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Effects of intrahepatic arterial and intraportal administration of FK506 on liver allograft survival in rats

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Abstract Rejection is still the limiting factor for successful organ transplantation, and overdosage of immunosuppressive drugs often results in severe viral infection, side-effects and toxicity. Thus, more specific immunosuppression to lessen these side-effects is highly desirable. In this study, we compared the effects of FK506 administered by different routes (hepatic artery, portal vein and systemic circulation) on the inhibition of rejection. FK506 was given to recipient LEW rats with PVG liver grafts via the penile vein (systemic administration), portal vein or hepatic artery (local administration) for 3 or 7 successive days after liver transplantation. In control LEW rats without immunosuppression, the PVG liver allografts were rejected between 9 and 21 days after transplantation. Intravenous administration of FK506 for 3 days (0.32 and 1.28 mg/kg daily) only had a marginal effect on prolonging liver allograft survival (21.1 ± 12.5 and 32.0 ± 24.0 days, respectively; control 14.1 ± 4.1 days). However, systemic administration of FK506 (0.08–1.28 mg/kg daily) for 7 days suppressed liver allograft rejection markedly (42.3 ± 5.9 to 80.5 ± 53.4 days; control 14.1 ± 4.1 days), and 50% of the recipient rats survived for at least 60 days after liver transplantation. Moreover, when a low dose of FK506 (0.32 mg/kg) was infused into the hepatic artery or portal vein of the transplanted liver for 3 days only, liver allograft survival times were prolonged markedly, and 54% of rats with grafts survived for at least 60 days. This effect was almost equal to that after 7 days systemic treatment with FK506. In conclusion, 7 days' treatment with FK506 administered systemically was an effective regimen for the suppression of liver allograft rejection in rats. Furthermore, local immunosuppression with low-dose, short-term (3 days) FK506 treatment administered via the hepatic artery or portal vein of the transplanted liver dramatically improved allograft salvage.

Key words Liver transplantation
Allograft · FK506 · Local immunosuppression · Rat

Introduction

Local immunosuppression by administering an immunosuppressive agent directly into the afferent artery of the transplanted organ is considered to be an ideal anti-rejection therapy that produces a high concentration of immunosuppressive drug in the transplanted organ and a low concentration in the peripheral blood. This enables the total dose required to inhibit rejection to be reduced and thus results in fewer systemic side-effects. A new immunosuppressive agent, FK506, has been shown to inhibit liver allograft and xenograft rejection in animals [4, 12, 13, 17, 18] and man [2, 9, 16, 19]. The aim of this study was to determine whether the administration of low-dose FK506 directly to the transplanted liver via the hepatic artery or portal vein is as effective as systemic administration for the suppression of liver allograft rejection in rats.

Materials and methods

Animals

Male inbred PVG ($RT1^c$) and LEW ($RT1^l$) rats weighing 250–300 g were obtained from Seiwa Experimental Laboratory and Charles River Japan and used as donors and recipients, respectively.

Drug

FK 506 was obtained from Fujisawa Pharmaceutical Company, Osaka, Japan. All the drug concentrations used were made by dissolving in sterile normal saline, and administration was via the hepatic artery, portal vein or penile vein.

Operative techniques

Orthotopic liver transplantations were performed using cuff techniques for the portal vein, infrahepatic vena cava and biliary anastomoses and hand suturing for the suprahepatic vena cava, as described by Kamada and Calne [6]. For the local immunosuppression experiment, the hepatic artery or portal vein of the transplanted liver was cannulated via the aorta (Fig. 1 A) or mesenteric vein (Fig. 1 B), respectively, with a silicone tube (602–135 or 602–105, silastic tubing, Dow Corning, Midland Mich.), which was connected to a subcutaneously placed reservoir for intra-arterial and intraportal infusion. Arterial flow to the liver was not re-established. The portal cross-clamping times ranged from 18 to 20 min. Rats that died within 3days of the operation were excluded from this study. After liver transplantation, survival times were recorded and compared among the groups. The rats that were sick or died within 3 days after transplantation were autopsied to determine the cause of death, and all liver specimens were subjected to histological studies.

