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Differential Survival of Hamster-to-Rat Liver and Cardiac Xenografts Under FK 506 Immunosuppression

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THE major barrier in clinical transplantation has been the limitation of donor organs.¹ Xenotransplantation has been considered an alternative source of organs, however strong immunologic barriers are thought to make this option impractical.

The immunosuppressant FK 506 has been shown to be several hundred times more potent than cyclosporine (CyA)^{2,3} and it is capable of suppressing antibody production.⁴ These qualities would justify a trial of this drug in experimental organ xenografts. Indeed, Nakajima et al⁵ failed to prolong hamster cardiac xenografts in rat recipients treated with FK 506. However, it has been demonstrated that untreated livers survive longer than the hearts in the same xenograft combination.^{6,7} Thus, in this study we investigated the effect of FK 506 in the difficult hamster-to-rat liver and cardiac xenotransplant model and compared the results with those of CyA.

MATERIALS AND METHODS

Male LVG hamsters (100 to 150 g) and male Lewis rats (240 to 280 g) served as donors and recipients, respectively. Orthotopic liver transplantation (OLT) was performed according to the cuff technique.⁸ Donor cholecystectomy was carried out at the time of grafting and the preparation lacked anastomosis of the hepatic artery. Heterotopic heart transplantation (HT) was performed by a microsurgical technique as described by Ono and Lindsey.⁹ Treatment groups were given either FK 506 or CyA by IM injection for 14 days (short-term immunosuppression), or FK-506 1 or 2 mg/kg/d for the first posttransplant month followed by dose reduction to 0.5 mg/kg every other day up to day 100 (long-term immunosuppression). Graft survival and liver function were monitored. Antihamster cytotoxic antibody levels were determined by

complement-dependent cytotoxicity assay (CDC) as previously described.⁸ Biopsies, postmortem specimens, or those taken at sacrifice were fixed in 10% formalin and stained with hematoxylin and eosin.

RESULTS

Xenograft survival is shown in Table 1. Liver xenograft recipients given low-dose FK 506 (0.25 mg/kg) rejected their grafts in 7 days, as controls. Short-term treatment with a dose of 0.5 mg/kg/d FK 506 increased hepatic xenograft survival to a mean of 47 days. On the other hand, a high dose of CyA resulted in moderate prolongation to 13 days, with five out of seven rats rejecting while still under treatment. Long-term immunosuppression with 1 mg/kg/d FK 506 for 30 days, followed by 0.5 mg/kg every other day up to day 100, kept five out of eight recipients alive for more than 80 days after grafting. The same long-term protocol with 2 mg/kg/d FK 506 prolonged liver xenograft survival for more than 100 days in 8 out of 12 rats. At the time of writing five recipients are still alive, the longest survivor alive at 200 days after transplantation and 100 days after FK 506 withdrawal. Unlike liver xenografts neither FK 506 nor CyA was able to produce clinically significant survival of HTx. Figure 1 shows the results of

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Table 1. Liver and Cardiac Xenograft Survival Under FK 506 or CyA Immunosuppression

Group	Treatment	No. Organ Transplants	Survival Days	Mean Survival Time (days)	P
1	None	8 OLT	6, 7, 7, 7, 7, 7, 7, 8	7.0 ± 0.5	
2	FK 506: 0.25 mg/kg/d (short-term)	5 OLT	7, 7, 7, 8, 8	7.4 ± 0.5	NS
3	FK 506: 0.5 mg/kg/d (short-term)	5 OLT	(20), 43, 51, 58, 64	47.2 ± 17.0	<.01*
4	CyA: 30 mg/kg/d (short-term)	7 OLT	7, 10, 11, 12, 12, 21, 22	13.5 ± 5.6	<.05*
5	FK 506: 1 mg/kg/d (long-term)	8 OLT	(12), (20), 27 [†] , >80 [‡] , >80 [‡] , >80 [‡] , >80 [‡]	>80	
6	FK 506: 2 mg/kg/d (long-term)	12 OLT	(23), (27), (37), 50 [†] , >100, >100, >100, >100 × 5 [§]	>100	
7	None	6 HT	3, 3, 3, 3, 3, 3	3.0	
8	FK 506: 0.5 mg/kg/d	6 HT	3, 3, 3, 3, 3, 3	3.0	NS
9	FK 506: 1.0 mg/kg/d	6 HT	3, 3, 3, 3, 3, 4	3.1 ± 0.4	NS
10	FK 506: 1.5 mg/kg/d	5 HT	3, 3, 3, 3, 4	3.2 ± 0.4	NS
11	FK 506: 2.0 mg/kg/d	6 HT	4, 4, 4, 4, 5, 5	4.3 ± 0.5	<.01 [¶]

Note: Days in parentheses indicate biliary obstruction.

