Heterotopic Heart Transplantation in the Rat Receiving FK-506 Alone or With Cyclosporine

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IN THE FIRST IN VIVO demonstration of immunosuppression by FK-506 (FK), Ochiai et al[1] used inbred rats. The potency and safety of the agent was confirmed in rats by Lee et al[2] who provided further information about dose-effect relationships and about dose timing. In the present study, longer follow-up will be provided for Lee's experiments, evidence will be presented that FK and cyclosporine (CyA) are synergistic, and preliminary experiments will be described that test the hypothesis that partial tolerance induction may be achieved with a brief course of properly timed FK administration.

MATERIALS AND METHODS

Animals

Inbred male rats were used in all experiments. Lewis rats ($RT1^l$) weighing 200 to 250 g were always used as recipients. ACI rats ($RT1^a$) weighing 150 to 200 g were used as heart donors except for a few experiments studying specific tolerance induction in which Brown Norway (BN) rats ($RT1^n$) were used as donors. Lewis (LEW) rats and BN rats were purchased from Harlan Sprague Dawley (Indianapolis). ACI rats were purchased from Simonsen Laboratories (Gilcoy, CA).

Operative Procedures

Anesthesia was with 40 mg/kg phenobarbital intraperitoneally. Hearts usually were transplanted heterotopically by anastomosing the donor aorta and pulmonary artery to the recipient aorta and infrarenal inferior vena cava.[3] The transplanted hearts were palpated daily through the abdominal wall. Rejection was diagnosed by the cessation of heart beat and confirmed visually by laparotomy and histological examination. Recipient rats that died with living grafts were excluded from statistical analysis; these exclusions accounted for less than 5% of the experiments. All animals are accounted for individually in the tables.

In the tolerance induction studies in which two heart transplants were performed serially, the second graft was inserted in the neck by a modification of Heron's method[4] whereby the aorta is anastomosed to the carotid artery and the pulmonary artery is anastomosed to the jugular vein.

Drug Administration

The Fujisawa Pharmaceutical Co, Ltd, Osaka, Japan, provided FK as a crystalline powder. It was dissolved in saline and was injected in the thigh once a day.

Experimental Designs

Dose-response studies. These experiments with shorter follow-up were reported previously by Lee et al.[2] Forty-two recipient rats were divided into seven groups of six to test the effectiveness of FK in doses from 0.02 to 1.28 mg/kg/d. In all seven groups, treatment was for 2 weeks (Table 1), starting just after recovery from anesthesia. Graft survival times were compared by using Wilcoxon rank sum testing.

Treatment timing studies. The most effective dose was judged from the dose-response studies to be 1.28 mg/kg/d. With this daily dosage, six groups of animals were treated at various time intervals before transplantation (Table 2). Another four groups were treated at different times after transplantation (Table 3). From one to 4 doses were given (Tables 2 and 3). The experiments in Tables 2 and 3 were the same as reported by Lee et al[2] but with a longer follow-up.

Studies of possible tolerance induction. The most striking results in the treatment timing studies were with intramuscular doses of 1.28 mg/kg on days 4, 5, and 6 after grafting (see Table 3). The grafts were not only rescued from rejection, but they survived for long times thereafter it not permanently.

The possibility of donor-specific antigen nonresponsiveness was analyzed with the experiments in Table 3. The actual experiments were in group 22 in which ACI rats provided serial heart grafts 14 days apart and in group 23 in which the second graft was provided by a third party (BN) donor. The other groups were controls, with the meanings summarized in Table 4.

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Table 1. Effect of Intramuscular FK Dose for 14 Days Starting the Day of Heterotopic Heart Transplantation

Group	Dose (mg/kg)	n	Graft Survival (d)	Median Survival (d)	*P* Value *v* Group 1
1	0.00	7	6, 6, 6, 6, 7, 7, 7	6.0	—
2	0.02	6	7, 7, 8, 8, 9, 9	8.0	<.05
3	0.04	6	9, 10, 10, 11, 24, 34	10.5	<.01
4	0.08	6	12, 14, 32, 36, 40, 10*	32.0	<.01
5	0.16	6	27, 30, 32, 33, 55, 14*	32.0	<.01
6	0.32	6	27, 30, 37, 39, 39, 49	38.0	<.01
7	0.64	6	33, 34, 36, 42, 43, 84	39.0	<.01
8	1.28	6	56, 71, 80, 94, 96, >200	87.0	<.01

*Died with a living graft.

