

Induction of Graft Acceptance After Dog Kidney or Liver Transplantation

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WE have reported that a short delayed course of intramuscular FK 506 can induce a donor strain-specific immunologic unresponsiveness to cardiac allograft in rats.¹ Further studies have been performed to determine if this agent can induce graft acceptance after canine renal (KT) or hepatic (OLT) allotransplantation. Preliminary descriptions of these efforts have been published.²

MATERIALS AND METHODS

Animals and Transplant Procedures

Female beagle dogs were used for recipients. The donors were mongrel dogs, except for groups 2 and 4 in the OLT trials, in which a beagle to beagle combination was used. Kidney grafts were implanted into the right iliac fossa and livers implanted were into the orthotopic position using a previously described technique.³

Drug Administration

FK 506 for intramuscular use was supplied by the Fujisawa Pharmaceutical Co. Ltd, Osaka, Japan. This powder was dissolved in saline. To prevent vomiting caused by FK 506, 2.0 mg/kg of atropine sulfate was given intramuscularly twice per day for 1 week.

Experimental Designs

The protocols are summarized in Table 1 for renal transplantation and in Table 2 for liver transplantation. An FK 506 bolus at a dose

of 1.0 mg/kg/d was given as rescue pulse therapy if rejection occurred. FK 506 was never given beyond 90 days after surgery.

Biochemistry

Blood samples were taken at 3-day intervals for measurements of serum creatinine, total bilirubin, SGOT, SGPT, glucose, and amylase.

Mixed Lymphocyte Reaction

About 10 ml of blood was drawn every 2 weeks from chronically surviving recipients for mixed lymphocyte reaction (MLR) studies.⁴ Their peripheral lymphocytes were cocultured with

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Table 1. Prolongation of Renal Allograft Survival in Dogs

Group	Intramuscular FK 506 (mg/kg/d)	Postoperative Days of FK 506 Administration	n	Survival Time (d)	Mean	P Value*
1	0	0	6	8, 9, 12, 14, 17, 18	13	—
2	0.5	4, 5, 6	4	20, 21, 22, 33	24	< 0.005
3	1.0	4, 5, 6	4	31, 46, 66, 67	52.5	< 0.005
4	2.0	4, 5, 6	6	8, 20, 21, 43, 54, 125	45.2	< 0.02
5	5.0	4, 5, 6	6	11, 16, 26, 60, 60, 79	42.3	< 0.015
6	10.0	4, 5, 6	6	11, 20, 31, 55, 68, 75	43.3	< 0.013
7	5.0	5	6	14, 16, 20, 23, 25, 53	25.2	< 0.015
8	10.0	5	6	7, 8, 10, 11, 41, 58	22.5	NS
9	25.0	5	4	6, 11, 12, 96	31.3	NS
10	0.5	4, 5, 6 plus every 2 weeks	2	127, 129	128	—
11	1.0	4, 5, 6 plus every 2 weeks	4	24, 26, 113, 132	73.8	< 0.04

* Generalized Wilcoxon test.

† Dogs died from intussusception with low serum creatinine level.

‡ Dogs died from emaciation with low serum creatinine level.

Table 2. Prolongation of Hepatic Allograft Survival in Dogs

Group	Intramuscular FK 506 (mg/kg/d)	Postoperative Days of FK 506 Administration	n	Survival Time (d)	Mean	P Value*
1 (M/B)	0	0	6	7, 8, 19, 26, 41	18.2	—
2 (B/B)	0	0	6	7, 7, 8, 13, 35, 38	18.0	—
3 (M/B)	1	4, 5, 6	7	9,† 16, 24, 28, 360, >620, 630	241.0	<0.05
4 (B/B)	1	4, 5, 6	8	21,† 26, 34, 58, 60, 62, 75, 193	66.1	<0.01

* Generalized Wilcoxon test.

† Dogs died from intussusception without signs of rejection.

donor spleen cells or spleen cells of third-party donors, as described in a previous report.⁴

Histology

When animals died or were killed, autopsy was performed immediately. Tissues were fixed with 10% formalin and stained with hematoxylin and eosin.

Statistics

The Student's *t* test and generalized Wilcoxon test were applied for the statistical analysis of group means. A probability of <0.05 was considered to be significant.

RESULTS

As seen in Tables 1 and 2, significant prolongation of renal or hepatic graft survival could be obtained by FK 506 treatment, when compared with nontreated animals. Two OLT dogs survived over 600 days after surgery, and one of these is still alive in good condition at 620 postoperative days, suggesting tolerance induction by FK 506 delayed treatment was achieved. In KT recipients, there were no permanent survivors, in spite of the fact that the rejection-free period was prolonged, in proportion to increases in the FK 506 intramuscular dosage (Fig 1). Four of six dogs treated by intermittent FK 506 administration (groups 10 and 11) survived for 113, 127, 129, and 132 days, respectively. FK 506 rescue treatment could reverse 80% of ongoing rejection episodes in renal allografts (Fig 2).

