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# Renal transplantation in baboons under FK 506

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*FK 506 was tested in unrelated baboons submitted to renal transplantation and bilateral native nephrectomy. Untreated baboons died after 9.2 ± 4.0 SD days. When FK 506 was given orally for 90 days, survival with the optimum dose was 74.6 ± 28.9 days; this allowed maximum credit for each individual animal of 90 days. A 3-day course of intramuscular FK 506 started on postoperative day 4 allowed 1- to 2-month survival. Delayed rejection in these baboons as well as in those treated daily for 90 days with FK could sometimes be reversed temporarily with a second 3-day course. The doses required for a good therapeutic effect were 10 times greater in baboons than in dogs, a finding that may reflect a species difference of lymphocyte sensitivity to this drug. FK appeared to be relatively nontoxic in subhuman primates, and it remains a promising drug for clinical trial. (SURGERY 1989;106:444-51.)*

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ALTHOUGH THE REMARKABLE immunosuppressive potency of FK 506 has been established in rodents and dogs,[1-6] prohibitive toxicity has been described by one group of investigators in dogs[7] and in baboons.[8,9] We have observed moderate toxicity in dogs[4,6] but few side effects in rats[10] and in baboons or cynomolgus monkeys.[6] However, the prevention of renal homograft rejection in our subhuman primate studies was incomplete and unreliable,[6] suggesting the possibility of underdosage. Consequently the safety and efficacy studies reported here were undertaken in baboons.

## METHODS

**Animals and operative procedures.** Half of the outbred unrelated baboons *(Papio anubis)* were bred and raised at the Southwestern Foundation for Biomedical Research, San Antonio, Texas. The other half were purchased from the Charles River Primate Corporation, Port Washington, N.Y. All of the recipients, as well as the weight-matched donors, were females weighing 8 to 14 kg. Blood types were determined and all donor-recipient combinations were ABO identical (88%) or compatible (12%). Donor-recipient cytotoxic antibody crossmatches were negative in all of the experiments.[11] One-way mixed lymphocyte reactions were determined.[12] The results were not used for donor selection, but these will be summarized in the present report and described separately in detail.

After an overnight fast, the animals were anesthesized with 10 mg/kg of intramuscular (IM) ketamine, intubated, and placed on a ventilator. Anesthesia was maintained with a mixture of oxygen, nitrous oxide, and halothane. Renal transplantation was performed intraabdominally, with end-to-side anastomoses of a renal artery Carrell patch to the abdominal aorta and of the renal vein to the inferior vena cava. Ureteroneocystostomy was performed, and the native kidneys of the recipients were removed. One gram of cephalosporin was given during the operation and daily for 3 days. Animals were fed beginning the next morning. A total of 30 renal transplantations were performed to obtain 23 complete experiments. Within 5 days the other seven animals died of technical complications such as graft vessel thrombosis or bleeding. These seven animals were not included in the analyses.

**Drug administration.** FK 506 for oral or IM administration was supplied as a powder by the Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan. The FK for IM use was suspended in normal saline solution, 4 mg/ml. Oral FK for morning administration was sus-

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**Table I.** Experimental groups and treatment regimens of FK 506 for baboons after renal transplantation

| Group | n | IM FK* (mg/kg/day), on days 1, 2, 3 | Oral FK* (mg/kg/day), after day 4 until day 90 | IM FK (mg/kg/day), on days 4, 5, 6 | IM FK (mg/kg/day), when creatinine for 3 days elevated >3 mg/dl |
|---|---|---|---|---|---|
| *Chronic treatment* | | | | | |
| 1 | 4 | — | — | — | — |
| 2 | 2 | 0.5 | 2.0 | — | — |
| 3 | 4 | 1.0, DD | 6.0, DD | — | — |
| 4 | 5 | 2.0, DD | 12.0, DD | — | 2.0* |
| 5 | 5 | 2.0, DD | 18.0, DD | — | 2.0* |
| *Brief treatment* | | | | | |
| 6 | 2 | — | — | 2.0 | 2.0 |
| 7 | 1 | — | — | 10.0 | 10.0 |

*Legend:* DD, Divided dose.
*With animals who lived for more than 90 days with discontinuance of oral FK treatment.

pended in 20 ml of normal saline solution and given through a gastric tube after the animals were anesthesized with 5 mg/kg of ketamine. For evening administration, FK was mixed with fruit paste or dispersed onto pieces of fruit. Complete acceptance of the FK was carefully observed (until animals ate them all).

