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THE IMMUNOSUPPRESSIVE EFFECTS OF FR 900506 IN RATS RECEIVING HETEROTOPIC CARDIAC ALLOGRAFTS

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The immunosuppressive effects of FR 900506 were studied in Lewis rats given ACI heterotopic heart allografts. Intramuscular doses of 0.02 mg/kg daily for 2 weeks extended graft survival slightly, and with doses up to 1.28 mg/kg for 2 weeks, there is long-term graft survival. If only 3 daily doses were given starting on day 4 after transplantation, graft survival for almost 3 months was accomplished in 4 of 6 animals who still bear functioning hearts.

FR 900506 (FR) is a new immunosuppressive agent with a molecular weight of 822.05 Daltons which was isolated from Streptomyces tsukubaensis in Japan (1). By in vitro testing it inhibits interleukin 2 (IL-2) production, inhibits mixed mouse lymphocyte culture cellular reactions, stops the generation of mouse cytotoxic T cells, and inhibits the appearance of IL-2 receptors on human lymphocytes. These effects of FR are about 500 to 1000 times more potent than those of cyclosporin A (CsA).

Pilot in vivo studies (1) have shown that FR can prolong the survival time of skin and heterotopic cardiac allografts in rats and renal allografts in dogs. We report here studies in rats on the optimal dosage of FR, the timing of drug administration, and the effectiveness of FR in reversing ongoing rejection.

MATERIAL AND METHODS

Animals

Inbred male rats were used in all experiments. Lewis rats (RT 1^l) weighing 200 - 250 gm. were used as recipients. ACI rats (RT 1^a) weighing 150 - 200 gm. were used as heart donors. Lewis rats were purchased from the Harlian Sprague Dawley (Indianapolis, Indiana), and ACI rats were purchased from the Simonsen Lab. (Gilcoy, California).

Operative Procedures

Heterotopic cardiac transplantation was performed by anastomosis of the donor aorta and pulmonary artery to the recipient aorta and infrarenal inferior vena cava with standard microvascular techniques (2). Impulses of the transplanted heart grafts were palpated daily through the abdominal wall of the recipients. Rejection was diagnosed by the cessation of heart beat and confirmed visually by laparotomy and histological examination. Of the 78 recipient rats treated with FR, 3 died with living grafts and were excluded from statistical analysis. All animals are accounted for individually in Tables 1-3.

Drug Administration

FR for intramuscular use was kindly supplied by Fujisawa Pharmaceutical Co., Osaka, Japan, as a crystalline powder. It was dissolved in saline and was injected in the thigh once a day.

Experimental Designs

Recipient rats were divided into seventeen groups. Another seven recipient rats without immunosuppressive treatment were used as control group.

Dose response studies --- Forty-two recipient rats were divided into seven groups of 6 to test the effectiveness of FR in doses from 0.02 mg/kg/day for two weeks to 1.28 mg/kg/day for two weeks (Table 1).

Treatment timing studies --- The best dose from the above experiments was judged to be 1.28 mg/kg/day. Using this daily dose, 6 groups of animals were treated at various time intervals before transplantation (Table 2). Another 4 groups were treated at different times after transplantation (Table 3). From one to 4 doses were given (Tables 2 and 3).

Statistical Analysis

Graft survival times were compared using Wilcoxon rank sum test.

RESULTS

Dose response studies --- All allografted hearts without FR treatment were rejected within 7 days (median survival time 6 days) (Table 1). All of the cardiac allograft survival times were significantly prolonged with two weeks treatment starting on the day of operation, compared with the control group (Table 1). Daily doses as small as 0.02 mg/kg and 0.04 mg/kg had a slight but significant effect (Table 1). Between doses of 0.08 mg/kg and 0.64 mg/kg for 2 weeks, the median survival times were between 32 and 39 days. At I.M. doses of 1.28 mg/kg/day for two weeks, the survival times were remarkably prolonged (median survival time 87 days).

