.0–15.0 ng/ml, and serum creatinine levels of 0.2–1.1 mg/dl were considered to be within normal range. Histopathological examination of 2- μ m sections of biopsy tissue was performed after routine formalin fixation and paraffin embedding. Identification of fibrin thrombi was based on evaluation of slides stained with hematoxylin-eosin or Masson's silver trichrome. Tissue was not available for immunofluorescence or electron microscopy.

RESULTS

The clinical characteristics of these cases are summarized in Table 1. The recipient age ranged from 21 to 76 years (median 49), with a male:female

ratio of 4:6. Donor-specific cross-match performed by standard and antihuman globulin microcytotoxicity assays gave negative results. Panel-reactive antibody testing by the Amos modified cytotoxicity assay showed <10% reactivity in eight cases and <40% reactivity in all cases. The mean donor organ cold ischemia time was 32.1 h (range 18.2–56.2). The native renal disease varied, but no cases of hemolytic uremic syndrome or thrombotic thrombocytopenic purpura were included. Biopsies were performed for clinically significant episodes of allograft dysfunction. At the time of biopsy, the FK506 dosage was 10–45 mg/day, the plasma FK506 level 1.1–5.4 ng/ml (median 2.5), and the serum creatinine level 1.7–8.0 mg/dl (median 3.6). In case 10, the whole blood FK506 level was monitored and measured to be 34.8 ng/ml. The FK506 levels were in the toxic range in eight of 10 cases, whereas the serum creatinine level was elevated in all patients. Cases 1 and 6 have been mentioned in earlier work (19,20).

A pretransplant donor biopsy was felt to be clin-

TABLE 1. Clinical characteristics of patients with microvascular toxicity

					Pretreatment parameters
	Age (yr)	Sex	Native kidney disease	Clinical onset*	FK506 (mg/day)
1	29	F	Undetermined	24	20
2	54	M	IgA nephritis	44	45
3	75	M	Polycystic kidney	25	18
4	47	F	Diabetes	37	10
5	51	F	Diabetes	107	36
6	36	F	Systemic lupus	270	14
7	76	M	Glomerulonephritis	20	20
8	21	M	Renal tubular acidosis	545	12
9	33	F	Glomerulonephritis	240	10
10	60	F	Diabetes	78	12

Pretreatment parameters		Posttreatment parameters			Clinical follow-up (days)
FK506 ^b (ng/ml)	Serum creatinine (mg/dl)	FK506 (mg/day)	FK506 ^b (ng/ml)	Serum creatinine (mg/dl)	
5.4	3.0	16	3.6	2.0	Mild acute rejection (45)
2.7	2.5	36	1.9	2.0	Borderline acute rejection (120)
2.3	6.1	14	1.3	4.2	No follow-up biopsy
					Creatinine 1.9 mg/dl (50)
2.2	3.1	8	1.2	2.7	Moderate acute rejection (84)
3.3	2.2	32	1.5	1.7	Arteriolar hyalinosis (910)
2.1	8.0	0	NA	4.5	No follow-up biopsy
					Creatinine 2.0 mg/dl (1,650)
4.4	4.6	14	1.0	4.9	Perinephric abscess
					Allograft nephrectomy (86)
1.1	4.0	10	0.7	1.7	No follow-up biopsy
					Creatinine 2.1 mg/dl (590)
1.1	1.7	8	1.4	2.1	Arteriolar hyalinosis (300)
34.8 ^b	4.3	5	6.6 ^b	3.2	Creatinine 3.2 mg/dl (84)

* Onset of clinical symptoms is recorded in days since transplantation.

^b FK506 levels were generally measured in the plasma (normal range 0.5–2.0 ng/ml), except in case 10 in which whole blood values were estimated (normal 10.0–15.0 ng/ml.).

ically indicated only in case 7, which involved a 65-year-old donor. This specimen showed mild arteriosclerosis and interstitial fibrosis. Posttransplant allograft biopsies showed fibrin thrombi in the glomerular capillaries in eight of 10 cases and within arterioles in six of 10 cases (Figs. 1-3). The percentage of glomeruli/arterioles involved varied from 5% to 40% in different specimens. The arteriolar walls showed deposition of fibrin within the intima and/or media in four of 10 cases (Fig. 4). One case showed globules of an eosinophilic material lying in the medial coat (Fig. 5). Myocyte vacuolization in the arteriolar media was universally present, and endothelial hypertrophy was seen in four of 10 cases. A single case showed rare subendothelial lymphocytes, nuclear fragmentation, and focal coagulative necrosis of the media (Fig. 6). Clinical response to reduction in dosage of FK506 excluded rejection and led to its inclusion in the study. The tubular epithelium showed cytoplasmic vacuolization in eight of 10 cases; generally, fine isometric and coarse irregular vacuoles were present concurrently within the same biopsy sample. The tubular changes were mild and focal in four of eight cases.