**Experimental groups.** The 23 recipients (Table I) included 4 untreated control animals (group 1); three animals that were treated for 3 days only with intramuscular FK beginning on day 4 (brief treatment, groups 6 and 7), and 16 animals treated for a maximum of 90 days (long-term treatment, groups 2 to 5).

Most of the animals treated for the longer period were given FK in divided doses (Table I). Animals in which late rejection occurred when therapy was discontinued in both the long-term (groups 4 and 5) and brief treatment groups (groups 6 and 7) were given 3-day rescue courses of IM FK.

**Biochemical studies.** Blood samples were collected routinely on the mornings of operations, before FK administration on postoperative days 4, 7, 10, and 14 and once a week thereafter. More sampling was added as needed, while the animals were under IM treatment or during rejection. Creatinine, blood urea nitrogen, aspartate aminotransferase, alanine aminotransferase, total bilirubin, and blood glucose levels were determined. FK plasma levels were measured by enzyme immunoassay with a monoclonal antibody to FK.[13]

**Pathologic studies.** Animals that became deeply lethargic were killed for humane reasons with 25 mg/kg of pentobarbital and 0.2 ml/kg of Beuthanasia solution. Animals that lived for more than 90 days with daily oral FK treatment were anesthetized and biopsy specimens were taken from the kidney graft, liver, and pancreas. All of the samples were fixed with neutral buffered formalin. Histopathologic analyses were performed blindly without knowledge of postoperative course, type or dosage of immunosuppressive therapy, or length of survival. Histologic severity of acute cellular graft rejection was scored according to subjective scales, from none to severe.

**Statistics.** The values were described as the mean plus or minus standard deviation. When survival was computed, the maximum credit given to any animal was 90 days. The Mann-Whitney U test, the Student $t$ test, and the $\chi^2$ test were used for statistical analyses. The difference in group means was considered significant if the probability was less than 0.05.

## RESULTS

### General clinical behavior

All of the animals became inactive and depressed for the first 3 to 5 days after their operations, but those treated with effective doses of FK recovered soon afterward. Severe diarrhea or vomiting was not seen in the treated animals. There was variable loss of body weight for the first several weeks after the operations. This did not correlate with the FK dose, and ultimately weight gain occurred toward or beyond the preoperative level in eight of the ten baboons that survived for more than 1 month.

### Untreated controls

Survival was for 5 to 14 days (Fig. 1, Table II), for a mean of 9.2 ± 4 (SD) days. Renal failure developed rapidly in the baboons (Fig. 2, Table II); this was shown by the histopathologic studies (Table II) to have been

**Table II.** Survival, severity of graft rejection, and cause of death of animals with no treatment and chronic treatment with FK (observations limited to first 90 days)

