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SUPPRESSION OF ALLOGRAFT REJECTION WITH FK506

I. PROLONGED CARDIAC AND LIVER SURVIVAL IN RATS FOLLOWING SHORT-COURSE THERAPY[1]

NORIKO MURASE, DONG-GOO KIM, SATORU TODO, DONALD V. CRAMER, JOHN J. FUNG, AND THOMAS E. STARZL[2]

The Departments of Surgery and Pathology, University Health Center of Pittsburgh, University of Pittsburgh, and the Veterans Administration Medical Center, Pittsburgh, Pennsylvania

Heterotopic heart and orthotopic liver grafts from ACI donors were transplanted to Lewis rat recipients that were treated with a 3 (or 4) day course of FK506 IM that was started on postoperative day 0, 2, 3, 4, 5, or 6. Hearts, which rejected after a median of 6 days in untreated controls, always had prolonged survival (median 91 days) when treatment was started on postoperative day 4. The results were inferior when treatment was started earlier or later than this, but even when the first dose of FK506 was on postoperative day 5, one day before rejection was imminent in controls, the median survival was 50 days. The poorest results with a median graft survival of only 36 days were obtained when injections were on days 0–3. Results were similar with liver grafts that rejected after a median time of 10 days in nontreated controls but that usually survived permanently after a 3 (or 4) day FK506 course starting on day 0, 2, 3, or 4. Therapy started on day 6 was too late.

The novel immunosuppressive agent FK506 (originally FR900506) was discovered in 1984 (*1, 2*) and has become an object of considerable interest in the field of organ transplantation. Prolongation of survival times has been demonstrated for skin, heart, kidney, or liver allografts, in rodents (*3–7*), dogs (*8–11*), subhuman primates (*11, 12*), and humans (*13*). FK506 is more potent than cyclosporine and has a similar mode of action, including inhibition of T lymphocyte response to stimulation with mitogen, or stimulation with alloantigens in mixed lymphocyte reactions (*14, 15*). It inhibits the induction of allospecific human and mouse cytolytic T lymphocytes, and it suppresses IL-2 and gamma-IFN secretion and IL-2 receptor expression of the T lymphocytes (*2, 14–17*).

What FK506 does not do is of equal interest. It does not at any concentration inhibit the B-cell-stimulating factor 2 driven proliferation of cloned B cells, nor does it inhibit IL-2-driven proliferation of cloned T cells (*17*). Thus, FK506 has been said to inhibit the proliferation of lymphocytes during the recognition and induction phase of the allograft reaction and not the proliferation seen subsequent to the production and binding of lymphokines. If this were its complete profile, the effectiveness of FK506 in preventing allograft rejection would depend upon administration of the drug very early in the posttransplantation period.

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[2] Reprint requests should be sent to Thomas E. Starzl, M.D., Ph.D., Department of Surgery, 3601 Fifth AVenue, Falk Clinic 5C, Pittsburgh, PA 15213.

We have previously shown that a brief course of FK506 treatment can induce prolonged cardiac allograft survival in rats (*5*) and liver or kidney survival in dogs (*11, 12*). In the present study, we have examined in more detail the effect of a 3- or 4-day course of FK506 treatment on the subsequent survival of heart and liver allografts in rats. In a companion study (*18*), we have characterized the changes of the host immune response that result from this therapeutic approach.

MATERIALS AND METHODS

Animals. Inbred male rats were used in all experiments. Lewis rats (LEW) weighing 250–350 g (Harlan Sprague-Dawley Inc., Indianapolis, IN) were the recipients, and ACI rats weighing 150–250 g (Simonsen Laboratories, Gilroy, CA) were donors. All animals were maintained in conventional animal facilities with water and commercial rat chow provided ad libitum.

FK506 administration. FK506 was provided as a crystalline powder from the Fujisawa Pharmaceutical Company, Ltd. (Osaka, Japan). It was dissolved in saline and administered once a day by intramuscular injection. The date and timing of FK506 is noted for each experimental group in Tables 1 and 2.