*Compared with group 1.

[†]Pneumonia due to aspiration.

[‡]Recipient is still alive at the time of writing.

[§]Compared with group 7.

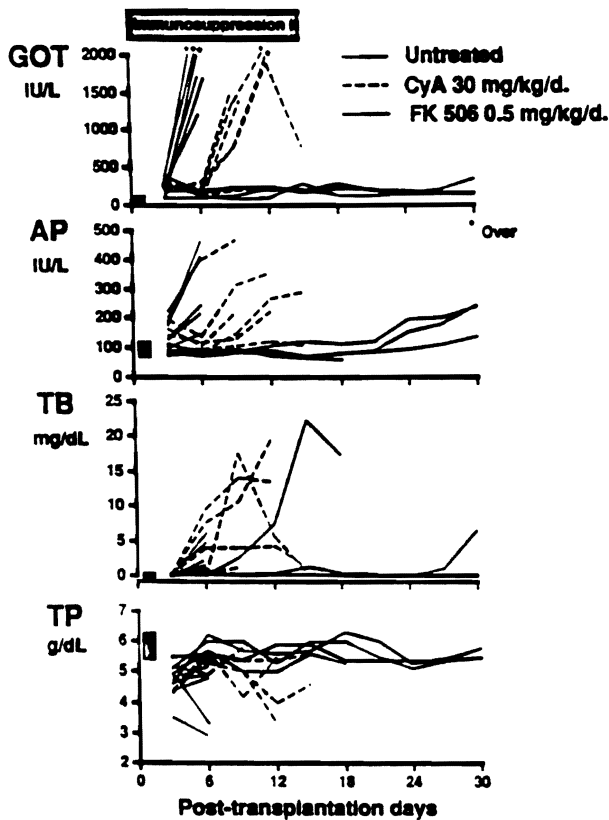


Fig 1. Biochemical profiles in liver xenograft recipients under short-term FK 506 or CyA immunosuppression.

liver functions in xenograft recipients under short-term immunosuppression. FK 506 kept glutamic oxaloacetic transaminase (GOT), alkaline phosphatase, total bilirubin, and total protein at normal or near normal levels when compared with those of controls or CyA-treated animals. The best indicator of rejection seems to be alkaline phosphatase, showing increasing values after FK 506 administration was stopped on day 14.

The antibody response to liver xenografts on short-term immunosuppression is shown in Fig 2. After an initial rise in cytotoxic antibody titers that was similar to that of CyA-treated recipients, FK 506-treated animals showed suppression of antibody formation. After FK 506 withdrawal on day 14, antibody titers started to rise again by the third week. Untreated liver xenograft recipients showed a rapid elevation in antibody titers from 1:1 on day 0 to around 1:8,192 at rejection time. Long-term treatment with either 1 or 2 mg/kg FK 506 (Fig 3) completely suppressed the initial humoral response by the third post-transplant week. This antibody suppression was long-lasting since rats surviving for more than 100 days do not have any cytotoxic antibody titer despite no longer receiving FK 506. There was no suppression of antibody response to cardiac xenografts by either low- or high-dose FK 506.

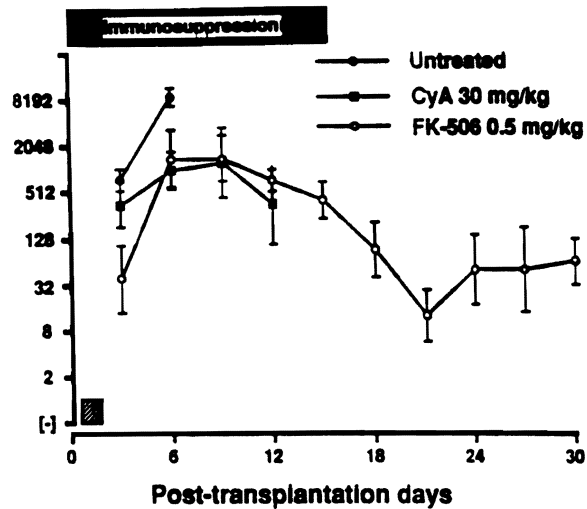


Fig 2. Antihamster cytotoxic antibody titers in liver xenograft recipients under short-term FK 506 or CyA immunosuppression. After 30 minutes incubation of the recipient's serum and complement with hamster lymph node cells (5×10^6 /mL), the percentage of cells stained with trypan blue was calculated. A score of 20% cell death was considered positive. Guinea pig serum diluted 1:10 served as the source of complement.

