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


TRANSPPLANTED 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.⁵ 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 (RT1a) 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 Hypanic-Ficol separation technique.⁶ 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, Cedarslane 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 2. Functional Period of Mixed Wi and Le Strain Rat Islet Allograft Under the KC of Diabetic ACI Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>FK 506</th>
<th>Survival (d)</th>
<th>Mean ± SEM</th>
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</thead>
<tbody>
<tr>
<td>1 None</td>
<td>4.5, 5.5, 7</td>
<td>5.2 ± 0.5</td>
<td></td>
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<tr>
<td>2 1 mg/kg/d IM, day</td>
<td>67, 85, 90, 132</td>
<td>95.4 ± 10.8</td>
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*Kidney containing the islet allograft was removed and normal-appearing islets were present at the graft site.

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. 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.

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

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.
REFERENCES