Orthotopic liver transplantation. Liver allografting was performed with a modification of the method described by Kamada and Calne (*19*) using the simplified cuff technique for the portal and infrahepatic vena cava anastomosis. Recipient animals received Cefamandole Nafate (20 mg/day i.m.) (Eli Lilly and Company, Indianapolis, IN) for 3 days postoperatively.

Animals that died within 3 days after surgery were considered technical failures and were excluded from the analyzed experimental data; however, the success rate for all liver transplants in this study was more than 95%. Rejection was monitored by body weight and blood chemistry values (SGOT, SGPT, and total bilirubin). Rejection of the grafts resulted in death of the recipients, and at the time of death all grafts were examined histologically.

Heterotopic heart transplantation. Cardiac allografting was performed as described by Ono and Lindsay (*20*) and consisted of anastomosis of the donor aorta and pulmonary artery to the recipient aorta and infrarenal vena cava. Heart grafts were palpated daily, and rejection was established by cessation of the heartbeat and confirmed by direct inspection at laparotomy and by histologic examination.

Statistical analysis. Results were analyzed for statistical significance by Wilcoxon rank-sum test, and differences considered statistically significant if $P<0.01$.

RESULTS

Survival in cardiac allografts. Untreated LEW recipients rejected ACI heart grafts with median graft survival time of 6 days (group 1, Table 1). As we previously reported in a study on FK506 dose-response relations (*5*), treatment with daily intramuscular FK506 (1.28 mg/kg) for the first 2 weeks after grafting significantly prolonged graft survival (group 2, MST:

TABLE 1. Effect of FK506 on survival of heart grafts from ACI donors to LEW recipients

Group	Treatment	N	Graft survival (days)	MST (days)[a]	P value[b]
1	—	7	6, 6, 6, 6, 6, 7, 7	6.0	—
2	1.28 mg/kg days 0–13	6	56, 71, 80, 94, 96, 213[c]	87.0	0.01
3	1.28 mg/kg days 0–13, then once a week for 5 weeks	12	8,[c] 81, 82, 86, 87, 88, 88, 89, 93, 101, 110, 122	88.0	0.001
4	1.28 mg/kg days 0, 1, 2	6	23, 35, 36, 36, 58, 75	36.0	0.01
5	1.28 mg/kg days 4, 5, 6	6	23, 51, 89, 93, 98, 146	91.0	0.01
6	1.28 mg/kg days 5, 6, 7	6	25, 48, 49, 51, 60, 61	50.0	0.01
7	1.28 mg/kg days 3, 4, 5, 6	6	71, 71, 72, 76, 79, 81	74.0	0.01
8	2.5 mg/kg days 4, 5, 6	6	38, 54, 75, 85, 101, 120	80.0	0.01

[a] Median graft survival time in days.
[b] Group 2 vs. 3, 4, 5, 7, 8, N.S.; group 2 vs. 6, $P=0.01$.
[c] Died with pulsating graft.

TABLE 2. Effect of FK506 treatment on survival of liver grafts from ACI donors to LEW recipients

Group	Days of treatment[a]	N	Graft survival (days)	MST (days)	P value
1	—	10	9, 9, 9, 9, 10, 10, 10, 11, 12, 13	10.0	—
2	Days 0, 1, 2	6	>100×6	>100	0.001
3	Days 2, 3, 4	6	9,[b] >100×5	>100	0.001
4	Days 4, 5, 6	14	9, 10, 10, 12, >100×10	>100	0.01
5	Days 6, 7, 8	6	9, 9, 10, 11, 12, 15	10.5	NS
6	Days 3, 4, 5, 6	10	13,[b] >100×9	>100	0.001

[a] FK506 was given by daily intramuscular injection (1.28 mg/kg/day).
[b] Death due to infection.

87.0 days, $P<0.01$). Five additional weekly injections of FK506 (1.28 mg/kg) (group 3) did not prolong the median graft survival time for the group, but it did reduce the time range in which rejection occurred (Table 1).