Treatment timing studies --- The optimum dose of 1.28 mg/kg was used. I.M. single dose treatment before operation was ineffective (Table 2). However, 2 to 4 doses on the days before grafting increased greatly the graft survival times (Table 2).

Therapy with 1.28 mg/kg/day after transplantation was even more effective (Table 3). Two or 3 daily doses just after operation prolonged survival to more than a month. The most striking results were when treatment was delayed to the fourth postoperative day. Then, a remarkable "rescue" was evident, and 4 animals from this group of 6 are approaching 3 months survival (Table 3).

DISCUSSION

In confirmation of the original work of Ochiai et al (1), the potency of FR 900506 was evident in this study. The dose range of demonstrable effect was very broad, from .02 to 1.28 mg/kg/day without a toxicity-imposed ceiling being reached. Furthermore, there was no obvious histopathologic or clinical evidence of drug toxicity, even at the highest I.M. dose (unpublished observations).

So potent is FR 900506 that 2, 3, or 4 doses of 1.28 mg/kg given I.M. before transplantation permitted prolonged survival without postoperative treatment. The most remarkable observation was that 3 daily doses started after 4 days, almost on the eve of anticipated rejection, allowed almost unlimited subsequent graft survival in 4 of 6 recipients. This ability to rescue heterotopic heart grafts from rejection was far greater than that reported for cyclosporine (3).

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TABLE 1
EFFECT OF FR 900506 IN VARIOUS DOSES UPON THE GRAFT SURVIVAL OF LEWIS RATS RECEIVING ACI HEART

Treatment	Rat No.	Graft Survival (Days)	Median (Days)	P-value @
Control	7	6, 6, 6, 6, 7, 7, 7	6	-
0.02 mg/kg I.M. daily for 14 days	6	7, 7, 8, 8, 9, 9	8	<0.05
0.04 mg/kg I.M. daily for 14 days	6	9, 10, 10, 11, 24, 34	10, 5	<0.01
0.08 mg/kg I.M. daily for 14 days	6	12, 14, 32, 36, 40, 10*	32	<0.01
0.16 mg/kg I.M. daily for 14 days	6	27, 30, 32, 33, 55, 14*	32	<0.01
0.32 mg/kg I.M. daily for 14 days	6	27, 30, 37, 39, 39, 49	38	<0.01
0.64 mg/kg I.M. daily for 14 days	6	33, 34, 36, 42, 43, 84	39	<0.01
1.28 mg/kg I.M. daily for 14 days	6	56, 71, 80, 94, 96, >150	87	<0.01

@ Compared with control group
*** Died with living graft**

TABLE 2

FR 900506 GIVEN I.M. BEFORE TRANSPLANTATION: GRAFT SURVIVAL OF LEWIS RATS RECEIVING ACI HEARTS

Treatment	Rat No.	Graft Survival (Days)	Median (Days)
1.28 mg/kg once on day -4	3	6, 6, 6	6
1.28 mg/kg once on day -2	3	8, 8, 8	8
1.28 mg/kg once on day -1	3	7, 8, 9	8
1.28 mg/kg on days -2, -1	3	18, 30, 37	30
1.28 mg/kg on days -3, -2, -1	3	33, 35, 48	35
1.28 mg/kg I.M. daily on days -4, -3, -2, -1	6	30, 31, 32, 35, 51, 6*	32

*** Died with living graft**

TABLE 3

FR 900506 GIVEN I.M. AFTER TRANSPLANTATION: GRAFT SURVIVAL OF LEWIS RATS RECEIVING ACI HEARTS

Treatment	Rat No.	Graft Survival (Days)	Median (Days)
1.28 mg/kg on days 0, 1	3	22, 31, 53	31
1.28 mg/kg on days 2, 3	3	30, 49, >48	49
1.28 mg/kg on days 0, 1, 2	3	23, 35, 36	35
1.28 mg/kg on days 4, 5, 6	6	23, 51, >84, >87, >87, >87	>87