Table 2. Preoperative Treatment With One to Four Doses of 1.28 mg/kg Intramuscular FK

Group	Days Preoperative FK −4	−3	−2	−1	n	Graft Survival (d)	Median Survival (d)
1	None				7	6, 6, 6, 6, 7, 7, 7	6.0
9				X	3	7, 8, 9	8.0
10			X		3	8, 8, 8	8.0
11	X				3	6, 6, 6	6.0
12			X	X	3	18, 30, 37	30.0
13		X	X	X	3	33, 35, 48	35.0
14	X	X	X	X	6	30, 31, 32, 35, 51, 6*	32.0

*Died with a living graft.

Intramuscular FK in combination with oral CyA. The experimental design using ACI-LEW heart transplants is summarized in Table 5. Minimally effective doses of intramuscular FK or oral CyA were combined.

RESULTS

Dose-Response Studies

Untreated rats rejected their hearts in six days (Table 1). Graft survival was significantly increased with doses as small as 0.02 mg/kg, and major survival increases were demonstrated at all doses greater than 0.04 mg/kg. The great range of dose effectiveness (to as high as 1.28 mg/kg) with little or no mortality was noteworthy, implying a favorable therapeutic index. Nalesnik et al[5] are reporting elsewhere in this symposium that weight loss and other potential complications of FK are minor in rats.

The most effective dose was 1.28 mg/kg. When this dose was given daily for 2 weeks, all hearts beat for at least 6 more weeks without therapy, and one is still going more than a half year later.

Treatment Timing Studies

Two or more intramuscular doses of 1.28 mg/kg FK prolonged heart graft survival

Table 3. Postoperative Treatment With Two or Three Doses of 1.28 mg/kg Intramuscular FK

Group	Days Preoperative FK 0	1	2	3	4	5	6	n	Graft Survival (d)	Median Survival (d)
1	None							7	6, 6, 6, 6, 7, 7, 7	6.0
15	X	X						3	22, 31, 53	31.0
16	X	X	X					3	23, 35, 36	35.0
17			X	X				3	30, 49, 53	49.0
18					X	X	X	6	23, 51, 89, 93, 98, >145	89.0

Table 4. Fate of Double Heart Transplants With Three-Day Course of 1.28 mg/kg/d Intramuscular FK

Group	Strain Combinations	Time Sequence Over 14-Day Period: 1st HTx on Day 0 and Survival (d)	FK on Days 4, 5, and 6	HTx on Day 14 and Survival (d)	Meaning of Group
1	1st HTx, ACI-LEW	Yes (6, 6, 6, 6, 7, 7, 7)	No	No	Untreated control, same as group 1, Table 1
19	1st HTx, BN-LEW	Yes (11, 11, 11, 12, 12, 13)	No	No	Untreated control, with BN-LEW strain combination
20	Delayed HTx, ACI-LEW	No	Yes	Yes (9, 10, 10, 11)	Measures effect of 3 days' treatment 7-10 days before delayed grafting
21	Delayed HTx, BN-LEW	No	Yes	Yes (27, 34)	Measures effect of 3 days' treatment 7-10 days before delayed grafting with easier BN-LEW combination
22	1st HTx, ACI-LEW	Yes (>37, 63, 64, 70)	Yes		Confirms group 18, Table 3, and conditions recipient for 2nd graft
	2nd HTx, ACI-LEW			Yes (25, 41, 49, 56)	Test of donor-specific tolerance without further FR treatment, ACI donor $P < .01$ *v* group 20
23	1st HTx, ACI-Lew	Yes (48, 60)	Yes		Same result with 1st Htx as in group 22
	2nd HTx, BN-LEW			Yes (14, 15)	Minimal survival advantage for 2nd graft using BN donor

Test groups are groups 22 and 23; all others are controls.
Abbreviation: HTx, heart transplant.

