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Low-Dose of FK 506 and Associated Blood Levels in Allotransplantation of Rat Liver, Heart, and Skin

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THE immunosuppressive properties of FK 506 have been documented in many studies both in vitro and in vivo.[1] Ideal immunosuppression of solid organ transplantation would produce immunologic suppression of the allograft recipient without adverse toxic effects. Decreasing the dose of FK 506 to the lowest effective level will minimize its side effects. This study attempts to elucidate the relationship between low doses of FK 506 and the corresponding blood value, and graft survival after treatment cessation in allotransplantation of liver, heart, and skin in rat models.

MATERIALS AND METHODS

Animals

Inbred male ACI ($RT1^a$) donor and LEW ($RT1^l$) recipient (weighing 250 to 300 g) rats were purchased from Harlan Sprague Dawley (Indianapolis, Ind). They were housed in a conventional animal facility, and fed commercial rat chow (Wayne Lab Blox F-6, Chicago, Ill) and tap water ad libitum. Rats were allowed to acclimatize for at least 1 week before investigation.

Operative Procedures

Orthotopic liver transplantation (OLTx) was performed according to the method of Kamada and Calne.[2] In brief, the donor liver was harvested after the portal vein, infra- and superhepatic venae cavae, and bile duct were dissected. Two cuffs were mounted onto the portal vein and infrahepatic vena cava, and cannulation of the bile duct was performed at the same time. The cuffed graft was then implanted into an orthotopic position followed by the anastomoses of three veins and the bile duct. Rejection was determined by recipient jaundice, weight loss, hunched position, hard and distended upper abdomen, and eventual death. If rejection was not confirmed by the usual criteria then histopathologic examination was used to verify the rejection process.

Heterotopic heart transplantations (HHTx) were performed by the method of Ono and Lindsey.[3] Briefly, after harvesting the graft, the donor's aorta and pulmonary artery were anastomosed to the recipient's abdominal aorta and inferior vena cava in an end-to-side fashion with continuous sutures of 10-0 Novafil by standard microvascular techniques. Grafted hearts were palpated every other day after transplantation and graded as one to four plus. Graft rejection was determined by complete cessation of heart beat and confirmed by gross necropsy. Histopathologic examination was carried out only when the gross diagnosis was uncertain.

Full thickness skin transplantation (FTSTx) was performed using the method of Barker and Billingham.[4] Briefly, a full thickness skin graft of 2 cm^2 was harvested from a donor's tail and transplanted onto a muscle-based graft bed of the lateral thoracic wall of the recipient. To prevent shearing, a bandage cast was applied around the thorax of the recipient. The inspection of grafts started on the 10th day postoperatively and was subsequently carried out every other day until the graft was rejected. Rejection was determined by the absence of residual viable graft.

Treatment

FK 506 was supplied by Fujisawa Pharmaceutical Company, Osaka, Japan. The FK 506 powder with HCO-60 and D-mannitol, was suspended in normal saline. The FK 506 suspension was prepared shortly before daily administration. Recipients were treated IM for 14 days after transplantation with FK 506 at doses ranging between 0.04 to 1.0 mg/kg.

Blood Sample Collection

The first blood sample was collected on day 14 before the last treatment, which represented the FK 506 blood value during treatment. Three more samples were drawn on days 18, 22, and 26 from three rats in each group. All samples were stored at −80°C until measurement.

FK 506 Assay

The blood level of FK 506 was determined by enzyme-linked immunosorbent assay (ELISA) using a mouse monoclonal anti-FK 506 antibody (Fujisawa Pharmaceuticals Co Ltd, Osaka, Japan), developed by Tamura et al[5] and modified by Cadoff et al.[6] To obtain consistent results, all samples were measured at the same time. The lowest detectable limit of FK 506 in rat blood was 0.8 ng/mL by this assay.[7]

Experimental Groups

Three separate control animal groups (n = 7) were established consisting of no immunosuppression. FK 506 (0.04 mg/kg and 0.08 mg/kg) were given to the OLTx and HHTx recipients for 14 days. FK 506 doses varying from 0.16 mg/kg to 1.0 mg/kg were given to the FTSTx recipients for the same duration as described above.

Result Analysis

Student's t test was used for statistical comparison between control and treatment groups. Results are expressed as mean ± SD. Statistical significance was defined when P values were smaller than .001.

