Effect of FK506 in Experimental Organ Transplantation

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THE FIRST report on FK506 (FK) was made by Ochiai et al[1] in Helsinki in 1986. Since then, our efforts have been designed to clarify the efficiency and the toxicity of this agent. In vitro systems and experimental organ transplantation models have been used for testing. Several reports of this work have been published.[2-10] We describe here the cumulative information and further follow-up of the whole organ transplantation studies.

MATERIALS AND METHODS

Animals and Operative Procedures

FK was tested in three different models of experimental organ transplantation: heterotopic heart transplantation in the rat (HTX),[2,5,8] kidney transplantation in the dog (KTX),[3,6] and orthotopic liver transplantation in the dog (OLTX).[3,7]

Animal species and operative procedures were described in the previous reports. In brief, HTX[2,5,8] was performed intra-abdominally from ACI rats (RT 1ᵃ) to Lewis rats (RT 1ˡ). Brown Norway rats (RT 1ⁿ) were used as donors where third-party grafts were involved. KTX was performed using mongrel dogs as donors and beagle dogs as recipients, using conventional intra-abdominal techniques[3,6]; native kidneys were removed at the end of operation. In dogs under a pump driven veno-venous bypass, OLTX was carried out from beagle donors to beagle recipients (B/B) or from mongrel donors to beagle recipients (M/B).[3,7]

Nephrotoxicity of FK was tested in rats whose test kidneys were submitted to one-hour ischemia by cross clamping, followed by contralateral nephrectomy.

Drug Administration

FK was supplied by the Fujisawa Pharmaceutical Company, Ltd, Osaka, Japan. It was dissolved in saline for intramuscular use or placed in commercial capsules for oral use. Cyclosporine (CyA) in the commercial oil carrier that is used clinically was given orally by syringe. Prednisone (Pred) for oral use in dogs was given in 5-mg tablets. To prevent vomiting in the dog caused by FK or other postoperative factors, 2 mg of atropine sulphate was given intramuscularly twice a day for the first postoperative week and once a day for the second postoperative week.

Dose Effectiveness Studies

The effectiveness of FK in HTX was reported previously by Lee et al[2] and Murase et al.[5] Seven different intramuscular doses of FK from 0.02 mg/kg/d to 1.28 mg/kg/d were tested. FK was given for 2 weeks starting just after recovery from anesthesia.

The effect of oral doses on canine KTX was tested at 0.5, 1.0, 1.5, and 2.0 mg/kg/d. FK was started on the morning after the operation and continued for 3 months. The initial doses were reduced to 75% after 2 months.

The effect of FK alone was further evaluated in OLTX, starting with 1.0 mg/kg/d of oral FK. The dose was adjusted to 75% of the initial dose after 1 month.

Drug Combination Studies

In HTX, minimally effective doses of intramuscular FK (0.02 mg/kg/d and 0.04 mg/kg/d) and cyclosporine (CyA) (2.5 mg/kg/d) were used together. In canine KTX, oral FK (0.5 mg/kg/d), CyA (5 mg/kg/d) and Pred (5 mg/kg/d) were used in five combination groups (see RESULTS for groups).

Delayed Treatment

FK was given intramuscularly for three days starting at different times postoperatively. Rats with HTX were treated with an intramuscular dose of 1.28 mg/kg/d and dogs with KTX were treated with an intramuscular dose of 1.0 mg/kg/d.

Toxicity Studies

Nontransplanted Lewis rats and beagle dogs were subjected to toxicity studies. FK was given orally to the rat for 5 weeks at doses of 0.5, 1.0, 2.0, and 4.0 mg/kg/d. Dogs were treated for 4 weeks with oral FK at doses of 1.0, 2.0, and 4.0 mg/kg/d. Functional and histopathologic changes in rats were reported earlier by Nalesnik et al.[10]

Nephrotoxicity of FK and CyA was compared in rats by using a renal ischemia model in which the right renal pedicle was crossclamped for one hour, followed by contralateral nephrectomy. Animals were orally treated for ten days starting two days prior to the ischemia. Doses of FK tested were 1.0, 2.0, and 4.0 mg/kg/d. The CyA dose was 25 mg/kg/d.