Table 5. Effect of Intramuscular FK Combined with Oral CyA for 2 Weeks Starting After Recovery From Anesthesia

Group	Treatment	n	Graft Survival (d)	Median Survival (d)	*P* Value
1	Untreated control	7	6, 6, 6, 6, 7, 7, 7	6.0	—
2	FK, 0.02 mg/kg/d	6	7, 7, 8, 8, 9, 9	8.0	<.05 *v* 1
3	FK, 0.04 mg/kg/d	6	9, 10, 10, 11, 24, 34	10.5	<.01 *v* 1
24	CyA, 2.5 mg/kg/d	6	7, 9, 9, 11, 15, 19	10.0	<.01 *v* 1
25	FK, 0.02 mg/kg/d, +CyA, 2.5 mg/kg/d	6	24, 25, 26, 26, 38, 51	26.0	<.01 *v* 2, 3, and 24
26	FK, 0.04 mg/kg/d, +CyA, 2.5 mg/kg/d	6	15, 21, 34, 36, 48, 53	35.0	<.01 *v* 2, 3, and 24

remarkably (Table 3). In group 18, three injections beginning almost on the eve of anticipated rejection rescued all six of the grafts. Furthermore, the subsequent survival was 2½ to 20 weeks, and the longest surviving graft is still beating (Table 3). These observations prompted the experiments in the next section.

Studies of Possible Partial Tolerance Induction

After four ACI-to-LEW transplants, the striking effect of 1.28 mg/kg FK on days 4, 5, and 6 after grafting was confirmed (group 22, Table 4). Placement eight days later of another ACI heart without further therapy did not precipitate rejection of the first organ, and the second grafts survived for 25 to 56 days. Rejection was the usual eventual cause of loss of both the first and second heart. When the second heart donor was of the BN strain (group 23, Table 4), most but not all of the foregoing potentiation of second-graft survival was lost. Control studies in groups 20 and 21 explained why the third-party BN grafts of group 23 had a slight and unexpected survival prolongation. In these controls, treatment of the LEW recipients for three days and ending eight days before transplantation of either ACI (group 20) or BN hearts (group 21) resulted in a still-perceptible immunosuppressive effect compared with animals with no pretreatment at all (groups 1 and 19, Table 4).

FK Combined With CyA

Small doses of FK alone or CyA alone had caused barely detectable increases in graft survival. When used together, the drugs had a synergistic effect (Table 5).

DISCUSSION

The studies of dosage and timing have completed the profile already published in part by Lee et al.[2] At the optimal dose tested so far in a 2-week course of therapy, graft survival far outlasts discontinuance of treatment, but survival of the now-treated graft is not usually permanent. The same applies if FK administration is delayed for several days after transplantation and then delivered in a burst that could theoretically lead to specific clone deletion as was speculated by Lee et al.[2]

The possibility of creating selecting nonresponsiveness was directly examined with drugs given delayed FK treatment and then challenged eight days later with a second cardiac graft from the same donor species while the original heart was still beating. The second heart graft had a great survival advantage in this conditioned environment, and the original heart transplant was not thereby harmed. The immunologic specificity of this effect was suggested by the fact that a survival advantage for third-party (BN) hearts was very minor in comparison.

The other new rat data in this ongoing study were concerned with the synergism between FK and CyA. Minimally therapeutic doses of these agents had a greatly amplified effect when the drugs were used together, which adds to the theme of multiple-drug therapy on which our attention has been focused in the event of clinical trials of FK.

SUMMARY

In rats, FK significantly prolonged heterotopic heart graft survival over a wide dose range when given for 2 weeks starting on the day of the operation. Brief courses of FK for one to four days preoperatively, and especially beginning four days postoperatively, allowed long subsequent survival of heart grafts in otherwise untreated recipients. The seeming acceptance of the grafts with postoperative FK treatment was largely but not exclusively donor specific when tested eight days after the last FK dose by second grafts from the same donor *v* third-party donor grafts. FK in minimally therapeutic doses was synergistic with suboptimal doses of CyA.

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