The timing of drug administration influenced the effectiveness of treatment. Doses at postoperative days 4, 5, and 6 (group 5) was as effective as a course of 2 or more weeks (groups 2 and 3). Earlier (days 0, 1, 2; group 4) or later treatment (days 5, 6, 7; group 6) was less effective (Table 1). Increasing the length of treatment (days 3, 4, 5, 6; group 7) or increasing the amount of drug administered (2.5 mg/kg/day; group 8) did not result in additional prolongation of graft survival (Table 1).

Survival in liver allografts. The untreated control ACI liver grafts were acutely rejected in nontreated LEW recipients in 9–13 days (group 1, Table 2). The longer survival with untreated liver versus untreated heart grafts was significant ($P<0.001$). Short-term treatment with 1.28 mg/kg i.m. FK506 was highly effective in prolonging liver survival when given on days 0–2 (group 2); 2–4 (group 3); 4–6 (group 4); and 3–6 (group 6). Permanent liver graft acceptance was the rule in all groups in which treatment was started before day 5. Treatment started on day 6 (group 5) was too late, and survival was reduced to that in the untreated control group.

Histopathology. In both the heart and liver grafts, the short course of FK506 treatment early in the posttransplantation period markedly reduced the number of inflammatory cells. The allograft reaction in the heart consists of a diffuse, rapidly progressive mononuclear infiltration of the myocardium. By day 6, the inflammatory reaction had caused sufficient damage to the myocardium so that the graft ceased to function (Fig. 1A). In contrast, the hearts in FK506-treated animals only had a mild scattering of lymphocytes in the myocardium and no myocardial damage (Fig. 1B).

A short course of FK506 also greatly reduced the intensity of the cellular infiltration in liver allografts. By day 7, the untreated animals had an intense mononuclear infiltration of the periportal areas and a diffuse infiltration of the sinusoids (Fig. 1C). The allografts in the treated animals had a mild-to-moderate periportal inflammation (Fig. 1D) that decreased spontaneously with time.

DISCUSSION

The ACI and LEW strain combinations used for these experiments are strongly histoincompatible and differ for both MHC (RT1) and non-MHC histocompatibility genes. The effect of allograft acceptance under a longer course of FK506 therapy has been shown to be specific for the donor strain (*5, 6*). The same kind of short-course treatment, even as late as 4 days after transplantation, induced permanent acceptance of practically all liver grafts. The greater ease with which liver graft survival could be accomplished compared to the heart is consistent with much work showing a slight biologic advantage for hepatic grafts (*21–24*).

With either the liver or heart, the ability of FK506 to switch off the destructive immune process, practically on the eve of rejection, was extraordinary. The same thing has been accomplished occasionally in dog liver recipients with a few doses of antilymphocyte serum (*25*), and rather easily in canine recipients of livers and kidneys under FK506 treatment (*11*). However, liver graft acceptance in larger animals usually is not permanent. An explanation for permanent graft acceptance without further treatment may require revision of some of the classic theories of rejection and immunosuppression as has been discussed in another communication (*18*).

A conventional view might hold that FK506 disrupts the recognition and/or the early induction phase of the allograft

