Sequential kidney/islet transplantation: efficacy and safety assessment of a steroid-free immunosuppression protocol

TOSO, Christian, et al.

Abstract
The aim of this study was to assess the efficiency and safety of the Edmonton immunosuppression protocol in recipients of islet-after-kidney (IAK) grafts. Fifteen islet infusions were administered to 8 patients with type 1 diabetes and a functioning kidney graft. Immunosuppression was switched on the day of transplantation to a regimen associating sirolimus-tacrolimus-daclizumab. Insulin-independence was achieved in all patients for at least 3 months, with an actual rate of 71% at 1 year after transplantation (5 of 7 patients). After 24-month mean follow-up, five have ongoing insulin independence, 11-34 months after transplantation, with normal HbA1c, fructosamine and mean amplitude of glycemic excursions (MAGE) values. Results of arginine-stimulation tests improved over time, mostly after the second islet infusion. Severe adverse events included bleeding after percutaneous portal access (n=2), severe pneumonia attributed to sirolimus toxicity (n=1), kidney graft loss after immunosuppression discontinuation (n=1), reversible humoral kidney rejection (n=1) and fever of unknown origin (n=1). These data indicate that the [...]
Sequential Kidney/Islet Transplantation: Efficacy and Safety Assessment of a Steroid-Free Immunosuppression Protocol


The aim of this study was to assess the efficiency and safety of the Edmonton immunosuppression protocol in recipients of islet-after-kidney (IAK) grafts. Fifteen islet infusions were administered to 8 patients with type 1 diabetes and a functioning kidney graft. Immunosuppression was switched on the day of transplantation to a regimen associating sirolimus-tacrolimus-daclizumab. Insulin-independence was achieved in all patients for at least 3 months, with an actual rate of 71% at 1 year after transplantation (5 of 7 patients). After 24-month mean follow-up, five have ongoing insulin independence, 11–34 months after transplantation, with normal HbA1c, fructosamine and mean amplitude of glycemic excursions (MAGE) values. Results of arginine-stimulation tests improved over time, mostly after the second islet infusion. Severe adverse events included bleeding after percutaneous portal access (n = 2), severe pneumonia attributed to sirolimus toxicity (n = 1), kidney graft loss after immunosuppression discontinuation (n = 1), reversible humoral kidney rejection (n = 1) and fever of unknown origin (n = 1). These data indicate that the Edmonton approach can be successfully applied to the IAK setting. This procedure is associated with significant side effects and only patients with stable function of the kidney graft should be considered. The net harm versus benefit has not yet been established and will require further studies with larger numbers of enrolled subjects.

Key words: Complications, graft function, immunosuppression, insulin independence, islet transplantation

Received 18 November 2005, revised and accepted for publication 25 January 2006

Introduction

Type 1 diabetes can lead to end-stage kidney failure. In such a condition, it is generally accepted to perform combined pancreas/kidney (SPK) rather than kidney transplantation alone, because of improved recipient and graft survival (1,2). In case of contraindication to SPK, combined islet/kidney transplantation is a good alternative (3). Results of islet transplantation have been continuously improving, but still have to match those of whole pancreas transplantation, especially in the long term (4). According to registry data, about 60% of islet recipients are free of insulin 1 year after transplantation and increasing numbers of centers worldwide are offering the procedure to their patients (5).

While one should promote combined transplantation, many patients with type 1 diabetes have received a kidney transplant alone or have experienced pancreas graft failure. Such patients with a functioning kidney graft are candidates for whole pancreas or islet transplant, in an attempt to improve glucose regulation and prolong kidney graft survival.

In the steroid-free era, the effort has been concentrated on islet transplant alone (ITA), in nonuremic patients with metabolic lability (3,6–11). No study has specifically addressed the impact of a sirolimus-based, steroid-free immunosuppression regimen in a series of islet-after–kidney (IAK) transplantation so far. The aims of the present study are to investigate whether the Edmonton approach could be successfully applied to the IAK setting and whether the procedure is safe for the kidney graft.