| Groups | Animal No. | Survival (days) | MLR† | Histologic severity‡ of graft rejection | Latest graft function* Creatinine (mg/dl) | Latest graft function* BUN (mg/dl) | Cause of animal death |
|---|---|---|---|---|---|---|---|
| 1 | 87 | 11 | None | 3 | 24.3 | 300 | Rejection |
| | 90 | 7 | Low | 2 to 3 | 16.5 | 154 | Rejection |
| | 106 | 5 | Middle | 1 to 2 | 14.2 | 98 | Rejection |
| | 112 | 14 | NT | 3 | 13.0 | 100 | Rejection |
| 2 | 95 | 14 | NT | 2 | 19.3 | 273 | Rejection |
| | 97 | 18 | NT | 3 | 17.5 | 264 | Rejection |
| 3 | 104 | 23 | NT | 3 | 23.3 | 84 | Rejection |
| | 107 | 76 | None | 2 to 3 | 8.4 | 229 | Rejection |
| | 100 | 12 | NT | 1 | 5.5 | 114 | Renal vein thrombosis (?) |
| | 110 | 7 | Low | 1 | 14.4 | 97 | Rejection |
| 4 | 10 | >90 | High | 2 | 0.8 | 68 | — |
| | 21 | 30 | High | 1 to 2 | 11.1 | 448 | Rejection |
| | 23 | >90 | High | 0 | 0.7 | 22 | — |
| | 28 | 54 | Low | 2 | 15.8 | 263 | Rejection |
| | 6 | >90 | Middle | 1 | 1.0 | 21 | — |
| 5 | 29 | 23 | High | 2 to 3 | 22.1 | 289 | Rejection |
| | 9 | >90 | High | 1 to 2 | 1.5 | 71 | — |
| | 24 | 84 | High | 0 | 3.9 | 225 | Unknown (anemia?) |
| | 5 | 86 | High | 0 | 24.1 | 39 | Pyelonephritis |
| | 26 | >90 | High | 1 | 1.1 | 22 | — |

*Graft function at the latest measurement or at the 90th postoperative day.
†MLR (mixed lymphocyte reaction): none (no response), low (<5,000 cpm), middle (<10,000 cpm), high (>10,000 cpm), NT (not tested)
‡Histologic severity; 0 (none), 1 (mild), 2 (moderate), and 3 (severe) at the time of sacrifice or biopsy at the 90th day.

**Table III.** All postoperative AST, ALT, total bilirubin, blood glucose, and FK levels in baboons of groups 1 to 5 (mean ± SD)

| Groups | Dose of oral FK (mg/kg/day) | n | Serum AST (U/L) | Serum ALT (U/L) | Serum total bilirubin (mg/dl) | Blood glucose (mg/dl) | Plasma FK (ng/ml) |
|---|---|---|---|---|---|---|---|
| 1 | 0 | 4 | 49.5 ± 49.5 | 12.0 ± 13.7 | 0.7 ± 0.4 | 103 ± 4 | — |
| 2 | 2 | 2 | 38.7 ± 19.7 | 37.4 ± 40.5 | 0.5 ± 0.5 | 87 ± 78 | 1.26 ± 1.20 |
| 3 | 6 | 4 | 45.0 ± 20.3 | 25.4 ± 17.6 | 0.4 ± 0.4 | 104 ± 20 | 4.27 ± 2.87 |
| 4 | 12 | 5 | 51.0 ± 28.8 | 29.7 ± 28.6 | 0.2 ± 0.2 | 86 ± 76 | 3.26 ± 1.85 |
| 5 | 18 | 5 | 49.4 ± 30.9 | 36.4 ± 64.6 | 0.4 ± 0.8 | 102 ± 43 | 5.24 ± 5.00 |

*Legend:* AST, Aspartate aminotransferase; ALT, alanine aminotransferase.

caused by rejection. Liver function tests and fasting blood glucose values are shown in Table III.

**Long-term FK treatment**

*Survival.* Consistent prolongation of survival was achieved only with the high doses given to the animals of groups 4 and 5 (Fig. 1, Table II). Nine of the 10 animals in these two groups lived for at least 1 month, seven lived for at least 84 days, and five completed the full 90 days of treatment. There was no correlation between mixed lymphocyte reactions and the outcome (Table II). A survival effect was discernible in the lower-dosage groups 2 and 3, but this was minor (Fig. 1, Table II). In group 4, survival was 70.8 ± 27.6 (SD) days, giving a maximum credit to any animal of 90 days. Group 5 animals survived for 74.6 ± 28.9 (SD) days.