Experimental design

In order to determine the effect of treatment duration on liver allograft survival, various doses of FK506 (0.08, 0.16, 0.32 and 1.28 mg/kg) were given once daily to the LEW recipient rats via the

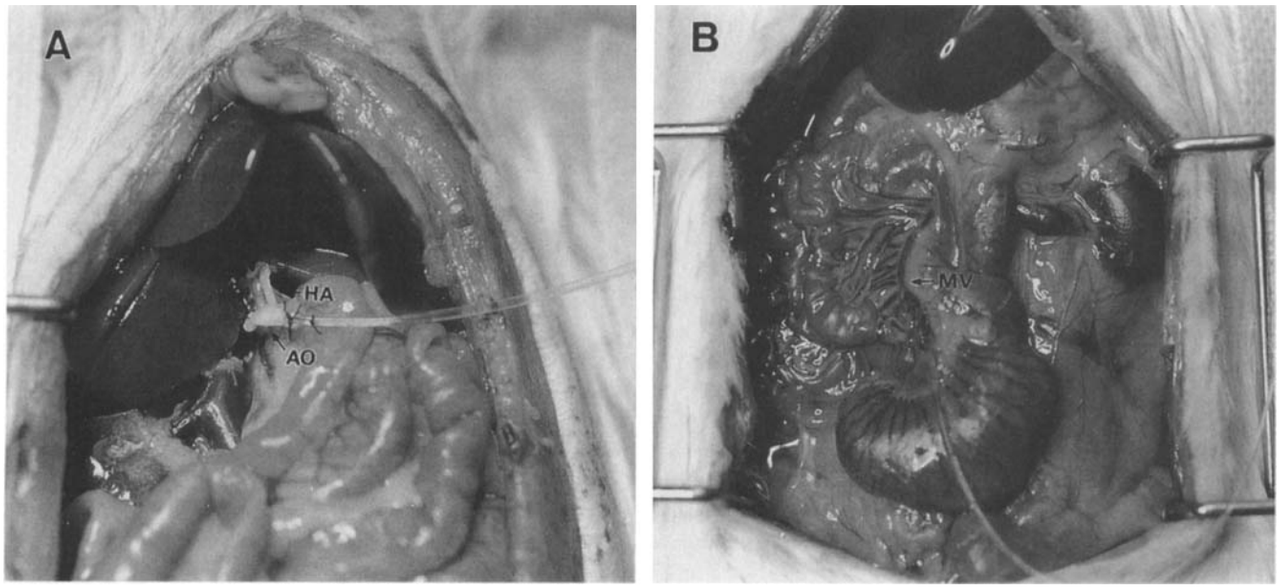


Fig. 1 A, B Orthotopic liver transplantation and drug delivery via the hepatic artery of the grafted liver (**A**) and via the portal vein into the mesenteric vein of the recipient rat (**B**). A silicone tube was introduced into the aorta bearing the hepatic artery of the transplanted liver and into the mesenteric vein of the recipient rat and ligated with silk suture. The other end of the tube was connected to a reservoir containing drug solution, which was placed in a subcutaneous pocket. (*HA* hepatic artery, *AO* aorta, *MV* mesenteric vein)

penile vein for 3 or 7 days after the transplant operation. In order to determine the effects of different administration routes, the recipients were divided into four groups: I, no immunosuppressive therapy was given (control group, $n = 8$); II, FK 506 (0.32 mg/kg daily) was administered via the penile vein (I.V.) once daily for 3 days (systemic administration group, $n = 7$); III, FK 506 (0.32 mg/kg daily) was given via the hepatic artery (I. H. A) once daily for 3 days (arterial administration group, $n = 7$); and IV, FK 506 (0.32 mg/kg daily) was administered via the portal vein (I. P. V.) once daily for 3 days (portal administration group, $n = 6$).