Patients and Methods

Study design

This is a pilot study designed to assess the efficacy and safety of islet transplantation in patients with type 1 diabetes already transplanted with a kidney. The primary endpoint was insulin independence at 1 year. The following secondary outcome markers of islet graft function and efficacy were assessed at regular time points: insulin requirements, basal C-peptide,
response to arginine stimulation test, HbA1c, fructosamine and mean amplitude of glycaemic excursions (MAGE). The following safety indicators were also assessed at regular time points: occurrence of adverse events, markers of kidney function, autoantibodies, anti-HLA lymphocytotoxic alloantibodies, PCR for CMV and EBV viremia.

**Patients**

All patients had C-peptide negative (<166 pmol/L) type 1 diabetes and had a functioning kidney graft. Inclusion criteria were: creatinine clearance >50 ml/min, proteinuria <0.5 g/day, daily insulin requirement <0.7 U/kg/day, BMI <27 kg/m², body weight <80 kg (male) or <75 kg (female). Metabolic liability or hypoglycemia unawareness was not considered as a prerequisite for inclusion.

This trial was approved by the Ethical Committee for Clinical Research of Geneva University Hospitals and was performed according to the rules of good clinical practice. Although the protocol is part of the Swiss-French GRAGIL collaborative network (12), this study only addresses patients transplanted in Geneva, without islet shipment.

**Organ selection and harvesting**

Pancreata were obtained from brain-dead multiorgan deceased donors from Switzerland or France. They were selected from donors between 18 and 65 years of age, with an ICU stay shorter than 7 days. Donors with a prolonged hypotensive episode inducing biochemical abnormalities (>50% increase in creatinine or >100% in liver function tests), those having had a cardiac arrest without professional cardiorespiratory resuscitation as well as donors with a history of diabetes, pancreatitis or alcohol abuse were excluded. All islet preparations were isolated within a 35-month period. During this time period, 406 pancreata were offered, 143 accepted for islet transplantation. Exclusion criteria were a functioning kidney graft. Inclusion criteria were: creatinine clearance >5000 IEQ/kg body weight of the recipient (for the first islet infusion) and >10 000 cumulated IEQ/kg for the second), >30% purity, >70% viability, <10 mL packed cell volume, ABO compatibility, negative T- and B-cell cross-match and negative Gram staining of the islet culture medium. Transplantation was not performed when islets had repeat HLA antigen mismatches with respect to a previous kidney graft. However, one repeat in a HLA class A antigen was tolerated (Table 2). HLA matching of the islet donor with the recipient was not taken into consideration.

At the time of transplantation, islets were washed and pooled in CMRL 1066 medium supplemented with human albumin (4%) and heparin (35 U/kg body weight of the recipient). Syringes were used for the first 11 infusions and transfer bags (Baxter, Maurepas, France (17)) for the last four infusions. Transplantation was done under local anesthesia with ultrasound approach of the portal vein and through a 6 French catheter for the first 13 infusions or a 4.5 French catheter thereafter. At the end of the procedure, gelatin sponge particles (Gelfoam, Pfizer, Zurich, Switzerland) were embolized into the liver parenchymal tract and an ultrasonographic control was performed (18).

**Islet isolation and culture**

Islet isolations were performed as previously described according to a modified Ricordi method (14,15). Briefly, after trimming the organ, the pancreatic duct was opened and the pancreas was distended by intraductal infusion of a cold collagenase solution. Liberase and Serva NB1 (15) were indifferently used. After digestion at 37°C in a modified Ricordi chamber, separated cells were washed and purified in a continuous Biocoll gradient (Biochrom, Berlin, Germany) using a refrigerated cell separator (COBE 2991, Cobe, Lakewood, CO, USA). Prior to transplantation, islets were cultured in serum-free CMRL 1066-based medium, supplemented with 0.6% human albumin (16) for 12–36 h. No xenogeneic protein was used at any time during isolation or culture.

