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


SUCCESSFUL islet transplantation (TR) has been shown to restore normoglycemia and prevent the development of chronic complications in diabetic animals. 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. The efficacy of FK 506 in the prolongation of islet allograft survival has been found to be influenced by the dosage of the compound. It is known that the FK 506 concentration in the blood, which is determined by the dosage administered, is critical to 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^b), 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. 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 < 0.05 was considered statistically significant.

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 donor.

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survival of islet allografts in rats

Group 1
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.

Table 1 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, i.e., 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.

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, i.e., 2 weeks, FK 506 immunosuppression was found to be ineffective. Nevertheless hyperacute rejection was not observed.

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Donor</th>
<th>Site</th>
<th>FK Rx*</th>
<th>n</th>
<th>Days</th>
<th>Mean ± SEM</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Wi</td>
<td>KC</td>
<td>No</td>
<td>5</td>
<td>7, 7, 8, 8</td>
<td>7.4 ± 0.25</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Wi</td>
<td>IPo</td>
<td>No</td>
<td>6</td>
<td>5, 6, 7, 8, 8</td>
<td>6.7 ± 0.50</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Wi + Le</td>
<td>KC</td>
<td>No</td>
<td>5</td>
<td>4, 5, 5, 7</td>
<td>5.2 ± 0.50</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Wi</td>
<td>KC</td>
<td>I</td>
<td>10</td>
<td>41, 45, 46, 47, 52, 73, &gt;120, &gt;122, &gt;124</td>
<td>&gt;71.8 ± 11.3</td>
<td>.003</td>
</tr>
<tr>
<td>5</td>
<td>Wi</td>
<td>KC</td>
<td>II</td>
<td>8</td>
<td>&gt;112, &gt;114, &gt;182, &gt;185, &gt;191, &gt;192, &gt;195, &gt;200</td>
<td>&gt;171.4 ± 12.9</td>
<td>.002</td>
</tr>
<tr>
<td>6</td>
<td>Wi</td>
<td>IPo</td>
<td>II</td>
<td>9</td>
<td>4 x &gt;122, &gt;167, &gt;190, &gt;191, &gt;193, &gt;290</td>
<td>&gt;166.6 ± 18.6</td>
<td>.008</td>
</tr>
<tr>
<td>7</td>
<td>Wi + Le</td>
<td>KC</td>
<td>II</td>
<td>7</td>
<td>67, 85, 90, &gt;98, 103, &gt;115, 132</td>
<td>&gt;98.6 ± 8.0</td>
<td>.055</td>
</tr>
<tr>
<td>8</td>
<td>Wi + Le</td>
<td>IPo</td>
<td>II</td>
<td>7</td>
<td>96, 102, 110, 120, &gt;172, &gt;182, &gt;183</td>
<td>&gt;138.2 ± 14.7</td>
<td></td>
</tr>
</tbody>
</table>

*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.

![Table 2](image)

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Donor</th>
<th>Days 1st to 2nd TR</th>
<th>FK Rx†</th>
<th>n</th>
<th>Days</th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Wi</td>
<td>No</td>
<td>No</td>
<td>4</td>
<td>5, 6, 7, 7</td>
<td>6.25 ± 0.48</td>
</tr>
<tr>
<td>2</td>
<td>Wi</td>
<td>104.0 ± 3.2</td>
<td>No</td>
<td>4</td>
<td>7, 7, 7, 8</td>
<td>7.25 ± 0.25</td>
</tr>
<tr>
<td>3</td>
<td>Wi</td>
<td>115.6 ± 21.8</td>
<td>II</td>
<td>7</td>
<td>59, 78, 99, 113, &gt;141, &gt;198, &gt;210</td>
<td>&gt;128.3 ± 21.9</td>
</tr>
<tr>
<td>4</td>
<td>Wi</td>
<td>14.2 ± 0.2</td>
<td>II</td>
<td>5</td>
<td>5, 5, 10, 11, 34</td>
<td>13.0 ± 5.4</td>
</tr>
</tbody>
</table>

*Presensitization 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.

REFERENCES