Graft function and body weight

The rats were bled on the 4th, 7th and 14th postoperative days, weekly thereafter for 1 month and then every 3 weeks for a further 2 months, for the measurement of liver function including serum glutamic-oxaloacetic transaminase (SGOT) and total bilirubin. The rats were weighed on the 4th, 7th and 14th postoperative days and weekly thereafter for 4 months, and the mean values for the groups were compared.

Histology

Liver biopsy specimens from each group were obtained 4 days after tansplantation and from the long-term (250 days) surviving rats treated with FK 506 administered via the hepatic artery. All the specimens were subjected to histological examination.

Statistical analysis

Statistical analysis was performed using the Wilcoxon rank test, and differences were considered to be statistically significant at $P < 0.05$ using a two-tailed test.

Results

Survival

All the LEW rats which had received PVG livers without immunosuppressive therapy died between 9 and 21 days after transplantation due to acute graft rejection (control group 1: 14.1 ± 4.1 days, $n = 8$). Therefore, this donor-recipient combination is a rejection combination. Administration of 0.08 mg FK 506/kg via the penile vein for 3 days (group 2) had no effect on the mean survival time (14.3 ± 1.5 days, $P > 0.05$). Administration of 0.32 (group 3) and 1.28 (group 4) mg FK 506/kg to the recipient rats via the penile vein for 3 days only produced marginal effects on prolonging liver graft survival (21.1 ± 12.5 and 32.0 ± 24.0, respectively; Table 1). However, there were no statistically significant differences between the mean graft survival rates of groups 1, 2 and 3, but there was between groups 1, 2 and 4 ($P < 0.05$).

Administration of FK 506 (0.08–1.28 mg/kg) via the penile vein for 7 days suppressed liver allograft rejection markedly, and 6 of the 12 recipients in each group survived for at least 60 daxs after transplantation (Table 2). Thus, systemic administration of FK 506 for 7 days was more beneficial for prolonging liver graft survival than 3 days of treatment.

The mean survival times of the groups given FK 506 via the penile vein, hepatic artery and portal vein were compared. Administration of 0.32 mg FK 506/kg via the penile vein for 3 days (group II) had no effect on the mean survival time (21.1 ± 12.5 days). However, when FK 506 (0.32 mg/kg) was administered via the hepatic artery (group III), survival was prolonged markedly (72.9 ± 42.9 days) compared with the controls (group I: 14.1 ± 4.1 days), and two of the seven recipients survived for more than 120 days. Intraportal administration of the same dose of FK 506 (group IV) also extended the mean survival to 67.0 ± 46.6 fays, and one of the six animals survived for more than 120 days (Fig. 2). The mean survival of groups III and IV differed significantly from that of groups I and II ($P < 0.01$).

Table 1 Survival times of liver allografts after various doses of FK 506 administered intravenously via the penile vein for 3 days (*MST* mean survival time ± SD)

Group	*n*	Treatment	Survival times (days)	MST ± SD (days)
1	8	None	9, 9, 13, 14, 17, 17, 21	14.1 ± 4.1
2	3	FK 506, 0.08 mg/kg	13, 14, 16	14.3 ± 1.5[a]
3	7	FK 506, 0.32 mg/kg	10, 13, 15, 15, 21, 28, 46	21.1 ± 12.5[a]
4	4	FK 506, 1.28 mg/kg	12, 14, 39, 63	32.0 ± 24.0[a]

[a] Wilcoxon rank test for difference between group 2 and 1 (NS), group 3 and 1, 2 (NS), group 4 and 1, 2 ($P < 0.05$)

Table 2 Survival times of liver allografts after various doses of FK 506 administered intravenously via the penile vein for 7 days

