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# Long-Term Survival of Islet Allografts in Diabetic Rats Treated With FK 506

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SUCCESSFUL islet transplantation (TR) has been shown to restore normoglycemia and prevent the development of chronic complications in diabetic animals.[1,2] Immune rejection is currently the major hindrance in the application of allo- and xeno-TR of pancreatic islets for the treatment of diabetes. We have shown earlier that FK 506 is an effective antirejection agent for fresh islet allografts across major histocompatibility barrier.[3] The efficacy of FK 506 in the prolongation of islet allograft survival has been found to be influenced by the dosage of the FK 506 and the site of the islet graft.[3,4] In anticipation of the use of FK 506 as an immunosuppressive agent in human islet TR, its efficacy in various conditions similar to clinical TR needs to be examined. The present study was undertaken to determine whether FK 506 is effective in the prolongation of fresh islet allograft in recipients of islet grafts from one or two donor strains in two commonly used TR sites and also in previously sensitized diabetic recipient rats.

## MATERIALS AND METHODS

### Animals

Male rats of outbred Wistar (Wi) and inbred Lewis (Le) strains (*RT-1*$^l$), with body weights of 350 to 500 g were used as donors of pancreatic tissue, and rats of inbred ACI (*RT-1*$^a$) strain were used as streptozotocin (STZ)-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.

### Islet Isolation and Transplantation

Pancreatic tissue was digested with collagenase, and the islets were hand-picked under a dissection microscope. Contaminating acinar tissues and blood vessels were removed from the islets by the single-layer Hypaque-Ficoll (H-F) separation technique.[5] For kidney subcapsular (KC) TR, approximately 2000 freshly isolated islets, suspended in a total volume of 70 μL Hanks' balanced salt solution (HBSS), were injected. For intraportal (IPo) TR, 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.

### FK 506 Treatment Protocol

An 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 TR. Protocol I consisted of FK 506 at 1 mg/kg per day IM for 2 weeks. Protocol II consisted of FK 506 IM administration at 1 mg/kg per day for 2 weeks followed by 1 mg/kg per week.

### Assessment of Graft Function

Daily serum glucose and body weight of recipient rats were determined for 2 weeks after TR, and then twice weekly thereafter. Rejection was considered to have occurred when the serum glucose level exceeded 200 mg/dL for 3 successive days. Survival time for each recipient group is reported as mean ± SEM. Statistical evaluation was performed using the Mann-Whitney *U* test. $P < .05$ was considered statistically significant.

### Assessment of the Efficacy of FK 506 in Sensitized Recipients

After the rejection of the first islet allograft in a diabetic rat, a second islet graft from the same donor strain was performed in the contralateral kidney of the recipient. The second TR was performed either a short or a long period after the first TR. The animals were treated with FK 506 protocol II, and their serum glucose monitored.

## RESULTS

Table 1 shows the functional period of Wi and admixed Wi and Le islet allograft survival in two TR sites in diabetic ACI rats treated with two different FK 506 protocols. FK 506 treatment at 1 mg/kg per day for 2 weeks significantly prolonged the survival of islet allograft transplanted both under KC and IPo. The survival was significantly longer in the IPo site than the KC site. (group 6 vs 4: 9 of 9 vs 3 of 10 functioned for over 4 months). In the KC site, the islet survival in recipients treated with FK 506 for 2 weeks was >71.8 ± 11.3 days (group 4), and this increased significantly to >171.4 ± 12.9 days (group 5) when an additional IM weekly injection of FK 506 was given. In the groups transplanted with a mixture of Wi and Le islets, FK 506 treatment significantly improved the islet allograft functional period over the untreated recipients (group 7 vs 3). Also, the group transplanted with the mixed islet allograft IPo had a longer functional period than the group transplanted under KC (group 8 vs 7). The efficacy of FK 506 was less for mixed islet allograft than islets from a single

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0041-1345/91/$3.00/+0

**Table 1. Functional Period of Wi and Admixture of Wi and Le Islet Allografts in Diabetic ACI Rats Immunosuppressed with FK 506**

| Group | Donor | Site | FK Rx* | *n* | Days | Mean ± SEM | *P* Value |
|---|---|---|---|---|---|---|---|
| 1 | Wi | KC | No | 5 | 7, 7, 7, 8, 8 | 7.4 ± 0.25 | |
| 2 | Wi | IPo | No | 6 | 5, 6, 6, 7, 8, 8 | 6.7 ± 0.50 | |
| 3 | Wi + Le | KC | No | 5 | 4, 5, 5, 5, 7 | 5.2 ± 0.50 | |
| 4 | Wi | KC | I | 10 | 41, 45, 46, 47, 48, 52, 73, >120‡, >122†, >124† | >71.8 ± 11.3 | .003 |
| 5 | Wi | KC | II | 8 | >112†, >114†, >182, >185, >191, >192, >195, >200 | >171.4 ± 12.9 | .002 |
| 6 | Wi | IP0 | I | 9 | 4×>122, >167, >190, >191, >193, >290 | >168.6 ± 18.6 | .008 |
| 7 | Wi + Le | KC | II | 7 | 67, 85, 90†, >98, 103, >115, 132 | >98.6 ± 8.0 | .055 |
| 8 | Wi + Le | IPo | II | 7 | 98‡, 102, 110, 120, >172, >182, >183 | >138.2 ± 14.7 | |