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Table 1. Efficacy of Low-Dose FK 506 Treatment With OLTx, HHTx, and FTSTx

Transplant	FK 506 (mg/kg)	Animal No.	Survival time (d)	MST ± SD (d)	FK Undetectable Time (d)	Days Surviving* After FK 506 Undetectable	*P* Value†
Heart	Control	7	6, 7 × 5	6.86 ± 0.38	—	—	—
	0.04	6	8 × 2, 9 × 2, 14, 18	11.0 ± 4.10	—	N/A	.0107
	0.08	6	20, 41, 61, 63, 64, 65	52.3 ± 18.2	—	N/A	.0001
Liver	Control	8	5, 8, 9, 10, 11 × 2, 12, 14	10.0 ± 2.73	—	—	—
	0.04	6	10 × 2, 12, 13, 16, 21	13.7 ± 4.20	—	N/A	.0357
	0.08	6	26, 63, >100 × 5	81.5 ± 31.0	—	N/A	.0001
Skin	Control	7	12 × 2, 13 × 5	12.7 ± 0.49	—	—	—
	0.16	7	12, 13 × 2, 15 × 4	14.0 ± 1.29	14	0	.0149
	0.2	7	14 × 4, 15, 17, 21	15.6 ± 2.64	14	1.6	.0077
	0.24	7	14 × 2, 15, 18 × 2, 19, 21	17.0 ± 2.71	14	3	.0007
	0.3	7	25 × 7	25.0 ± 0.00	18	7	.0001
	0.6	4	26, 32, 36, 39	33.5 ± 5.57	22	11.5	.0001
	1	4	29, 36, 46, 46	39.3 ± 8.30	26	13.3	.0001

Note: FK 506 was given IM for 14 days (day 0→13).
*Days surviving after FK undetectable = MST − the day at which FK 506 value was not detectable. FK 506 blood values were not detectable when the concentrations were 0.04 to 0.24 mg/kg/d.
†All *P* values were determined with Student's *t* test by comparing treatment group with control group.

RESULTS

Liver allografts (n = 8) in the control group were rejected around the 10th day (mean survival time [MST] = 10.0 ± 2.7). When allografted recipients were treated with 0.04 mg/kg of FK 506 (n = 6), the MST (13.7 ± 4.2 days) was almost the same as that in the control group. But, when the allografted recipients were treated with 0.08 mg/kg of FK 506 (n = 6), the MST was remarkably increased to 81.5 ± 31.0 days ($P < .0001$) (see Table 1). The steady-state trough FK 506 blood concentrations, however, were too low to be detected when the recipients were treated with FK 506 doses of either 0.04 mg/kg or 0.08 mg/kg.

In HHTx, the pattern of graft survival was the same as that in OLTx. The heartbeat of grafts stopped on day 6.9 ± 0.4 in the control group (n = 7). When the recipients received 0.04 mg/kg of FK 506 (n = 6), the allografted hearts stopped beating on day 11.0 ± 4.1. But, when the recipients were treated with 0.08 mg/kg of FK 506 (n = 6), the heart beating time was significantly prolonged to 52.3 ± 18.2 days ($P < .0001$) (see Table 1). The steady-state FK 506 levels were below assay detection limits.

The grafts in FTSTx control group (n = 7) were rejected on day 12.7 ± 0.49. After treatment with FK 506 at 0.16, 0.20, and 0.24 mg/kg (n = 7 in each group), the graft survival time was slightly extended to 14.0 ± 1.3, 15.6 ± 2.6, and 17.0 ± 2.7 days, respectively ($P > .0001$). However, when the dose of FK 506 was increased to 0.3, 0.6, and 1.0 mg/kg (n = 4 in the last two groups), the MST of skin grafts was significantly prolonged to 25.0 ± 0.0, 33.5 ± 5.6, and 39.3 ± 8.3 days, respectively ($P < .0001$) (see Table 1).

FK 506 blood concentration was measurable only when the treatment dosage was 0.3 mg/kg or more. Animals treated with 0.3 mg/kg of FK 506 exhibited a blood concentration of 2.9 ± 0.5 ng/mL during the treatment, which was then undetectable on day 18. In the 0.60 mg/kg group, FK 506 blood concentration was 6.9 ± 1.5 ng/mL during the treatment, 1.8 ± 0.7 ng/mL on day 18, and undetectable on day 22. In the 1.0 mg/kg group, it was 7.3 ± 1.2 ng/mL during the treatment, 2.3 ± 1.4 ng/mL on day 18, 0.8 ng/mL (one sample was 0.8 ng/mL, the other two were unmeasurable) on day 22, and undetectable on day 26 (see Fig 1).

The skin allografts were not rejected for a period after the FK 506 blood concentration decreased from a detectable level to an undetectable one. The graft survival time after the levels of FK 506 were no longer detectable (see Table 1) was 7 days in the 0.3 mg/kg group, 11.5 days in the 0.6 mg/kg group, and 13.3 days in the 1.0 mg/kg group.