Group	*n*	Treatment	Survival times (days)	MST ± SD (days)
1	8	None	9, 9, 13, 13, 14, 17, 17, 21	14,1 ± 4,1
5	3	FK 506, 0.08 mg/kg	40, 58, > 120	72.7 ± 42.0[a]
6	3	FK 506, 0.16 mg/kg	38, 40, 49	42.3 ± 5.9[a]
7	3	FK 506, 0.32 mg/kg	74, > 120, > 120	80.5 ± 54.4[a]
8	3	FK 506, 1.28 mg/kg	48, 63, > 120	77.0 ± 38.0[a]

[a] Wilcoxon rank test for difference group 1, and 5, 6, 7, 8 ($P < 0.05$)

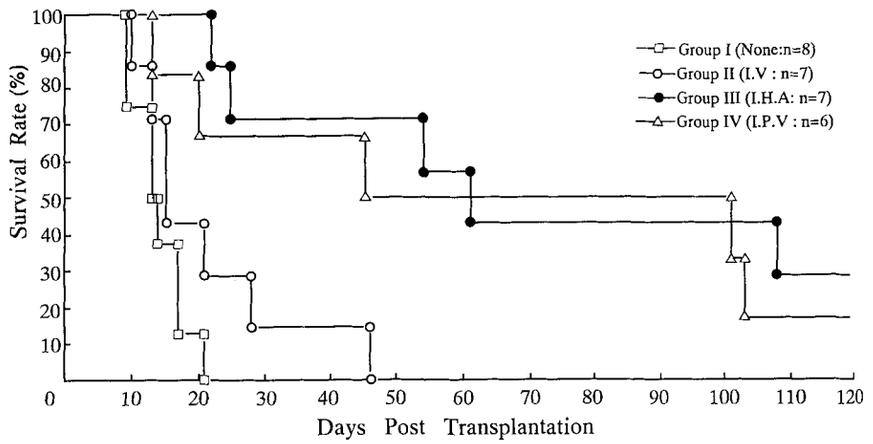
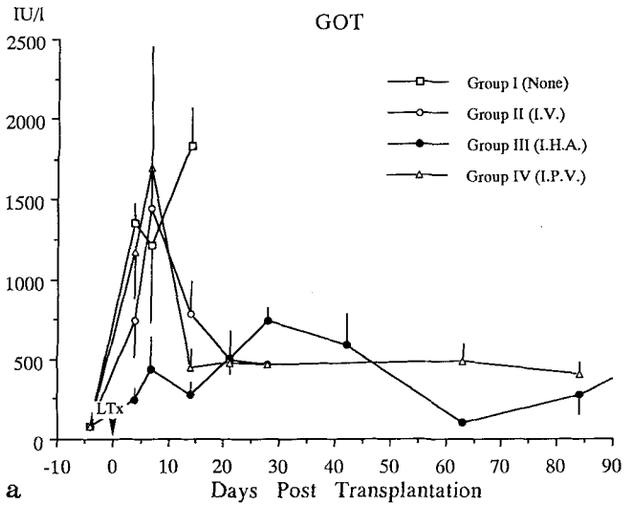


Fig. 2. Kaplan-Meier survival curves of liver allografts after administration of FK 506 by various routes. Wilcoxon rank test for difference between groups III and I, II ($P < 0.01$), groups IV and I, II ($P < 0.01$), groups II and I (NS)



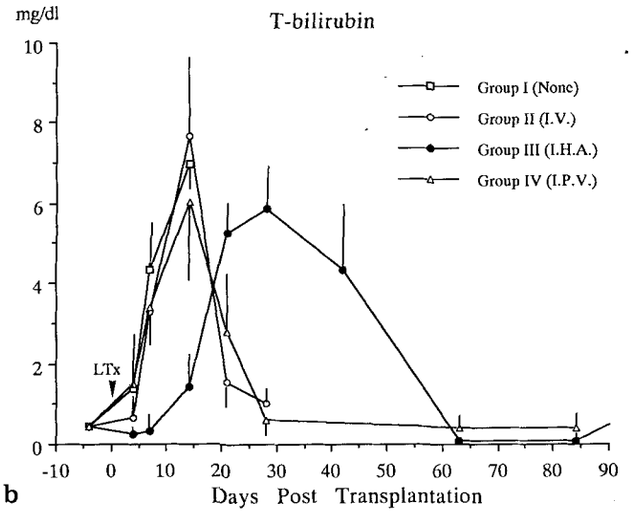


Fig. 3 Postoperative serum glutamic-oxaloacetic transaminase (*GOT*) and total (T-)bilibrubin concentrations in LEW rats with PVG liver grafts after administration of FK 506 by various routes