*Protocol I: FK 1 mg/kg per day for 2 weeks IM; protocol II: FK 1 mg/kg per day for 2 weeks + weekly IM.
†Nephrectomy.
‡Died of anesthesia.

donor strain when TR was under KC but not IPo (group 7 vs 5; 8 vs 6). Nephrectomy of the graft-containing kidney resulted in the return to hyperglycemia in 5 of 5 cases in ACI rats with long-term Wi ($n = 4$) or Wi + Le ($n = 1$) islet allograft.

Table 2 shows the efficacy of IM FK 506 at 1 mg/kg per day for 2 weeks plus weekly treatment on islet allograft survival in diabetic ACI recipients which had previously rejected a Wi islet graft in the first TR performed 50 to 177 days previously. The immunosuppression was effective in achieving long-term survival in 7 of 7 recipients with 3 of 7 recipients achieving indefinite graft survival. This was not significantly different from that achieved in nonsensitized animals (Table 2 group 3 vs Table 1 group 5, $P = .10$). In contrast, when the interval between the first and second TR was of shorter duration, ie, 2 weeks, FK 506 immunosuppression was found to be ineffective. Nevertheless hyperacute rejection was not observed.

## DISCUSSION

The present results show that FK 506 is an effective immunosuppressant in prolonging fresh islet allograft survival in rats across the MHC barrier. Significant prolongation of allograft survival was observed both under KC and IPo with 14 injections of 1 mg/kg FK 506. The efficacy of FK 506 treatment was improved by additional weekly injection in the group with islets transplanted under KC. This intermittent treatment regimen was effective in prolonging islet allograft survival composed of tissues from two donor strains. The observation is important, since islet tissue from more than one donor is needed to reverse the diabetic state of the recipients in clinical TR.[6,7] Though FK 506 prolonged islet allograft from a single and two donor strains transplanted under KC, the result achieved in the former group was significantly better. One possible explanation is the higher immunogenicity of the mixed-islet preparation from two donor strains.

Earlier, Yasunami et al[4] failed to achieve prolonged islet allograft under KC. The discrepancy could be due to the high dosage of FK 506 for a longer duration used by us. The route of TR seems to be important as we observed the superiority of the IPo over the KC site in the islet TR model. This finding confirms previous observations.[4] The suggestion that FK 506 is metabolized extensively in the liver before excretion may contribute to the superiority of the IPo site.[8]

Despite voluminous literature on the effect of various immunosuppressive agents and islet pretreatment on the successful outcome of islet allo-TR in nonsensitized rodents, relatively little information is available for sensitized animals. The present data demonstrated that FK 506 was very effective in prolonging the second islet allograft survival in sensitized rats provided the interval between

**Table 2. Effect of FK 506 Treatment on Functional Islet Allograft Survival Under Kidney Capsule in Presensitized ACI Rats***

| Group | Donor | Days 1st to 2nd TR | FK Rx† | *n* | Days | Mean ± SEM |
|---|---|---|---|---|---|---|
| 1 | Wi | No | No | 4 | 5, 6, 7, 7 | 6.25 ± 0.48 |
| 2 | Wi | 104.0 ± 3.2 | No | 4 | 7, 7, 7, 8 | 7.25 ± 0.25 |
| 3 | Wi | 115.6 ± 21.6 | II | 7 | 59, 78, 99, 113, >141, >198, >210 | >128.3 ± 21.9 |
| 4 | Wi | 14.2 ± 0.2 | II | 5 | 5, 5, 10, 11, 34 | 13.0 ± 5.4 |

*Sensitization was achieved by fresh Wi islet allograft under kidney capsule.
†Protocol II: FK 1 mg/kg per day for 2 weeks + weekly IM.

the initial and second islet transplants was sufficient for the antidonor immune activities to subside. Though FK 506 treatment failed to prolong the rat islet allograft in recently sensitized recipients, in no instance was hyperacute rejection observed in the animals, with or without FK 506 immunosuppression.

In conclusion, FK 506 treatment is effective in prolonging islet allograft survival in recipients of islets from a single- or dual-donor strain. In sensitized recipients, the long interval between the initial and second transplant is crucial to the survival of the second transplant using FK 506 as immunosuppressive agent. Further modifications of the treatment protocol are needed to improve the efficacy of the drug in highly sensitized recipients.

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