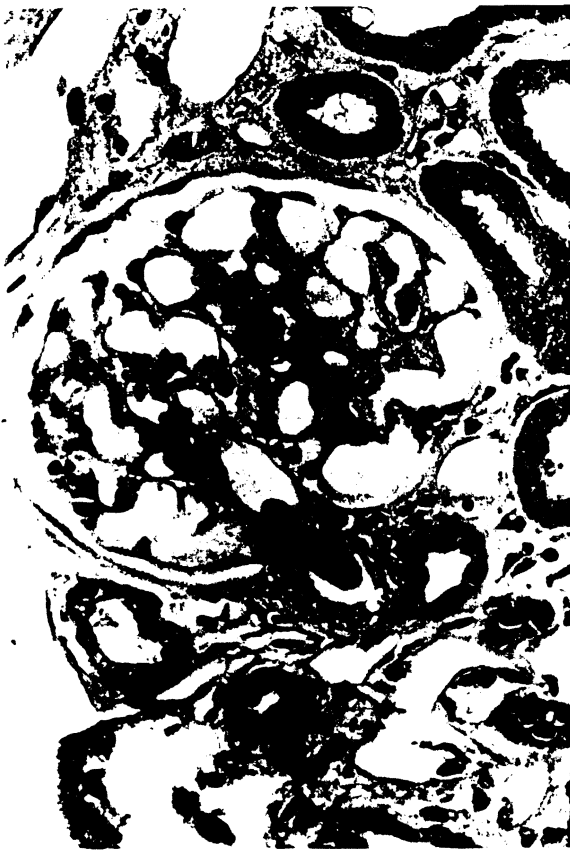


FIG. 1. A partially occlusive fibrin thrombus seen within the afferent arteriole of a glomerulus. The capillary loops distal to the thrombus appear to be dilated.



FIG. 2. An isolated glomerular capillary loop thrombus (arrow) visualized by the Masson's silver trichrome technique.

Arteriolar hyaline change was seen in two cases at the time of initial biopsy (case 3 and 4) and in two other cases on subsequent follow-up (cases 5 and 9). The juxtaglomerular cells showed no increase in number or cytoplasmic granularity, as assessed by routine silver staining.

In the absence of rejection, the histopathological changes observed were clinically assumed to represent drug toxicity, and the dose of FK506 was reduced. The median reduction in dose was 20% of baseline, but in case 6, in which the patient presented with frank hemolytic uremic syndrome, FK506 was discontinued completely (100% reduction). These dose adjustments led to a 0.3- to 3.4-ng/ml (median 1.0) decrease in the plasma FK506 level. In eight of 10 cases, a decrease in serum creatinine varying from 0.5 to 3.5 mg/dl (median 1.1) was observed over a period of 4-21 days (median 6.0). Episodes of acute rejection were documented in cases 1, 2, and 4 by biopsies performed respectively 45, 120, and 84 days after diagnosis of microvascular injury. There was no evidence of persistent capillary thrombi or mural fibrinoid change in these repeat specimens. Cases 5 and 9 showed ar-



FIG. 3. A diffuse deposition of fibrin involving nearly all capillary loops was seen in this glomerulus.

tertiolar hyaline change in follow-up biopsies obtained 300 and 910 days after initial documentation of drug toxicity. Patient 7 had a complicated course with persistent nonfunction of the graft, urinary leak, and perinephric abscess formation. Allograft nephrectomy performed on day 86 showed mild to moderate acute cellular rejection and persistent glomerular capillary thrombi. Follow-up biopsies were not available in cases 3, 6, 8, and 10, but stable serum creatinine was documented respectively 50–1,650 days posttransplantation.