Histologic examination of liver xenografts in untreated controls at rejection time showed prominent portal and central venulitis with marked portal edema and moderate infiltrate of histiocytes, plasma cells, and lymphocytes (in order of predominance). CyA treatment did not change these findings. One liver xenograft who received low-dose FK 506 for 2 weeks and was killed on the last day of treatment presented focal mild portal infiltrate with blastic cells. Essentially, the liver was normal except for focal rejection infiltrate. Open liver biopsies (day 24) were performed in two xenograft recipients under long-term immunosuppression with FK 506 (1 and 2 mg/kg). Except

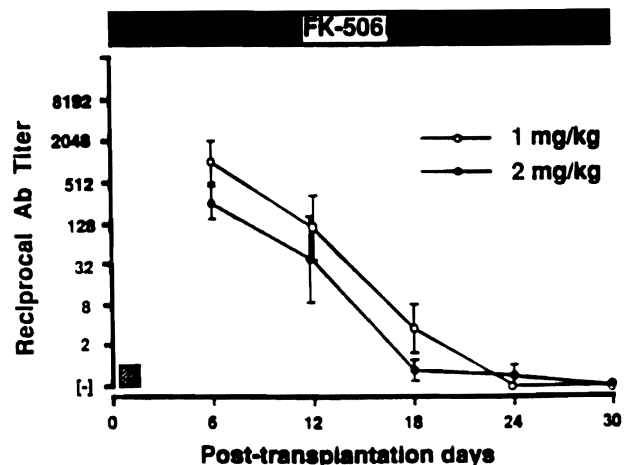


Fig 3. Antihamster cytotoxic antibody titers in liver xenograft recipients under long-term FK 506 immunosuppression.

for mild bile duct proliferation there was no acute cellular rejection. The same features were observed in one rat that died from aspiration pneumonia 50 days after transplantation, and in another recipient that died on day 109 of a cardiac condition due to widespread calcification of the left atrium. One liver xenograft recipient underwent liver biopsy on day 150. This animal had no antibody titers in the serum, however, histologic findings revealed that a brisk cellular acute rejection is ongoing, with biliary duct damage but no lobular necrosis. This particular recipient is still alive at 200 days after transplantation and it is doing clinically well.

Untreated cardiac xenografts presented characteristic humoral-mediated rejection and these findings could not be changed by FK 506.

DISCUSSION

The first attempts to prevent hepatic xenograft rejection in the hamster-to-rat model using CyA combined with splenectomy,⁹ or the addition of total lymphoid irradiation (TLI) to the former therapy,¹⁰ resulted in only a moderate prolongation but not in long-term recipient survival. The difficulty in this xenograft model is the fact that antibody-complement mechanisms are primarily responsible for rejection of extrahepatic xenografts,^{11,12} while liver xenografts undergo a combination of both humoral and cellular rejection.⁸ In this context, our results with FK 506 alone producing long-term survival of liver xenografts, even with clinically applicable dosing, are remarkable. Notably, we have demonstrated that only the liver is favored by this immunosuppressant because the heart succumbs to the early vigorous humoral rejection. An interesting development would be to investigate if a liver xenograft could protect the heart or other organs from rejection, as it has been demonstrated with liver allografts protecting kidneys from the same donors in presensitized humans.¹³

There are several important observations to be made in this study. Naive Lewis rats do have preformed antibodies against hamster lymphocytes, though in very low titers. Because of this they would fail to induce hyperacute rejection. In a matter of days, however, cytotoxic antibodies are induced in extremely high titers. Although both FK 506 and CyA suppressed moderately this initial humoral response, antibody titers were still above the level that is usually found at the time of cardiac xenograft rejection in this model. Thus, not only hepatic allografts¹⁴ but hepatic xenografts are unusually resistant to humoral injury. On the other hand, we have found that CyA-treated animals presented more cellular infiltrate than untreated xenografts, while in the recipients receiving FK 506 there

were only minimal portal inflammatory cells. Thus, the difference between both drugs might be in their ability to suppress cellular-mediated rejection.

A striking finding in this study is that under FK 506 treatment the humoral and cellular responses from the xenograft recipient waned by the end of the third week, provided that the treatment was continued. No animal presented cellular rejection or cytotoxic antibody titers while under long-term immunosuppression, no matter that the FK 506 dose was reduced. Only 50 days after FK 506 administration was stopped, cellular but not humoral rejection is under way. This lack of antibodies would explain, in part, how a xenograft recipient does not develop immune complex-related diseases, such as serum sickness and arthritis.⁷

In conclusion, this is the first report of a successful liver xenograft in a model that is immunologically difficult to overcome. FK 506 is largely more effective than CyA in this xenograft system but its effect favors only the liver and not the heart xenotransplants. A successful clinical liver xenotransplantation with FK 506 immunosuppression looks highly possible, especially in a combination in which preformed antibodies are present in low titers and there is no hyperacute rejection, but in the end rejection is mainly humoral-mediated, in other words, the baboon-to-human system.

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