After dithizone staining, islet number and purity were manually assessed by 2 or 3 independent investigators on various samples. With such a technique, the mean coefficient of variation for 10 consecutive final islet counts was 8.7 ± 5.7%, reflecting low variability between investigators. The equivalent islet number (IEQ) was calculated by normalizing the islets to a standard diameter of 150 μm. Viability was assessed by propidium iodide and fluorescein diacetate staining.

In vitro islet function was evaluated by glucose-stimulated insulin release in static incubation assays run in triplicate. Two hundred IEQ were preincubated at low glucose concentration (2.2 mmol/L) for 60 min. Basal and stimulated insulin concentration was measured by ELISA in the culture medium after further incubation at low and high glucose concentration (2.2 and 22.2 mmol/L for two periods of 90 min). A stimulation index (SI) was calculated by dividing the stimulated by the basal insulin response.

Endotoxin concentration of the islet culture media was assessed by a Limulus Amebocyte Lysate (BioWhittaker, Walkersville, MD, USA). Bacteriological status of the islets was tested by performing Gram staining and cultures of the transplant medium of the islets.

**Islet transplantation**

Transplantation was performed when the following release criteria were fulfilled: >5000 IEQ/kg body weight of the recipient (for the first islet infusion) and >10 000 cumulated IEQ/kg for the second), >30% purity, >70% viability, <10 mL packed cell volume, ABO compatibility, negative T- and B-cell cross-match and negative Gram staining of the islet culture medium. Transplantation was not performed when islets had repeat HLA antigen mismatches with respect to a previous kidney graft. However, one repeat in a HLA class A antigen was tolerated (Table 2). HLA matching of the islet donor with the recipient was not taken into consideration.

At the time of transplantation, islets were washed and pooled in CMRL 1066 medium supplemented with human albumin (4%) and heparin (35 U/kg body weight of the recipient). Syringes were used for the first 11 infusions and transfer bags (Baxter, Maurepas, France (17)) for the last four infusions. Transplantation was done under local anesthesia with ultrasound approach of the portal vein and through a 6 French catheter for the first 13 infusions or a 4.5 French catheter thereafter. At the end of the procedure, gelatin sponge particles (Gelfoam, Pfizer, Zurich, Switzerland) were embolized into the liver parenchymal tract and an ultrasonographic control was performed (18).

**Immunosuppression and infection prophylaxis**

A tapering of steroids was started when patients were inscribed on the waiting list, and transplantation was considered only when daily prednisone intake was ≤5 mg. The other immunosuppression drugs were not modified while on the waiting list. On the day of islet transplantation, the established immunosuppression was replaced by the Edmonton regimen (6). Sirolimus (Rapamune, Wyeth, Zug, Switzerland) was started at a loading dose of 0.2 mg/kg and was further given at 0.1 mg/kg once daily, aiming for trough levels between 12 and 15 μg/L for the first 3 months and between 10 and 12 μg/L thereafter. Tacrolimus was given at a dose of 1 mg twice daily aiming for trough levels between 4 and 6 μg/L. Induction was performed with i.v. daclizumab (Zenapax, Roche, Basel, Switzerland) at a dosage of 1 mg/kg infused every other week for a total of five doses, starting immediately before islet infusion. For the second islet transplantation, a full daclizumab protocol was started again, regardless of time elapsed since the first transplantation. In two patients (IAK 5 and 6), the remaining steroid medication was tapered and stopped 3.5 and 4.5 months after islet transplantation. All other patients were off steroids at the time of islet infusion.

Antibiotic prophylaxis with 2 g i.v. single-shot ceftriaxone (Rocephine, Roche) was given 1 h prior to transplantation. *Pneumocystis carinii* prophylaxis was given for 6 months (trimethoprim/sulfamethoxazol, 400/800 mg/day po; Bactrim, Roche), and anti-CMV prophylaxis for 3 months, regardless of donor/recipient serological combination (valganciclovir, 900 mg/day po; Valcyte, Roche).