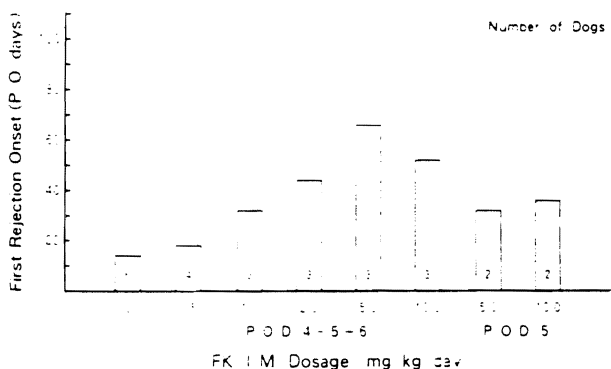


Fig 1. Rejection-free period in canine renal transplantation treated by dose-dependent, delayed intramuscular administration of FK 506.

unless the serum creatinine levels were higher than 5.0 mg/dl. As the intramuscular dosage of FK 506 was increased to more than 2.0 mg/kg/d, more of the dogs died from intussusception or emaciation, with well-functioning kidney grafts (Table 1).

The MLR responses were remarkably suppressed, at least for the first month after initial FK 506 delayed treatment, in both renal and hepatic recipients. As shown in Fig 3, the MLR inhibition of reactivity against donor spleen cells at the 11th postoperative week was stronger

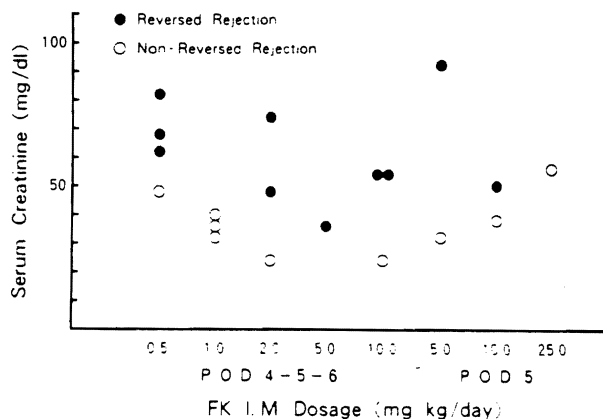


Fig 2. Outcomes of the FK 506 rescue treatment on the first rejection episode in canine renal allograft.

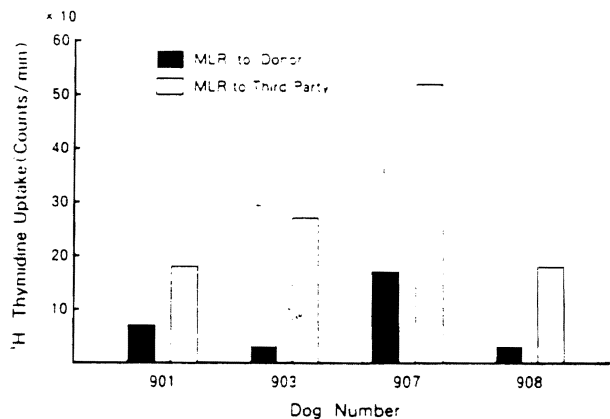


Fig 3. Comparison of MLR responses against donor and third-party spleen cells at the 11th postoperative week. The potency of responses were evaluated by ³H-thymidine uptake.

than that against third-party spleen cells, indicating that donor-specific immunosuppression was induced by FK 506 delayed and intermittent treatment.

DISCUSSION

As described in a previous report,² a 3-day course of intramuscular FK 506 at 1.0 mg/kg/d was the best dose for optimal graft protection without serious toxic side effects in dogs. For both rescue and intermittent FK 506 treatment, even less than 1.0 mg might be sufficient to reverse ongoing or recurrent rejection.

The mechanisms of FK 506-induced long-term allograft acceptance have been investigated by Murase et al¹ and Ochiai et al.⁵ These investigators concluded that there was specific immunologic unresponsiveness against the donor as a result of delayed treatment in rats with FK 506. Although we could not achieve permanent graft acceptance after renal allotransplantation in dogs, the MLR

study showed that antigen recognition was specifically reduced by recipient treatment, whether given transiently or repeatedly. Why unresponsiveness was easier to induce with livers than with kidneys is not presently known.

It is already known that FK 506-related toxicity is most prominent in dogs.^{2,6} This species peculiarity apparently is not relevant to the human situation.⁷

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