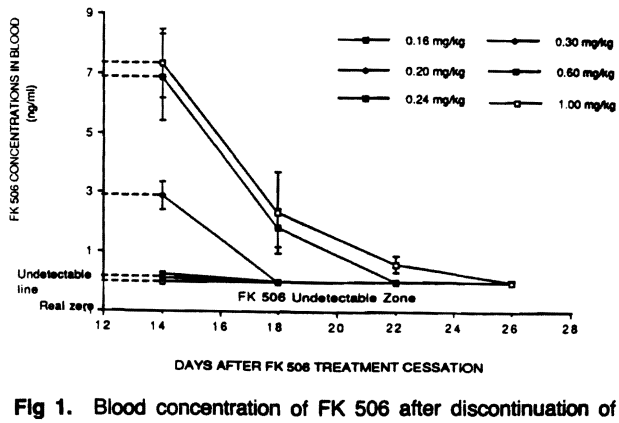

Fig 1. Blood concentration of FK 506 after discontinuation of treatment in FTSTx. Full thickness skin transplanted recipients (ACI→LEW) were treated IM by FK 506 at the concentrations ranging from 0.16 to 1.0 mg/kg for 14 days. Blood samples were drawn on days 14, 18, 22, and 26. FK 506 blood levels were determined by monoclonal antibody-based ELISA. The broken lines represented the FK 506 values during treatment.

DISCUSSION

Decreasing drug dosage to a lower effective dose is a method to limit the side effects of immunosuppression. FK 506 is a potent immunosuppressive agent, but, unfortunately, it exhibits side effects, such as nephrotoxicity.[8] Results from this study showed that in the rat OLTx and HHTx models, treatment with 0.04 mg/kg of FK 506 did not significantly improve the survival time compared with that in control group (only 3.7 days in OLTx and 4.1 days in HHTx, respectively). But, when the FK 506 dosage was increased slightly from 0.04 mg/kg to 0.08 mg/kg, the mean graft survival time was significantly prolonged by 71.5 days in OLTx and 45.4 days in HHTx, when compared to the control group ($P < .0001$ for both). These results suggest that the effective concentration of FK 506 could be reduced to as low as 0.08 mg/kg in liver and heart allotransplantation using the ACI to Lewis strain combination. Although the corresponding FK 506 blood level was undetectable, the lower dose of FK 506 was still effective in prolonging transplant liver and heart survival time in rats. This fact indicates a need to develop a more sensitive and precise method for measuring the lower effective blood concentration of FK 506. Bioassay of FK 506, based on the inhibition of an alloreactive T-cell clone proliferation, may be a better method, because the assay levels can measure lower levels of FK 506.[9] However, this method is time consuming and cannot be performed routinely.

Because the lower effective dose of FK 506 in OLTx and HHTx was too low to be detected, the skin transplant model was used to study the relationship between the lower dose of FK 506 and its corresponding blood level. The results from this experiment showed that 0.3 mg/kg of FK 506 was the comparatively lower effective dose in FTSTx. Meanwhile, this dose of FK 506 demonstrated the lowest detectable blood level of FK 506 in rats. When the doses of FK 506 were increased from 0.3 to 1.0 mg/kg, the blood levels of FK 506 were gradually elevated and the graft survival time was significantly prolonged. After cessation of treatment, the higher doses of FK 506 required a longer time to reach undetectable levels.

In addition, these results also showed that the graft survival time after the FK 506 blood level became undetectable and the grafts were totally rejected varied according to the treatment doses of FK 506. When the doses were increased from 0.3, 0.6 to 1.0 mg/kg, the graft survival time was prolonged from 7, 11.5 to 13.3 days (see Table 1). These results suggested that the improved skin graft acceptance paralleled increased steady state FK 506 concentrations, and the graft survival time was dose dependent.

CONCLUSIONS

To limit the side effects in FK 506 treatment, the effective dose of FK 506 can be reduced to as low as 0.08 mg/kg in liver and heart allotransplantation in rats when administered IM. It is known that at doses used in this study, FK 506 is not effective when administered orally. This may be related to the differences in the absorbtion of FK 506 from the gastrointestinal tract and the IM site of injection. Although improved survival is associated with increasing blood FK 506 concentrations, FK 506 is effective even at blood steady-state concentrations less than 0.8 ng/mL in OLTx and HHTx. Since lower concentrations are effective, it is necessary to find a more sensitive method for measuring the lower effective blood concentration of FK 506.

The minimal dose of FK 506 that resulted in detectable blood levels was 0.3 mg/kg in rats.

The comparatively lower effective dose of FK 506 in preventing skin allograft rejection was 0.3 mg/kg. The prolonged skin graft survival time was dependent on FK 506 dosage and its associated blood concentration.

Although there are no detectable levels of FK 506 in the blood after cessation of treatment, there is still a prolongation of graft survival. This effect is augmented with increased doses of FK 506.

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