DISCUSSION

The potential of FK506 to cause injury to blood vessels was the subject of several investigations during the developmental phases of this drug. Vascular necrosis described as vasculitis involving medium-sized arteries in the liver, pancreas, and heart was reported in FK506-treated dogs at the University of Cambridge (23). Work performed at two other laboratories raised doubts about the significance of these findings because vasculitis was found with equal frequency in control animals

(17,24). Investigators in other studies were unable to reproduce vasculitis lesions in rats, baboons, or monkeys treated with FK506 (16,25). Arteriolar-sized renal vessels in rats treated with FK506 can develop focal medial necrosis, accumulation of eosinophilic inclusions, and juxtaglomerular transformation, but not true arteritis (11,29). These noninflammatory lesions may be the result not of a drug-induced vasculitis but of arteriolar vasospasm similar to that reported with dopaminergic and adrenergic drugs (10). In human beings, focal necrosis of the pancreatic and peripancreatic arteries was first noted at autopsy in patients dying after organ transplantation (1). The concomitant presence of acute pancreatitis rendered an etiologic relationship with FK506 therapy unlikely. Subsequently, the Japanese FK506 study group demonstrated arteriolar fibrinoid necrosis and glomerular thrombi in human renal allograft biopsy samples obtained from patients maintained on FK506 (7,8). An improve-



FIG. 4. This afferent arteriole is completely occluded by a thrombus. The medial coat is permeated by a proteinaceous insudate and shows vacuolar change. A light periarteriolar infiltrate of lymphocyte is present, but there was no significant interstitial inflammation and no tubulitis. The patient responded to a reduction in drug dosage.



FIG. 5. Globules of a pink eosinophilic material were seen in the media of this small arteriole. The size of these globules and lack of proximity of this vessel to a glomerulus make it unlikely that the myocytes observed are a part of the juxtaglomerular apparatus. A similar lesion was reported in an experimental study on rats (11).

ment in serum creatinine after reduction in drug dosage was shown in only one of the reported cases. No information on plasma FK506 levels was provided.

The present study describes 10 patients with FK506-associated changes at the level of arterioles and glomerular capillaries, and correlates these changes with drug dosage, sequential plasma or whole blood FK506 levels, and serum creatinine levels. Such correlation is needed to provide firm evidence that FK506 can be toxic to the renal microvasculature. Plasma FK506 levels in cases 1–7 and 10 were indeed elevated at the time of initial kidney biopsy. Reduction of FK506 dosage led to lower drug levels and serum creatinine levels in cases 1–6 and 10, providing a clear diagnosis of FK506 toxicity. Case 7 showed no decrease in creatinine, but there was resolution of the glomerular thrombi on repeat biopsy.

Cases 8 and 9 had plasma FK506 levels within the normal range at presentation. In case 8, reduction in

drug dosage was followed by a decrease in serum creatinine from 4.0 to 1.7 mg/dl, strongly suggesting drug toxicity. In case 9, serial biopsies showed the glomerular thrombi to persist for several weeks, during which time the creatinine fluctuated between 1.6 and 2.1 mg/dl. After maintaining a constant dose of FK506 for about 4 weeks, the drug intake was reduced from 10 to 8 mg/day. This was followed by resolution of the thrombi over the next 3–4 weeks. No definite improvement in the serum creatinine was observed. However, there was no clinical or histologic evidence of hypertension, acute rejection, or recurrent glomerulonephritis. The late onset of renal dysfunction (240 days after transplantation) excluded the possibility of ischemic/harvesting injury as being the cause of the glomerular capillary damage. Hence, this case also was felt to be an example of drug toxicity. The lack of elevated plasma FK506 levels is not inconsistent with this impression because correlation between drug levels



FIG. 6. An interlobular artery with focal coagulative necrosis of the media and nuclear karyorrhexis. The interpretation of FK506 toxicity in this biopsy sample was supported by a concomitantly high FK506 level and improvement in renal allograft function when immunosuppression was reduced. This case has been previously reported, and this illustration is reproduced with permission from the publishers (19).

and clinical events in renal transplantation is not always perfect (2,8).

Case 6 presented as acute hemolytic uremic syndrome (HUS) after cadaveric transplantation for systemic lupus erythematosus (SLE). Several alternate explanations for the observed microvascular changes need to be considered. Spontaneously occurring HUS and thrombotic thrombocytopenic purpura (TTP)-like syndromes are described in SLE (5). Glomerular thrombosis is reported in 50% of biopsies obtained from patients with proliferative lupus nephritis (9). However, the patient under discussion had no clinical evidence for active SLE at the time HUS was documented. Antinuclear antibodies were undetectable, and serum complement levels were normal. A normal partial thromboplastin time was evidence against the presence of circulating lupus anticoagulants. Although we cannot definitely ascribe the clinical picture to FK506, no other satisfactory explanation for HUS was documented. Discontinuation of FK506 resulted in recovery over a period of several weeks. Another case of hemolytic uremic syndrome after FK506 therapy has been described in the literature (6), but rigorous proof that it was precipitated by the drug was again not provided. It is notable that HUS also has been reported to occur with CS (15,22,27), and these patients apparently can be safely switched to FK506 therapy without recurrence of HUS (13).

