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FK 506—An Effective Immunosuppressant in Achieving Long-Term Functional Islet Allograft Survival in Diabetic Rats

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TRANSPLANTED pancreatic islet tissue has been shown to restore normoglycemia and prevent the development of chronic complications in diabetic animals.[1] The application of allo- and xenotransplantation of pancreatic islets for the treatment of diabetes is hindered by immune rejection. FK 506, a new immunosuppressive agent, has been demonstrated to be effective in the prolongation of survival of heart, intestine, liver, and islet allografts in rats, and liver, kidney, and pancreas allografts in humans.[2-4] The efficacy of FK 506 in the prolongation of islet allograft survival has been found to be affected by the dosage of FK 506 and the site of the islet graft.[3] The present study is aimed at achieving long-term functional rat islet allograft across major histocompatibility barrier using FK 506 as an immunosuppressant.

MATERIALS AND METHODS

Male rats of outbred Wistar (Wi) and inbred Lewis (Le) strains, with body weights of 350 to 500 g, were used as donors of pancreatic tissue, and rats of inbred ACI (RT1^a) strain were used as streptozotocin-induced (55 mg/kg IV) diabetic recipients (Harlan Sprague Dawley, Indianapolis, Ind). An animal is defined as diabetic only when serum glucose is greater than 400 mg/dL over 10 days. Pancreatic tissue was digested with collagenase, and the islets were handpicked under a dissection microscope. Contaminating acinar tissues and blood vessels were removed from the islets by the single-layer Hypaque-Ficoll separation technique.[5] For kidney subcapsular (KC) transplantation approximately 2,000 freshly isolated islets, suspended in a total volume of 70 μL Hanks' balanced salt solution (HBSS), were injected. For intraportal (IPo) transplantation, the islets were suspended in 200 μL HBSS in a Monoject U-100 insulin syringe and injected over a 1-minute period into diabetic recipients. The syringe was flushed twice with the recipients' blood.

Injectable form of FK 506 (Lot 116393) was provided by Fujisawa Pharmaceutical Co (Osaka, Japan). The required amount of the compound was weighed out daily and prepared in saline within 10 minutes of IM injection. The administration of FK 506 was initiated on the day of transplantation. Protocol I consisted of FK 506 at 1 mg/kg/d IM for 2 weeks. Protocol II consisted of FK 506 at 1 mg/kg/d IM for 2 weeks plus 1 mg/kg/wk. Daily serum glucose and body weight of recipient rats were determined for 2 weeks after transplantation and then twice weekly thereafter. Rejection was considered to have occurred when the serum glucose level exceeded 200 mg/dL on 3 successive days. Survival time for each recipient group is reported as mean ± SEM.

Statistical evaluation was performed by Student's t test. IV glucose tolerance test (IVGTT, 1 g/kg IV through femoral vein in overnight fasted animals) was performed in some islet recipients 1 week after the last dose of FK 506. Blood samples were collected from the tail at 0, 1, 3, 5, 15, 30, 60, 90, and 120 minutes for glucose determination. Some functional grafts were removed for histologic and histochemical studies. Paraffin sections were stained for insulin and glucagon with immunoperoxidase staining (ABC Staining Kits, Vectastain, Cedarlane Laboratory, Hornby, Ontario) and frozen sections for cellular markers with monoclonal antibodies (clones OX1, OX6, OX8, and W 3/25; Daymar Laboratory, Toronto, Ontario).

RESULTS

Table 1 shows that fresh Wi islet allograft had a mean functional period of 7.4 ± 0.25 and 6.7 ± 0.5 days, respectively, when transplanted under the KC and IPo in diabetic ACI recipients. Diabetic ACI recipients of fresh Wi islets under the KC which received FK 506 1 mg/kg/d for 14 days had a much prolonged survival time. Seven of ten were rejected between 41 to 73 days following transplantation, while the remaining three achieved survival of over 120 days. In diabetic rats transplanted with islets IPo and treated similarly with FK 506, seven of seven grafts

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Table 1. Functional Period of WI Rat Islet Allograft Under the KC and IPo in Diabetic ACI Rats

Group	FK 506	Site	Survival (d)	Mean ± SEM
1	None	KC	7, 7, 7, 8, 8	7.4 ± 0.25
2	None	IPo	5, 6, 6, 7, 8, 8	6.7 ± 0.50
3	1 mg/kg/d IM, day 0-13	KC	41, 45, 46, 47, 48, 52, 73, >120,* >122,† >124†	>72.8 ± 11.1
4	1 mg/kg/d IM, day 0-13; weekly thereafter	KC	>112,† >114,† >142, >145, >151, >152, >155, >160	>141.4 ± 6.6
5	1 mg/kg/d IM, day 0-13	IPo	>119, >120, >137, >150, >161, >163, >250	>157.1 ± 16.9

*Died of anesthesia; normal-appearing islets were present at the graft site.
†Kidney containing the islet allograft was removed.