*Biochemical studies.* The dose/effect relationship was even more evident in the tests of renal function (Fig. 2, Table II). Uremia was a universal funding with the

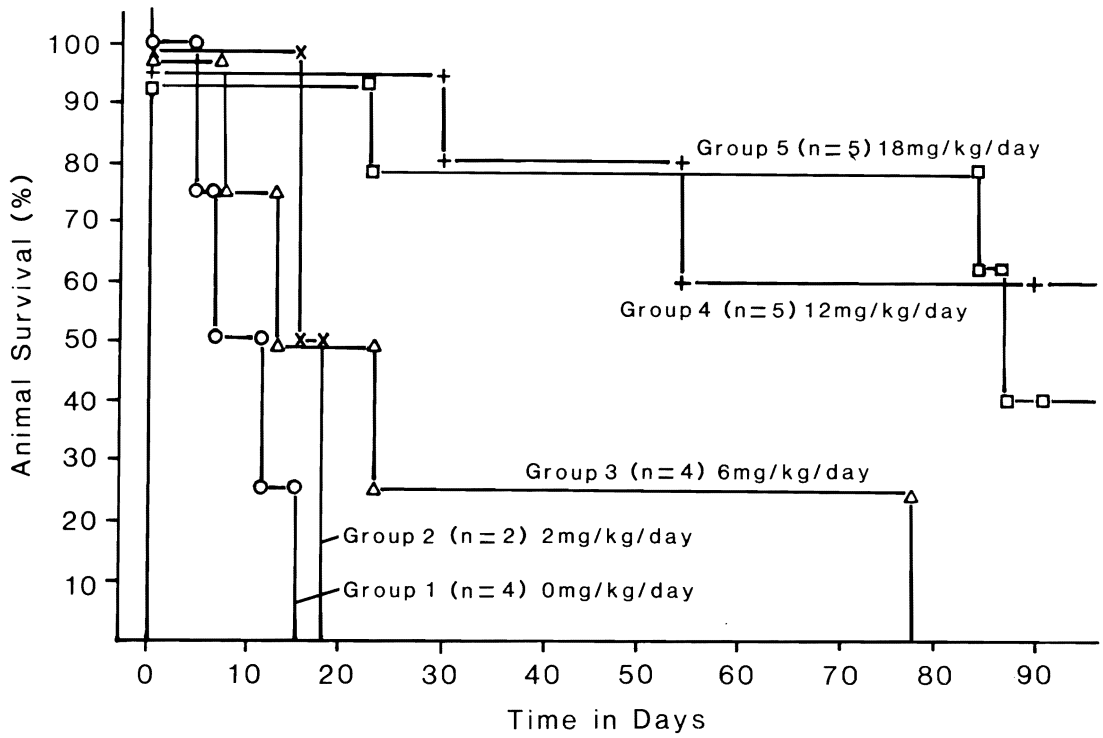


**Fig. 1.** Survival of baboons after renal transplantation under FK 506. Doses shown are oral FK given from the fourth postoperative day until the 90th day. Intramuscular doses were also given on days 1 to 3 (see Table I).

lower doses, but it was not found in the animals of groups 4 and 5.

Liver function and blood glucose levels were not significantly different than in the untreated control animals (Table III).

*FK plasma levels.* The mean trough plasma levels from all postoperative determinations in the chronically treated baboons are given in Table III. Serial measurements from these animals are shown in Fig. 3.

*Pathologic studies of the grafts.* Histopathologic signs of moderate or severe rejection were found in all of the grafts in the controls and in low-dose group 2 (Table II). The signs of rejection were slightly less developed in animals of group 3. Half of the 10 grafts in groups 4 and 5 had moderate rejection histopathologically, but these findings did not correlate well with renal function (Table II). The two grafts from animals that died while still under treatment after 84 and 86 days did not contain evidence of rejection. One of these animals died of pyelonephritis; the cause of death could not be determined in the other.

In four of the five animals that survived for 3 months and whose therapy was stopped rejection subsequently developed (Fig. 4). In two animals, the rejection was reversed with a 3-day course of IM FK (Fig. 4). Eventually, all four of these baboons died after a total survival of 116 to 233 days. At autopsy, three of the four grafts showed chronic rejection, a finding (mild interstitial fibrosis) that had been present in only one of them at day 90; the fourth graft infarcted between days 90 and 116 and could not be adequately evaluated histopathologically. One baboon is still alive more than 190 days after transplantation and 100 days after discontinuance of therapy. The 90-day biopsy specimen from this animal showed no rejection.