The renal allograft biopsy samples evaluated in this study demonstrated a number of other morphologic changes previously reported in the setting of FK506 toxicity (4,8,19). These changes provide further support for our belief that the microvascular pathology described in these patients is also drug mediated. Thus, isometric tubular cytoplasmic vacuolization was present in eight of 10 cases, and arteriolar myocyte vacuolization was present in 10 of 10 patients. Arteriolar hyalinosis was found at the time of initial biopsy in two patients (cases 3 and 4) and on follow-up biopsies in two additional patients (cases 5 and 9). Blood pressure was within the normal range in these patients, although maintenance antihypertensive therapy was needed in 2 of 4 cases. Donor disease was a potential contributing factor to the hyaline change in case 3, and diabetes was a complicating element in case 5. However, in both instances a nodular configuration to the hyaline deposits was observed similar to that previously reported in CS-associated arteriopathy (15). It is conceivable that the hyaline change observed in these vessels is drug induced and an aftermath of the acute microvascular injury documented earlier in the clinical course of these patients.

The spectrum of histopathological changes ob-

served in these biopsy samples is similar to that reported with CS (15). CS and FK506 are structurally unrelated compounds and bind to different cytosolic proteins in target cells. Nonetheless, both drugs have a closely-related mechanism of action, resulting ultimately from a block in the transcription of interleukin-2 and other cytokine genes (28). Given these basic similarities in the immunosuppressive action of FK506 and CS, the overlap in their nephrotoxicity profile is not surprising. The actual mechanism of the microvascular injury described in this study is unknown but may be related to FK506-induced vasospasm (12). Alternately, it may reflect direct endothelial injury and thrombosis secondary to alterations in the thromboxane A_2 -prostaglandin PGI_2 imbalance (18).

In summary, the data presented indicate that FK506 can cause microvascular injury in human renal allografts. The frequency of this occurrence is ~1% of renal transplant recipients if selection criteria similar to those described in this study are used. By way of comparison, fibrin thrombi are reported in 3% of diagnostic kidney biopsies obtained from CS-treated patients (15). Glomerular capillary and arteriolar thrombi fibrin also can be seen in biopsy samples showing ischemic injury, acute rejection with intimal arteritis, sepsis with disseminated intravascular coagulation, hemolytic uremic syndrome, recurrent lupus erythematosus, and malignant hypertension. Therefore, a diagnosis of FK506-induced microvascular toxicity should be made only after careful clinicopathologic correlation and reasonable exclusion of other cases of small vessel injury. □

Acknowledgment: Joyce Marcoz provided fine secretarial assistance in the preparation of this manuscript. Dr. K.K. Venkat at the Henry Ford Hospital, Detroit, Michigan, provided follow-up information on case 6. This work was supported by the Pathology Education & Research Foundation, Pittsburgh, Pennsylvania.

REFERENCES

1. Demetris AJ, Fung JJ, Todo S, Banner B, Zerby T, Sysyn G, Starzl TE. Pathologic observations in human allograft recipients treated with FK506. *Transplant Proc* 1990;22(suppl 1): 25-34.
2. Erden E, Warty V, Magnone M, Shapiro R, Demetris J, Randhawa P. Plasma FK506 levels in patients with histopathologically documented renal allograft rejection [Letter]. *Transplantation* 1994;58:397-8.
3. European FK506 Multicentre Liver Study Group. Randomized trial comparing tacrolimus (FK506) and cyclosporin in prevention of liver rejection. *Lancet* 1994;344:423-8.
4. Fries D, Rucay P, Samuel D, et al. Development of renal dysfunction and renal histological lesions in two patients treated with FK506 for acute rejection following liver transplantation. *Transplant Proc* 1991;23:3099-100.