During the first week after transplantation, patients received daily subcutaneous low-molecular weight heparin injections (nadoparine, 0.3 mg/day sc; Fraxiparine, Sanofi-Synthelabo, Meyrin, Switzerland).
Follow-up and metabolic tests
In order to rest islets, patients were started on an i.v. insulin pump immediately upon admission and until at least 5 days after transplantation. Subcutaneous insulin was then reintroduced and slowly tapered in order to keep blood glucose within normal values.

Full success was defined as insulin independence, with good glycemic control (HbA1c ≤ 6.5%). Partial success was defined as i.v. insulin function (C-peptide >166 pmol/L) and good glycemic control (HbA1c ≤ 6.5%) with a reduction of daily insulin requirements.

MAGE was calculated on 14 blood glucose values taken over 48 h (normal values <2 mmol/L, (19)). Arginine stimulation test was performed at 3, 6, 9 and 12 months and twice yearly thereafter. It was assessed by measuring serum insulin –10, 0, 2, 3, 4, 5, 7 and 10 min from i.v. injection of 5 g arginine. The area under the curve (AUC) for insulin was calculated as the AUC above the mean of values at −10 and 0 min. The acute insulin response (AIR) was calculated as the mean of the three highest values between 2 and 5 min minus the mean of values at −10 and 0 min. All blood samples were drawn in the morning, while patients were fasting and prior to any insulin injection. Arginine stimulation tests were performed in seven nondiabetic healthy individuals (4 males, 3 females; mean age: 39 ± 9 years). In these controls, mean AUC was 183 ± 57 mUmin/L and mean AIR 31.5 ± 9.5 mU/L.

HLA score was used to quantify matching between donor and recipient, as well as HLA repeats not carried by the recipient. A common HLA-A was rated one point, HLA-B two points and HLA-DR three points. Anti-HLA lymphocytotoxic, anti-GAD, anti-islet and anti-IA2 antibodies were monitored on a regular basis.

Results

Patients
From October 2002 to August 2005, eight consecutive patients with type I diabetes and a functioning kidney graft received an islet transplant. All completed a full transplantation, with two islet preparations (pooled in one infusion in IAK 2). Mean follow-up was 24 months. There were 5 females and 3 males with a mean age of 46 ± 9.5 years. Mean pretransplant weight was 61.6 ± 13 kg and BMI 23.8 ± 2.8 kg/m².

All patients were followed by a diabetes/endocrinology specialist at the time of referral for islet transplantation. They had a history of type I diabetes since 34.4 ± 7.2 years and had developed complications such as retinopathy (all patients), neuropathy (all patients) and macroangiopathy (1 patient). Insulin was administered by a subcutaneous pump in 1 patient. Poor metabolic control and hypoglycemia unawareness were present in five patients. Prior to transplantation, insulin requirements were 0.52 ± 0.1 U/kg body weight and HbA1c 7.7 ± 0.6%. All patients were C-peptide negative (<166 pmol/L) prior to transplantation.

Patient characteristics are shown on Table 1. Four patients had previously received a failed pancreas (n = 2) or islet (n = 2) graft simultaneously with the kidney transplant. Two additional patients were recipients of a living-related donor kidney transplant. Time between kidney and first islet transplantation was 9.8 ± 7.3 years.

Three patients were outside inclusion criteria at the time of transplantation (Table 1). One patient was below creatinine clearance criteria (IAK 1). The other two were above insulin intake criteria (IAK 2 and 4).

Transplantation
Pancreas donor characteristics as well as HLA matching and repeats are summarized in Table 2. One islet donor had a common HLA-A24 antigen mismatch with the kidney donor. Cold ischemia times were all under 12 h. A mean of 6263 IEQ/kg were injected for the first transplantation and a total of 12 530 IEQ/kg after the second. Islet purity, viability and cell tissue volume were always within release criteria (>30%, >70%, <10 mL; Table 2).