*Pathologic studies of the extrarenal organs.* The control animals and the animals of low-dose groups 2 and 3 did not have arteritis in the heart, liver, pancreas, lungs, stomach, intestine, and spleen. The five animals in the combined groups 4 and 5 that died before 90 days while undergoing higher-dose daily FK treatment orally did not have arteritis, with the exceptions of a single vessel with fibrinoid change in the appendix of animal 24 and a single similar vessel in the stomach of animal 29. The biopsy specimens of the liver and pancreas obtained from the other five animals that were alive at 90 days were free of arteritis.

Four of these five baboons died later, and autopsies were performed 26 to 143 days after daily FK treatment was stopped at 90 days (Fig. 4). One of these four ani-

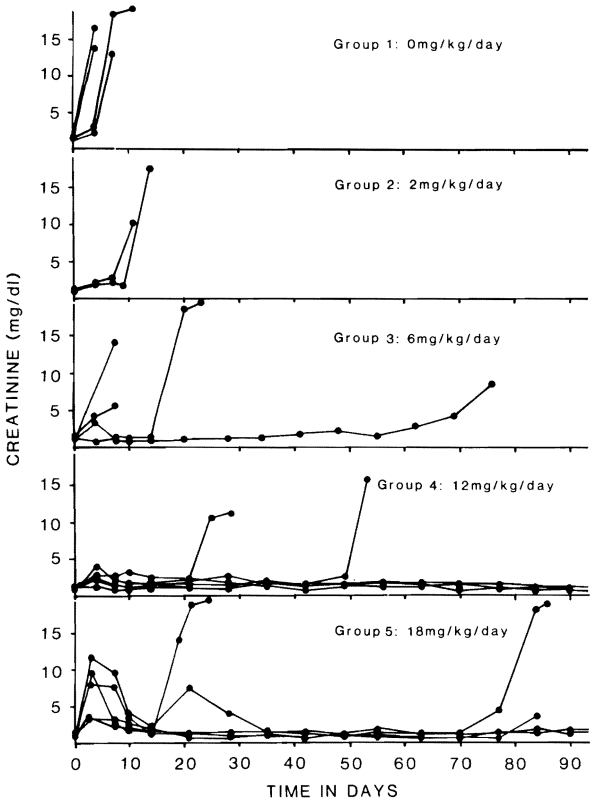


**Fig. 2.** Changes in serum creatinine. Doses of FK are as shown in Fig. 1.

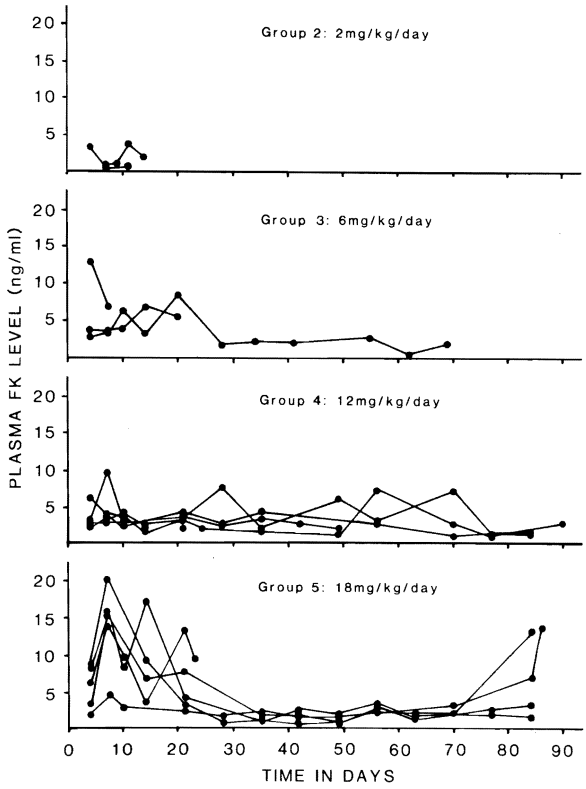


**Fig. 3.** Plasma trough levels of FK 506 after renal transplantation. Troughs in group 2 are at 24 hours, and in groups 3 to 5 troughs are at 12 hours.