Table 2. Functional Period of Mixed WI and Le Strain Rat Islet Allograft Under the KC of Diabetic ACI Rats

Group	FK 506	Survival (d)	Mean ± SEM
1	None	4, 5, 5, 5, 7	5.2 ± 0.5
2	1 mg/kg/d IM, day 0–13; weekly thereafter	67, 85, 90,* 103, 132	>95.4 ± 10.8

*Kidney containing the islet allograft was removed and normal-appearing islets were present at the graft site.

had prolonged survival. In the group given islet transplant under KC but treated with FK 506 at 1 mg/kg/d for 14 days followed by subsequent weekly injections, eight of eight recipients had prolonged functional islet allograft. Surgical removal of the kidney containing the allogeneic islet tissue resulted in the return to hyperglycemia in four of four recipients within 2 days. The body weight of the recipients remained constant during the FK 506 treatment period but increased when it was stopped or when changed to weekly injection. Following the surgical removal of the Wi islet graft, the body weight of the ACI recipients dropped rapidly.

To assess the effect of FK 506 immunosuppression on the survival of islet allograft composed of tissue from two donor strains, diabetic ACI rats were transplanted under the KC with a mixture of Wi and Le strain islets. Table 2 shows that the mixed strain islet allograft functioned for 5.2 ± 0.5 days (n = 5) in nonimmunosuppressed rats. FK 506 treatment significantly prolonged the allograft function to >95.4 ± 10.8 days (n = 5).

Figure 1 shows that the results of IVGTT performed in both groups of ACI recipients with functional Wi islets under KC, 1 week after the last injection of FK 506, were much improved over the diabetic controls.

Insulin-containing islets were abundant under the KC of all the islet allograft recipients treated with FK 506. CD4 positive and CD8 positive cells were present in the vicinity of the normal-appearing islets. However, they were not detected within the islets.

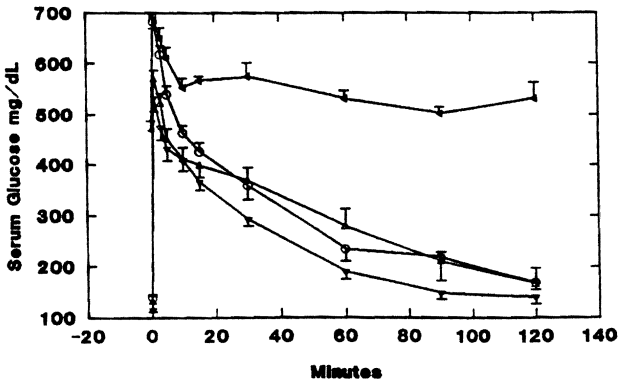

Fig 1. Serum glucose following IVGTT (1 g/kg body weight) in overnight fasted normal rats (▽--▽, n = 11), diabetic rats (◁--◁, n = 6), diabetic ACI recipient rats with functional islet allograft under KC treated with FK 506 (1 mg/kg/d IM, 2 weeks, ○--○, n=6; 1 mg/kg/d IM, 2 weeks plus 1 mg/kg wk, △--△, n = 7) 1 week after the last FK 506 injection.

DISCUSSION

Results of the present study show that FK 506 is an effective immunosuppressant in prolonging fresh islet allograft survival. At a daily dosage of 1 mg/kg administered IM for 14 days, starting on the day of transplantation, significant prolongation of fresh Wi islet allograft survival was observed both under the KC and IPo. The efficacy of FK 506 is improved by additional weekly injection in the group with islets transplanted under KC. Furthermore, this treatment regimen was effective in prolonging islet allograft survival composed of tissues from two donor strains. This observation is important as islet tissue from more than one donor is needed to reverse the diabetic state of the recipients in clinical transplantation.[6] Though FK 506 prolonged islet allograft from single and two donor strains transplanted under KC, the result achieved in the former group was significantly better. This may partly be explained by the higher immunogenicity of the islet preparation used in the latter group.

The present observation differed from that of Yasunami et al[3] who failed to achieve prolonged islet allograft under KC. This difference could be due to the use of a higher dosage of FK 506 for a longer duration by us. Similar to their observation, we also observed the superiority of the IPo over the KC site in the islet transplantation model we have used. The suggestion that FK 506 is metabolized extensively in the liver before excretion may contribute to the superiority of the IPo site.[7]

The islet allograft recipient animals had achieved normalization of random blood glucose that was similar to that of normal controls. In addition, the glucose clearance rates were significantly improved. The mildly abnormal glucose tolerance found in these diabetic recipient animals could be attributed to one or more factors, including the nonphysiologic implant site, renal subcapsular space, and inadequate islet mass being used for transplantation. Since the IVGTT was performed 1 week following the completion of a 2-week course of FK 506 administration, the abnormality might also be partly due to the effect of FK 506. Nalesnik et al[8] earlier also observed a trend for FK 506-treated rats to demonstrate higher levels of blood glucose than controls.

Histologically, the islet graft from the recipients treated for a 14-day course of FK 506, plus weekly injection for over 100 days appeared normal and stained strongly positive for insulin. This would be in keeping with our finding of the effect of FK 506 on islets in vitro.[9]

In conclusion, FK 506 is a potent, effective immunosuppressant in islet allograft transplantation in rats and has potential use for clinical islet transplantation in the treatment of human diabetics.

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