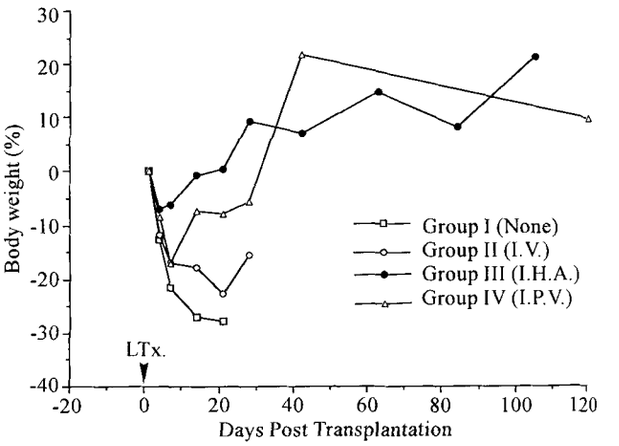


Fig. 4 Postoperative body weight with time curves after liver transplantation in rats given FK 506 by various routes

Liver function and body weight changes

The results of the serial SGOT and total bilirubin measurements are shown in Fig. 3. The mean SGOT values of groups I (control) and II (systemic route) rapidly increased until death, while that of group IV (I.P.V. route) reached a peak 1 week after transplantation and declined by 2 weeks post-grafting. Serum enzyme release in group III (I.H.A. route) was suppressed throughout the observation period. The mean total bilirubin in groups III and IV peaked at 1 months and 2 weeks post-grafting, respectively, then gradually declined by 2 months post-grafting.

The mean postoperative body weight with time curves are shown in Fig. 4. All rats lost 10% – 30% of their body weight shortly after the operation. Those in groups I and II lost weight progressively, and all died by 46 days after