mals had focal necrotizing arteritis in the gastric submucosa (animal 6) but nowhere else. This baboon, which died of renal failure, had been given a 3-day course of IM FK 506 on postoperative days 105, 106, and 107 and was given another single injection on the day before death (Fig. 4). Animal 9 had mild degenerative change in a single intramyocardial artery. Rejection was the cause of death in spite of two "rescue" courses of IM FK 506, of which the last was 34 days before death (Fig. 4).

Vacuolization of the parenchymal cells was seen occasionally but was not associated with dose. One baboon (No. 10) that had malaria died of intrahepatic bile duct necrosis 138 days after transplantation and 48 days after cessation of daily FK (Fig. 4). Another animal (No. 21) had severe acute cholangitis 30 days after transplantation.

An animal (No. 26) that died after 116 days of renal failure due to rejection (Fig. 4) had a small area of myocardial necrosis; this was the animal whose kidney was infarcted 26 days after cessation of daily FK treatment. Subendocardial fibrosis and focal calcification were present in an animal (No. 28) that died of rejection 54 days after transplantation.

### Brief FK treatment

The three animals of groups 6 and 7, who were given 3 days of IM FK on days 4, 5, and 6, retained good graft function for 1 to 2 months (Fig. 5). When renal function deteriorated, a further 3-day course reversed the rejection. However, a second bout of renal failure that ensued after another 20 days to 2 months was refractory to treatment (Fig. 5).

Autopsies of these three animals after 82, 106, and 122 days showed acute and chronic rejection of the grafts. The animal (No. 50) that survived 122 days with three 3-day IM treatment courses had extensive necrotizing arteritis in the small intestine, duodenum, and heart. Animal No. 25, treated in a similar way (Fig. 5), had parasitosis and necrotizing granulomas of the intestine. Animal 51 had hemorrhagic gastritis and closed-loop intestinal obstruction; there was mild fibrinoid change in one intramyocardial artery in this animal.

## DISCUSSION

The results from this study permit cautious optimism about impending clinical trials with FK 506. Particularly encouraging was the absence of major toxicity,

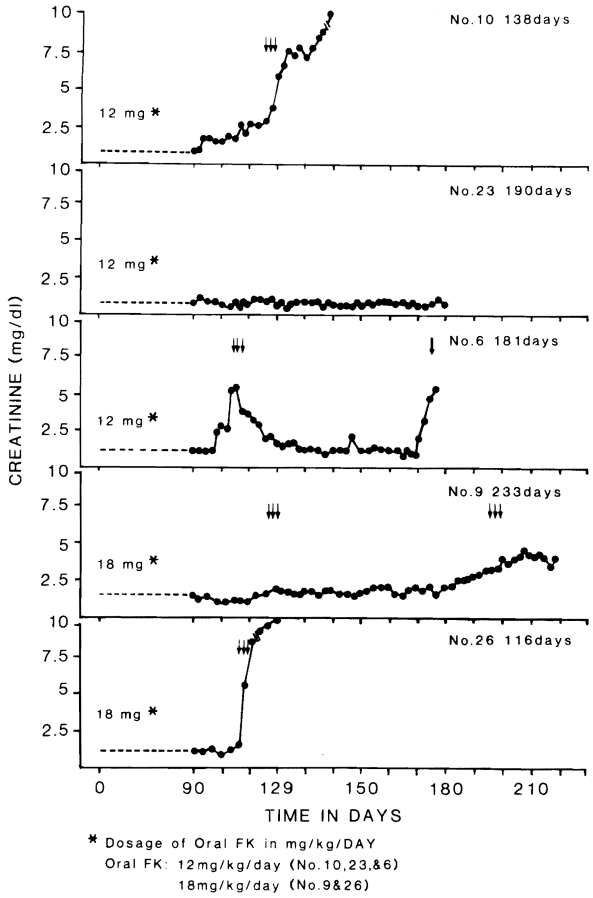


**Fig. 4.** Survival and graft function of five baboons who lived for 90 days and had daily FK stopped. Beyond 90 days, each arrow represents an intramuscular administration of 2 mg/kg FK 506.