RESULTS

Dose Effectiveness Studies

HTX. Grafts of untreated rats were rejected in six days. Graft survival was significantly increased by FK administration at all doses tested intramuscularly. The median survival was eight days with 0.02 mg/kg/d, 10.5 days with 0.04 mg/kg/d, 32.0 days with 0.08 mg/kg/d, 32.0 days with 0.16 mg/kg/d, 38.0 days with 0.32 mg/kg/d, 39.0 days with 0.64 mg/kg/d, and 87.0 days with 1.28 mg/kg/d.

KTX. Survival of animals in each group is shown in Fig 1. The best results were with 1.5 mg/kg/d of FK orally.

OLTX. The survival of ten dogs is shown in Fig 2. Two dogs died of intussusception and biliary peritonitis at day 6 and at day 10,

respectively. They had no rejection. The other eight dogs treated with oral doses of 1 mg/kg/d lived more than 1 month with good graft function. However, after reducing the FK dose to 0.75 mg/kg/d at the 31st postoperative day, rejection occurred in four dogs. Three were M/B combination and the other was B/B combination. Four dogs lived for more than 3 months. Three were B/B and one was M/B. After discontinuation of treatment at 90 days, one dog died at day 98 and another died at day 147. The other two are still alive after 150 days and 162 days. Both were B/B.

In comparison to a previous study of CyA in our laboratory,[11] the graft function during the first postoperative month was better in animals treated with FK.[7] Four of nine animals had rejection within 1 month when treated with 20 mg/kg/d of CyA, while no rejection occurred during the same period in animals treated with 1 mg/kg FK.

Drug Combinations

HTX: Small doses of FK alone caused a barely detectable increase in graft survival,[5] to 8.0 days with 0.02 mg/kg/d and to 10.5 days with 0.04 mg/kg/d. With CyA alone, survival was 10.0 days with 2.5 mg/kg/d. When both agents were used together, the graft survival was prolonged almost three times.

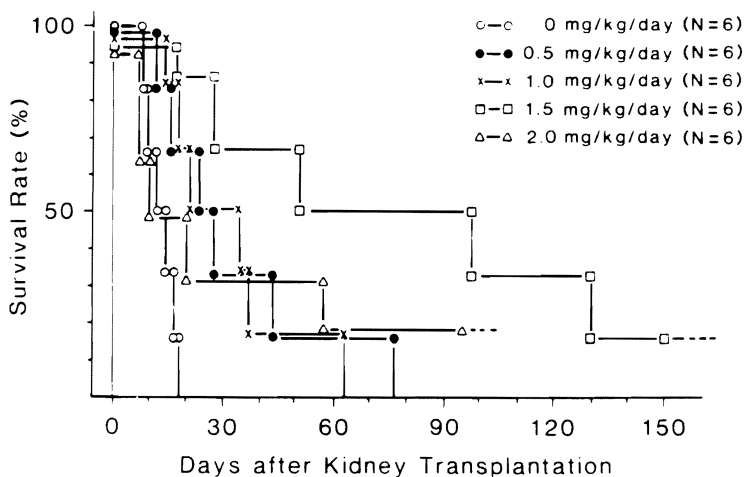*KTX:* The graft survival of KTX using

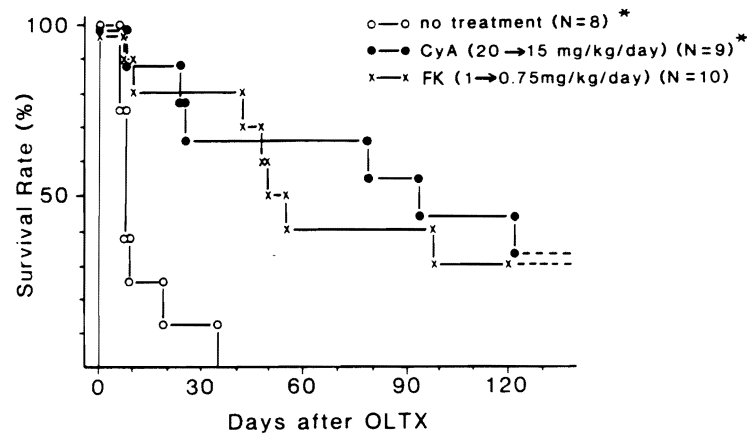Fig 1. Survival after KTX of dogs treated with different doses of FK.