There was very high variability in the static incubation assay SI of the transplanted islet preparations (Table 2). Values were always obtained after transplantation and therefore not taken into consideration for product release. There was no correlation between SI and graft outcome.

All transplantations were performed intraportal through a transhepatic percutaneous approach (17). Two cases were complicated with intraperitoneal bleeding (IAK 2 and IAK 4 prep 2). One of them (IAK 2) required emergency laparotomy. Portal pressure increased by 4.2 ± 2.7 mmHg over baseline. There have been no thrombosis, even in

Table 1: Recipient characteristics

<table>
<thead>
<tr>
<th>Patients</th>
<th>IAK 1</th>
<th>IAK 2</th>
<th>IAK 3</th>
<th>IAK 4</th>
<th>IAK 5</th>
<th>IAK 6</th>
<th>IAK 7</th>
<th>IAK 8</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>45</td>
<td>35</td>
<td>52</td>
<td>30</td>
<td>54</td>
<td>52</td>
<td>57</td>
<td>47</td>
<td>46 (9.5)</td>
</tr>
<tr>
<td>Gender</td>
<td>M</td>
<td>M</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>M</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>79</td>
<td>45.3</td>
<td>70</td>
<td>52.5</td>
<td>65</td>
<td>75</td>
<td>54.6</td>
<td>50</td>
<td>61 (12)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>26.1</td>
<td>21.5</td>
<td>25.7</td>
<td>25</td>
<td>25.2</td>
<td>25.4</td>
<td>24</td>
<td>17.9</td>
<td>23.8 (2.8)</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>36</td>
<td>21</td>
<td>41</td>
<td>27</td>
<td>40</td>
<td>37</td>
<td>32</td>
<td>41</td>
<td>34.4 (7.2)</td>
</tr>
<tr>
<td>Pretransplant insulin requirement (U/kg/day)</td>
<td>0.5</td>
<td>0.77</td>
<td>0.34</td>
<td>1</td>
<td>0.49</td>
<td>0.48</td>
<td>0.58</td>
<td>0.64</td>
<td>0.6 (0.2)</td>
</tr>
<tr>
<td>Pretransplant HbA1c (%)</td>
<td>8.5</td>
<td>7.5</td>
<td>8</td>
<td>7.1</td>
<td>8</td>
<td>6.6</td>
<td>8.1</td>
<td>7.4</td>
<td>7.7 (0.6)</td>
</tr>
<tr>
<td>Time between kidney and first islet transplantation (years)</td>
<td>15</td>
<td>3</td>
<td>13</td>
<td>4</td>
<td>23</td>
<td>11</td>
<td>1</td>
<td>8</td>
<td>9.8 (7.3)</td>
</tr>
<tr>
<td>Pretransplant creatinine clearance (mL/min)</td>
<td>42</td>
<td>50</td>
<td>52</td>
<td>60</td>
<td>52</td>
<td>64</td>
<td>57</td>
<td>55</td>
<td>54.7 (7.8)</td>
</tr>
</tbody>
</table>

F = female, M = male.
### Table 2: Donor, islet and transplantation characteristics