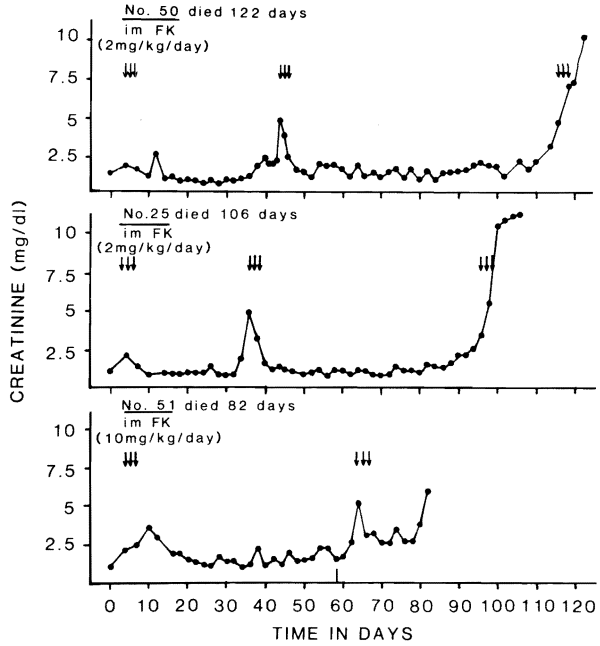


**Fig. 5.** Survival and serum creatinine of baboons after renal transplantation and 3-day intramuscular courses of FK 506. No other treatment was given. Each arrow represents one dose.

even when daily doses used over a 3-month period were 10 times higher than those that can cause lethal emaciation in dogs.[4, 6, 7] Although there were a few examples of arteritis in the liver, heart, and stomach, these usually were in animals that were undergoing acute and chronic rejection long after daily FK treatment had been stopped. Formal toxicology studies done by the Huntington Laboratories, Cambridge, England, have shown no such lesions with doses up to 36 mg/kg for 13 weeks (Nishiyama M, personal communication). Reports that FK 506 caused diabetes, lassitude, and other serious or lethal side effects in baboons[8, 9] could not be confirmed.

The unexpectedly high doses required for a therapeutic effect prompted an internal examination of several factors that might have been responsible. The quality of the drug was checked by scientists at the Fujisawa Corporation.[14] Degradation during storage was ruled out with stability tests, and the kinetics of absorption in baboons and dogs were studied and found to be similar (Venkataramanan R, personal communications). Eventually, Zeevi et al.[15] showed with mixed lymphocyte reactions testing that baboon lymphocytes are more resistant to FK than the lymphocytes of dogs. It is important to note that human lymphocyte sensitivity is closer to that of dogs than to that of baboons.[16]

The safeguards for the first clinical trials of FK 506 are unusually complete as exemplified by the availability of a highly specific system to assay plasma and tissue levels of this drug.[13] However, failure of the plasma trough levels to sensitively reflect large variations in dosage in the baboons was disappointing. It will be essential to carry out careful pharmacokinetic studies in the phase I clinical trials to see if the same pertains in human recipients.

In previous canine experiments with kidney and liver transplantation, an unusually prolonged biologic action of short courses of FK was noted.[6] The same thing was noted in the baboon kidney recipients herein reported who were given a 3-day course of IM FK 506 on days 4, 5, and 6 and after transplantation. The grafts functioned for 1 to 2 months before there were signs of rejection, and even then the rejections could be reversed with a second 3-day course. However, none of the animals was tolerant. Even after 3 months of daily treat-

ment with FK (groups 4 and 5), discontinuance of therapy was usually followed by rejection.