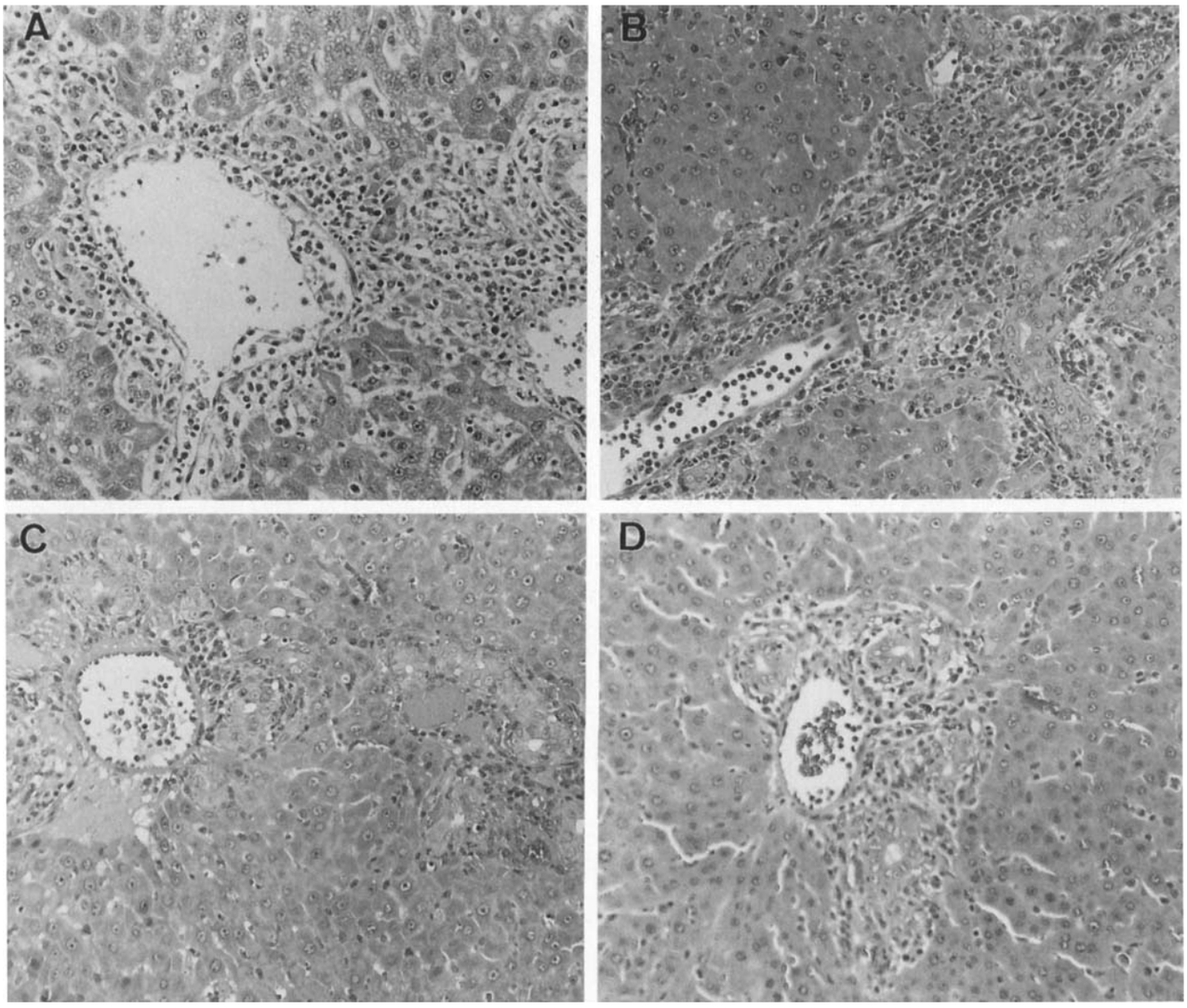


Fig. 5 A–D Histological findings of the grafted livers in rats given FK 506 by various routes on the 4th day after transplantation. Those with no immunosuppressive therapy (**A**) and intravenous administration (**B**) showed a marked portal infiltrate composed predominantly of lymphocytes, endothelialitis with bile duct damage including pleomorphism, anisocytosis and overlapping of nuclei, corresponding to moderate to marked acute rejection. Administration via the hepatic artery (**C**) resulted in minimal portal infiltrate without conspicuous damage to the bile ducts. Allografted liver received intraportal FK 506 (**D**) revealed less marked portal infiltrate and endotheliitis (HE, × 150 times)

transplantation, whereas the rats in groups III and IV gained weight after 10 days and grew normally thereafter.

Histological findings

Histopathological findings of the livers from the recipient rats treated with FK 506 by various routes are shown in Fig. 5. The allografted livers in groups I (Fig. 5 A) and II (Fig. 5 B) showed marked portal inflammation with many polymorphonuclear leucocytes, which had infiltrated into the liver parenchyma. Endotheliitis was conspicuous in both the portal vein and central vein. The bile duct demonstrated degenerative or reactive changes. The findings as a whole were diagnosed as acute rejection, moderate to marked grade. However, the degree of portal