Fig 2. Survival after OLTX of dogs treated with FK.

* Data from the previous study
(Todo, S. et al., Transplantation, 1986)

combination therapy is shown in Fig 3. Only one dog survived for more than 3 months when 5 mg/kg/d CyA and 5 mg Pred were given together; the mean survival was 31.6 days (12 to >115 days). In contrast, combination therapy with 0.5 mg/kg/d FK, 5 mg/kg/d CyA, and 5 mg Pred allowed five animals (80%) to live for more than 3 months, for a mean survival of 93.0 days (23 to 120 days). When the doses of the three drugs were cut in half, there was no thereapeutic effect. Using 0.5 mg/kg/d FK with 5 mg/kg/d CyA, three dogs (50%) are still alive after 3 months. The mean survival was 51.8 days (12 to >87 days).

Delayed Treatment

HTX. The administration of FK for three days only always prolonged graft survival compared to a mean survival of six days in untreated controls. The median graft survival was 35 days in rats treated on days 0, 1, and 2. It was 89 days in rats treated from days 4 to 6. A heart grafted to the same recipient from another ACI rat 2 weeks after the first grafting was accepted without further therapy[5] and both grafts were finally rejected at approximately 2 to 3 months. If the second grafts were from BN rats, these were always rejected at similar intervals as the control indicating

that temporary donor-specific unresponsiveness in recipient rats had been achieved.[5]

KTX. Mean survival was 13.0 days in untreated animals. The mean survival was prolonged to 26.3 days (21 to >55 days) in dogs given FK at postoperative days 1, 2, and 3; and to 27.8 days (8 to >55 days) in dogs given FK on days 4, 5, and 6 (Fig 4). Even when FK was given at seven, eight, and nine days after transplantation, three of seven animals are still alive for more than 48 days.

Toxicity in Rats

No significant histopathologic abnormalities suggesting drug toxicity were detected in rats.[10] There were dose-dependent changes in body weight, medullary atrophy of the thymus, perivascular eosinophilia in the lung, and single cell necrosis of pancreatic acinar cells. Blood glucose was elevated in these animals. There was no clinicopathologic evidence of vasculitis in the rat.[10]

Administration of FK and CyA to rats subjected to renal ischemia showed that FK had less nephrotoxicity than CyA. The peak creatinine at the first postoperative day was 2.18 ± 0.70 (SD) mg/dL in untreated group; 2.90 ± 0.44 in rats with 1 mg/kg/d FK; 3.00 ± 0.69 in rats with 2 mg/kg/d FK; 3.22 ± 0.70 in rats with 4 mg/kg/d FK, and 4.92 ±
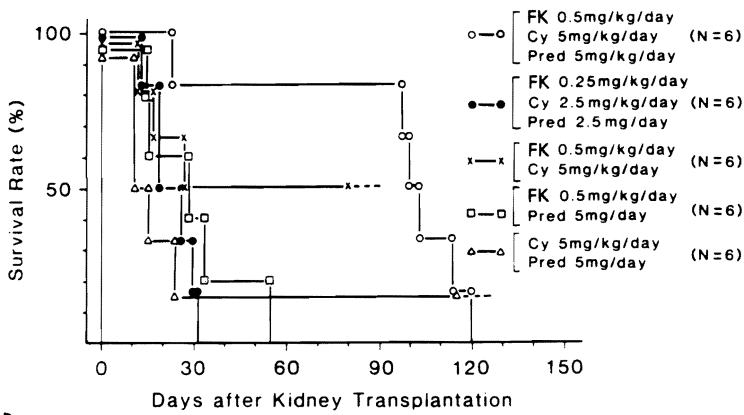
Fig 3. Survival after KTX of dogs treated with drug combinations.

0.57 in rats with 25 mg/kg/d of CyA. The rises were most significant in CyA treated rats.