These studies in baboons were conducted with a single agent. As with previously used immunosuppressive drugs, however, FK will probably be used clinically as part of a pharmacologic cocktail. The synergism of FK with other agents, particularly with cyclosporine, has been unequivocally shown both in vitro[16-18] and in vivo.[3, 4, 6] The possibility of exploiting this synergism in a clinical setting seems good.

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## DISCUSSION

**Dr. Ronald M. Ferguson** (Columbus, Ohio). I disagree with your last conclusion. I think it probably is not ready for clinical trials yet, and I have a number of questions about that. You have provided almost no evidence to suggest that FK 506 is any better than what we already have; nor have you provided any theoretical basis for slipping FK 506 into some rational theoretically based immunosuppressive protocol. Its mechanism of action is much like that of cyclosporine. How does this compare with our current immunosuppressive protocols? And why is it better? How do you see this if it were to go into clinical trials—being used as a single agent, or in combination with other agents? Is there any other theoretical basis for the immunosuppressive protocol to be used clinically that would include FK 506?

**Dr. Todo.** Our suggestion that a clinical trial would be proper is based on exhaustive animal studies carried out in rats, dogs, and subhhuman primates during the last 2½ years. These were summarized at the meeting last year of SUS and at other major meetings. By itself, FK 506 is at least as effective as cyclosporine in all three species, and probably more so. It has been demonstrated to be synergistic with cyclosporine and also with azathioprine. It is hoped, but not proved, that FK will be less nephrotoxic than cyclosporine. The work supporting these statements is extensively cited in the bibliography of our paper and in the bibliography of our SUS paper last year. The most likely use of FK will be with low doses of cyclosporine and steroids.

**Dr. Francis T. Thomas** (Greenville, N.C.). Dr. Ferguson has asked two key questions. We presented our results with FK testing at the Surgical Forum and at the Xeno 25 meeting last fall. In agreement with Dr. Todo, we have found the drug to be a unique immunosuppressive agent with quite a bit of potency. We used a difficult xenograft model that is not prolonged by any of the currently available suppressive agents. Even in combination with total lymphoid irradiation and cyclosporine as reported by others, we could not get significant prolongation in this model. It is a very difficult model. FK alone and in synergism with rat ATG was the only suppressive agent that seemed to work in this model. As for the toxicity, however, we do find significant toxicity of the drug in primates and in lower animals also. In our experience with more than 300 primates given kidney grafts, the perioperative mortality is essentially zero. I wonder if seven baboons of the 30 renal recipients in your study that died during the early postoperative period did not die of drug toxicity.

The second point, where I think we are probably at strongest variance with your findings, is that, in our experience, the use of cyclosporine in combination with FK produced a prohibitive mortality. This approached 60% to 80%, regardless of the combination of the drugs given, and also had a poor immunosuppressive effect. FK dose ranged from 0.5 mg/kg/day

to 12 mg/kg/day. We could not get immunosuppressive potency without significant toxicity with the cyclosporine in your clinical trials.

**Dr. Todo.** The seven aminals that died within 5 days after renal transplant included nontreated controls as well as treated animals. Thrombosis of the renal artery and intra-abdominal bleeding were the main causes of animal death. FK 506 with cyclosporine in rodents and dogs at subtherapeutic as well as therapeutic doses showed significant prolongation of graft and animal survival and was safe. This was the principal message of our SUS paper last year.

**Dr. K. Hockerstedt** (Helsinki, Finland). Although you have shown that FK 506 suppresses acute rejection without toxicity in your model, I would like to ask if you have tried to evaluate why this drug is so efficient in suppressing acute rejection, and if you have measured the T-cell levels in blood?

**Dr. Todo** (closing). The mechanism of the suppression of acute rejection by brief intramuscular FK treatment is now under study. With heterotopic heart and orthotopic liver transplantation in rats, we did not see any difference in the T-cell subpopulation in the blood before and after this treatment (unpublished data), although massive infiltration of lymphocytes in these organs subsided progressively in some animals.