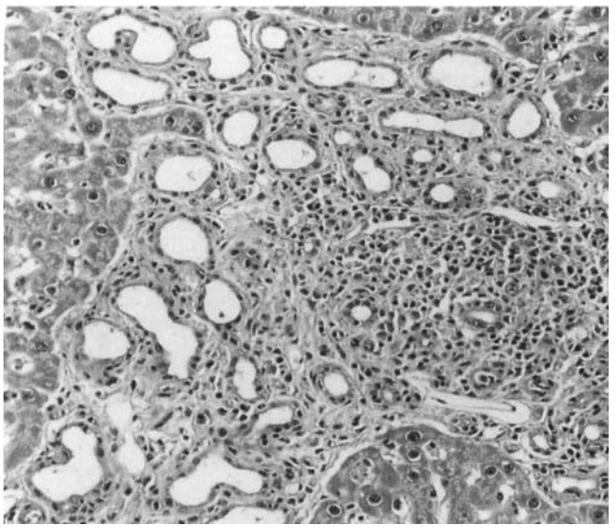


Fig. 6 Histological findings of grafted liver among the long-term (250 days) surviving rats treated with FK506 administered via the heapatic artery. Portal area shows little lymphocytic infiltrate. Loss of bile duct and obliterative endarteritis senot observed. In addition, small bile ducts proliferate in some portal tracts. These findings are not consistent with chronic rejection (HE, ×150 times)

infiltration, endotheliitis and bile duct damage was less in the allografted livers in groups III (Fig. 5C) and IV (Fig. 5D), which were diagnosed histologically as showing mild acute rejection.

There was mild portal inflammatory infiltration but no bile duct loss or endarteritis in the livers of long-term (250 days) surviving rats in group III, suggesting no evidence of chronic rejection (Fig. 6).

Discussion

Recently, we showed that intermittent intrahepatic arterial or intraportal infusion of the immunosuppressive drug 15-deoxyspergualin (DSG) to the transplanted liver was an effective anti-rejection regimen compared with systemic delivery of DSG and was effective with alower total dose and smaller incidence of subsequent side-effects [20]. In this study, we obtained similar results using FK506 as a local immunosuppressant.

FK506 is a powerful immunosuppressive agent which inhibits lymphokinetic factor synthesis by a mechanism apparently similar to that of cyclosporin A (CsA); it was discovered and isolated from the actinomycete *Streptomyces tsukubiensis* in Japan [3, 7] and first reported in the literuature in 1987 [8]. Since 1989, it has been shown to be an effective immunosuppressive drug for the prevention and treatment of liver allograft and xenograft rejection in animals [4, 12, 13, 17, 18] and man [2, 9, 19]. However, due to attempts to maximize its immunosuppressive effects by using high doses, FK506 has displayed a rage of side-effects (nephrotoxicity, hyperglycaemia, neurotoxicity, infectious and malignant complications) and has a narrower therapeutic windwo than CsA. In man, the primary side-effects are insomnia, tremours, headaches, tingling sensations, muscle ache, itching, fatigue, visual sensitivity to light and gastrointenstinal symptoms. However, these toxic effects usually disappear or their severity is reduced when low doses are used [1, 5]. Therefore, the concept of "local immunosuppression" enables higher tissue concentrations of the required immunosuppressive drug in the target organ to be achieved with a reduced total dose and thus minimizes drug-related toxicity while retaining its beneficial anti-rejection effect with reduced systemic levels.

A particularly interesting finding of this study is that the administration route used to deliver the immunosuppressive drug (arterial > portal > intravenous route) had beneficial effects on survival and postoperative liver function in that order. The reason for the different effects of the various administration modes is not clear but may presumably be attributable to the mechanisms involved in liver rejection. The bile duct epithelial cells are the principal target of immune attack during the process of liver rejection [10]. In view of the fact that the bile duct system has an arterial blood supply [13, 14, 15], it seems reasonable that direct delivery of immunosuppressive drugs into the allograft hepatic artery is a particularly effective regimen for the suppression of bile duct damage by immune attack due to liver rejection.

In conclusion, in this study treatment with FK506 in doses of 0.08–1.28 mg/kg daily administered systemically via the penile vein for 7 days suppressed liver allograft rejection. Moreover, short-term (3 days) local immunosuppression using a low dose (0.32 mg/kg) administered via the intrahepatic arterial and intraportal venous routes dramatically improved liver allograft survial. The results of this study suggest that this local immunosuppression technique may be useful in clinical practice.

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