Toxicity in Dogs

Dogs receiving oral doses of FK greater than 0.5 mg/kg/d showed remarkable emaciation and vomiting. In addition, 11 intussusceptions occurred among 96 operated animals, of which four were KTX dogs treated with the highest dose of FK. Vasculitis was the most important finding in dogs. Animals given 4 mg/kg/d of FK for toxicology studies showed medial necrosis of small arteries as early as 2 weeks after beginning treatment. Although slight but definite vascular changes were detected even in untreated KTX dogs, these findings were significantly increased by the treatment with FK. Single necrosis of pancreatic acinar cells was often seen. There was no liver dysfunction in KTX animals, nor was there Cr elevation in OLTX dogs. Complete histologic analyses of FK toxicity in rats and dogs is under investigation.

DISCUSSION

In confirmation of the previous observations by Ochiari et al,[1] and by us,[2,3,5-7] the potency of FK was evident in this study with longer follow-up. Treatment with FK alone allowed substantial prolongation of graft survival. The optimal therapeutic dose of this
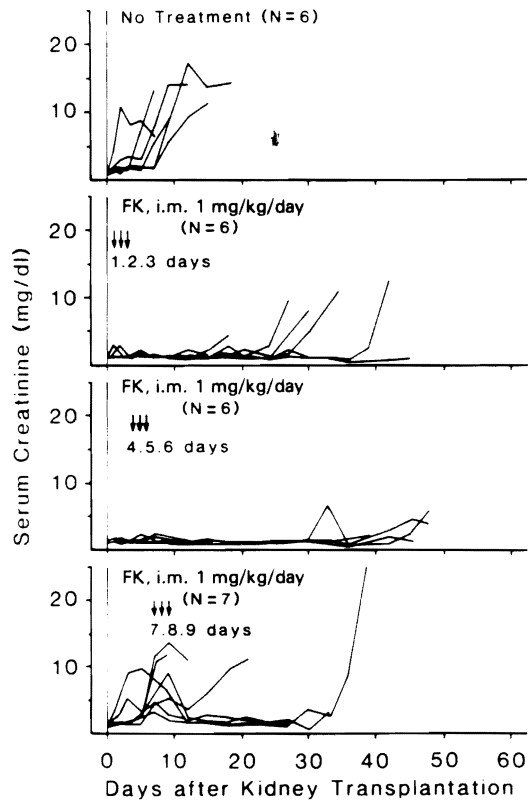

Fig 4. Changes in serum creatinine after KTX in dogs treated with only three daily doses of FK.

drug, when given orally to KTX dogs, was between 1.0 mg/kg/d and 1.5 mg/kg/d. In OLTX animals, the reduction of dose from 1.0 mg/kg/d to 0.75 mg/kg/d led to the loss of half of the animals from delayed rejection. Drug blood levels will be necessary to refine the therapeutic dose of FK.

Combination therapy with low doses of FK, CyA, and steroids was remarkably effective in all KTX groups. The finding was consistent with observations in the HTX model, indicating synergism of FK with CyA and steroids.[5] Zeevi et al[9] have reported a more than additive effect of FK and CyA in in vitro studies.

Another interesting result was the effectiveness of three postoperative doses of FK after KTX, even though the initiation of treatment was delayed for as long as seven days, when grafts were anticipated to have severe rejection. Delayed FK even at this late time allowed prolonged graft survival in half of the animals. This ability of FK suggests the possibility of minimizing the use of immunosuppressive drugs immediately after the operation and opens the possibility of using FK to rescue grafts from established rejection.

The toxicity of FK may be a species-specific phenomenon. Significant histopathologic changes seen in dogs, did not develop in rats except for minor changes in the pancreas. Single acinar cell necrosis was found in the pancreas of both rats and dogs, although it was mild in rats.

Nephrotoxicity of FK was less than that of CyA as revealed in an ischemia model. One of the most important side-effects of FK in the dog was the medial necrosis of small arteries, which was severe enough to lead to focal necrosis or infarction of the heart. This was never seen in rats receiving much higher doses of FK. Toxicology in subhuman primates is now under investigation.

SUMMARY

FK506 is the most potent immunosuppressive agent known. Its toxicity is substantial in dogs, minor in rats, and unknown in subhuman primates. In small doses that are nontoxic even in dogs, it can be used in synergistic combination with cyclosporine, steroids, and presumably other drugs.

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