5. Hill GS. Systemic lupus erythematosus and mixed connective tissue disease. In: Heptinstall RH, ed. *Pathology of the kidney*. Boston: Little Brown, 1992:884-5.
6. Holman MJ, Gonwa TA. FK506-associated thrombotic thrombocytopenic purpura. *Transplantation* 1992;55:205-6.
7. Japanese FK506 study group. Clinicopathological evaluation of kidney transplants in patients given a fixed dose of FK506. *Transplant Proc* 1991;23:3111-5.
8. Japanese FK 506 study group. Morphological characteristics of renal allografts showing renal dysfunction under FK506 therapy: is graft biopsy available to reveal the morphological findings corresponding with FK506 nephropathy? *Transplant Proc* 1993;25:624-7.
9. Kant KS, Pollak VE, Weiss MA, Glueck H, Miller MA, Hess EV. Glomerular thrombosis in systemic lupus erythematosus: prevalence and significance. *Medicine* 1981;60:71-86.
10. Kerns WD, Arena E, Morgan DG. Role of dopaminergic and adrenergic receptors in the pathogenesis of arterial lesions induced by Fenoldopam mesylate and dopamine in the rat. *Am J Pathol* 1989;135:339-49.
11. Kumano K, Wang G, Endo T, Kuwao S. FK506-induced nephrotoxicity in rats. *Transplant Proc* 1991;23:512-5.
12. Lieberman KV, Lin WG, Reisman L. FK506 is a direct glomeruloconstrictor as determined by electric resistance pulse sizing. *Transplant Proc* 1991;23:3119-20.
13. McCauley J, Bronsther O, Fung J, Todo S, Starzl TE. Treatment of cyclosporine induced hemolytic uremic syndrome with FK506 [Letter]. *Lancet* 1989;2:1516.
14. McCauley J, Fung JJ, Todo S, Jain A, Deballi P, Starzl TE. Changes in renal function after liver transplantation under FK506. *Transplant Proc* 1991;23:3143-5.
15. Mihatsch MJ, Ryffel B, Gudat F, Thiel G. Cyclosporine nephropathy. In: Tisher CC, Brenner BM, ed. *Renal pathology with clinical and functional correlations*. Philadelphia: JB Lippincott, 1989:1570-5.
16. Nalesnik MA, Todo S, Murase N, Gryzan S, Lee PH, Makowka L, Starzl TE. Toxicology of FK506 in the Lewis rat. *Transplant Proc* 1987;5:89-92.
17. Ochiai T, Sakamoto K, Gunji Y, et al. Effects of combination treatment with FK506 and cyclosporine on survival time and vascular changes in renal-allograft-recipient dogs. *Transplantation* 1989;48:193-7.
18. Peters DH, Fitton A, Plosker GL, Faulds D. Tacrolimus A review of its pharmacology, and therapeutic potential in hepatic and renal transplantation. *Drugs* 1993;4:746-94.
19. Randhawa PS, Shapiro R, Jordan ML, Starzl TE, Demetris AJ. The histopathological changes associated with allograft rejection and drug toxicity in renal transplant recipients maintained on FK506. *Am J Surg Pathol* 1993;17:60-8.
20. Schmidt RJ, Venkat KK, Dumler F. Hemolytic-uremic syndrome in a renal transplant recipient on FK506 immunosuppression. *Transplant Proc* 1991;23:3156-7.
21. Shapiro R, Jordan M, Scantlebury V, et al. FK506 in clinical kidney transplantation. *Transplant Proc* 1991;23:3065-7.
22. Shulman H, Striker G, Deeg HJ, Kennedy M, Storb R, Thomas ED. Nephrotoxicity of cyclosporine A after allogeneic marrow transplantation. Glomerular thrombosis and tubular injury. *N Engl J Med* 1981;305:1392-5.
23. Thiru S, Collier DSJ, Calne R. Pathological studies in canine and baboon renal allograft recipients immunosuppressed with FK506. *Transplant Proc* 1987;14:98-9.
24. Todo S, Demetris AJ, Ueda Y, et al. Canine kidney transplantation with FK506 alone or in combination with cyclosporine and steroids. *Transplant Proc* 1987;14:57-61.
25. Todo S, Ueda Y, Demetris AJ, et al. Immunosuppression of canine, monkey, and baboon allografts by FK506: with special reference to synergism with other drugs and to tolerance induction. *Surgery* 1988;104:239-49.
26. The U.S. Multicenter FK506 Liver Study Group. A comparison of tacrolimus (FK506) and cyclosporine for immunosuppression in liver transplantation. *N Engl J Med* 1994;331:1110-5.
27. Van Buren D, Van Buren CT, Flechner SM, Maddox AM, Verani R, Kahan BD. De novo hemolytic uremic syndrome in renal transplant recipients immunosuppressed with cyclosporine. *Surgery* 1985;98:54-62.
28. Wang SC, Morel PA, Wang Q, Jordan ML, Simmons RL, Twardy J. A dual mechanism of immunosuppression by FK506: differential suppression of IL-4 and IL10 levels in T helper 2 cells. *Transplantation* 1993;56:978-85.
29. Yamada K, Sugisaki Y, Akimoto M, Yamanaka N. Short-term FK506 induced morphological changes in rat kidneys. *Transplant Proc* 1991;23:3130-2.