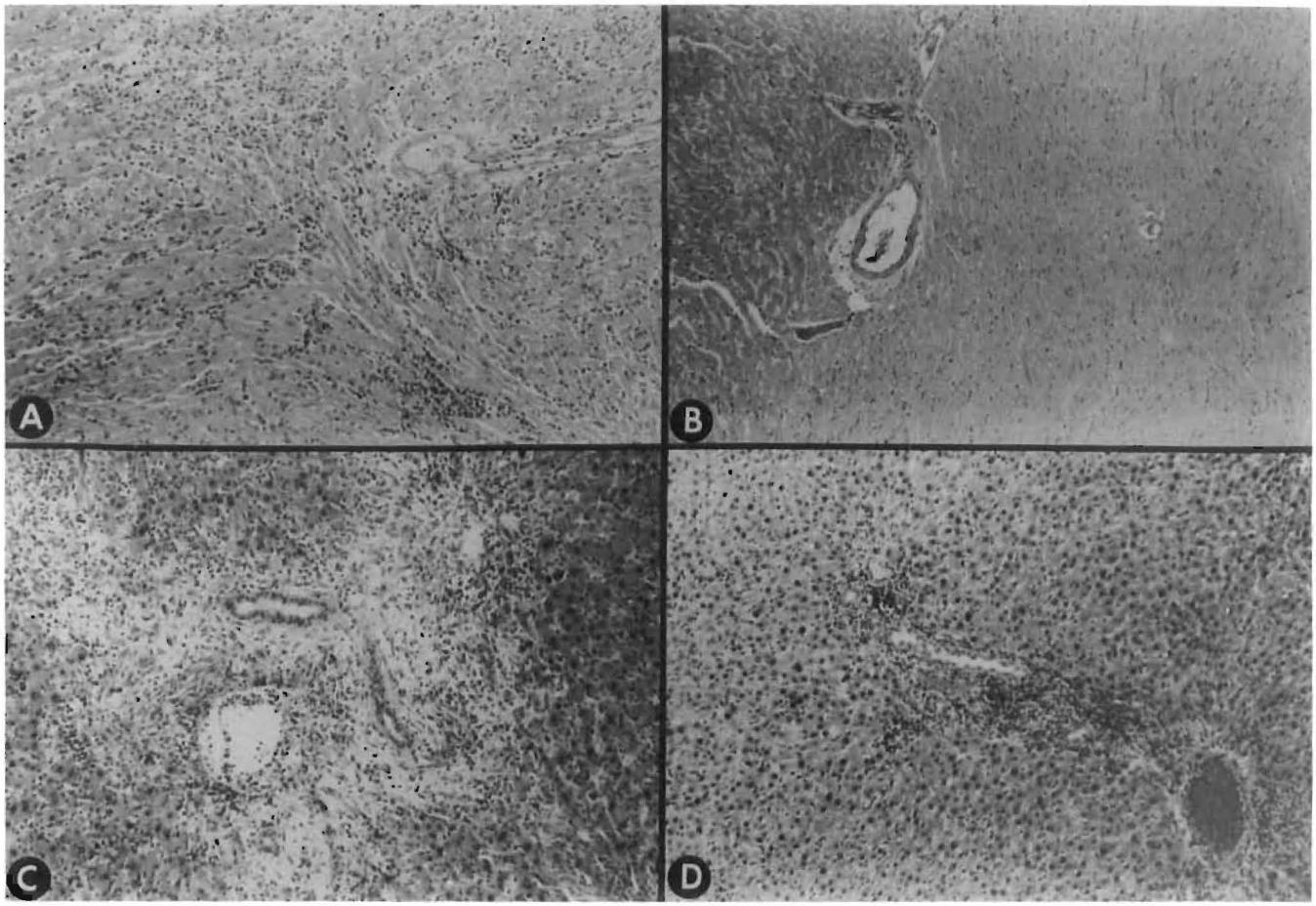

FIGURE 1. A. Untreated heart graft at day 5 showing mononuclear infiltration and interstitial edema of the myocardium. B. FK506-treated heart graft at day 5; i.m. FK506 was started on day 4; note mild scattered mononuclear infiltration. C. Untreated liver graft at day 7 with intense mononuclear cell infiltration of portal tracts and sinusoids with liver cell necrosis. D. FK506-treated liver graft at day 7; i.m. FK506 was given on days 4, 5, and 6. Although there are mononuclear cells in the portal tracts with slightly scattered cells through the sinusoids, the recipient had permanent acceptance of the graft.

response and does not effect the proliferation of activated T cells, preventing thereby an amplification effect by cytokines and other mediators. Recent molecular biological study reveals that FK506 inhibits mRNA accumulation and transcription of early-phase T cell activation genes, such as IL-2, IL-3, IL-4, and gamma-IFN (*26*). If this explanation were true, it is hard to understand how treatment begun 4 or 5 days after exposure to the alloantigens could be so effective. The astonishing ability of FK506 to stop advanced and refractory rejection in human liver grafts (*13*) may be a clinical analogue to the animal observations.

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