<table>
<thead>
<tr>
<th>Patients</th>
<th>IAK 1</th>
<th>IAK 2</th>
<th>IAK 3</th>
<th>IAK 4</th>
<th>IAK 5</th>
<th>IAK 6</th>
<th>IAK 7</th>
<th>IAK 8</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Islet preparation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Donors</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Donor age (years)</td>
<td>35</td>
<td>23</td>
<td>45</td>
<td>19</td>
<td>39</td>
<td>42</td>
<td>38</td>
<td>44</td>
<td>37</td>
</tr>
<tr>
<td>Donor weight (kg)</td>
<td>80</td>
<td>68</td>
<td>70</td>
<td>70</td>
<td>58</td>
<td>65</td>
<td>40</td>
<td>70</td>
<td>58</td>
</tr>
<tr>
<td>Donor body mass index (kg/m²)</td>
<td>23.4</td>
<td>21.3</td>
<td>28</td>
<td>24.2</td>
<td>20</td>
<td>18.8</td>
<td>23.8</td>
<td>24.3</td>
<td>27.7</td>
</tr>
<tr>
<td>Cause of death</td>
<td>CV</td>
<td>T</td>
<td>CV</td>
<td>IH</td>
<td>IH</td>
<td>IH</td>
<td>T</td>
<td>IH</td>
<td>CV</td>
</tr>
<tr>
<td>Donor vs. recipient HLA score</td>
<td>9</td>
<td>4</td>
<td>5</td>
<td>1</td>
<td>4</td>
<td>3</td>
<td>5</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Donor vs. kidney HLA repeat</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HLA repeat between islet donors</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Islets</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cold ischemia time (h)</td>
<td>7.2</td>
<td>6.5</td>
<td>8</td>
<td>9</td>
<td>8.75</td>
<td>9</td>
<td>7.5</td>
<td>11.5</td>
<td>10</td>
</tr>
<tr>
<td>IEq (×10³)</td>
<td>510</td>
<td>344</td>
<td>329</td>
<td>266</td>
<td>387</td>
<td>366</td>
<td>287</td>
<td>574</td>
<td>340</td>
</tr>
<tr>
<td>IEq per Kg body weight</td>
<td>6376</td>
<td>4407</td>
<td>7262</td>
<td>5871</td>
<td>5528</td>
<td>5228</td>
<td>5466</td>
<td>10933</td>
<td>5646</td>
</tr>
<tr>
<td>Tissue volume (mL)</td>
<td>7.8</td>
<td>2</td>
<td>3.5</td>
<td>2.5</td>
<td>4</td>
<td>3.6</td>
<td>2.5</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Islet purity (%)</td>
<td>50</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>30</td>
<td>39</td>
<td>70</td>
<td>70</td>
<td>65</td>
</tr>
<tr>
<td>Islet viability (%)</td>
<td>95</td>
<td>95</td>
<td>95</td>
<td>98</td>
<td>95</td>
<td>95</td>
<td>95</td>
<td>90</td>
<td>95</td>
</tr>
<tr>
<td>Endotoxin (EU/mL)</td>
<td>0.08</td>
<td>0.07</td>
<td>0.07</td>
<td>0.06</td>
<td>0.06</td>
<td>0.1</td>
<td>0.1</td>
<td>0.07</td>
<td>0.07</td>
</tr>
<tr>
<td>Static incubation stimulation index</td>
<td>14.8</td>
<td>3</td>
<td>11.4</td>
<td>5</td>
<td>3.9</td>
<td>2.33</td>
<td>0.95</td>
<td>2.24</td>
<td>6.6</td>
</tr>
<tr>
<td>Transplant</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal portal pressure (mmHg)</td>
<td>14</td>
<td>13</td>
<td>2</td>
<td>2</td>
<td>13</td>
<td>18</td>
<td>10</td>
<td>13</td>
<td>19</td>
</tr>
<tr>
<td>Peak portal pressure (mmHg)</td>
<td>16</td>
<td>21</td>
<td>10</td>
<td>10</td>
<td>15</td>
<td>25</td>
<td>12</td>
<td>18</td>
<td>22</td>
</tr>
</tbody>
</table>

CV = cerebrovascular, T = head trauma, IH = intracerebral hemorrhage, NA = not applicable.

HLA score calculated by adding HLA match points, HLA-A match being one point, HLA-B 2 points and HLA-DR 3 points.

HLA repeat: shared HLA between donors and not included in recipient HLA (HLA-A: one point, HLA-B: 2 points and HLA-DR: 3 points).
Steroid-Free IAK Transplantation

In arginine stimulation tests, the AUC of insulin increased after each islet infusion. This increase was higher after the second than after the first islet infusion. AUC kept on improving up to 6 months after the second infusion and remained stable in patients who retained islet function (Figure 4). Peak AUC were 26.9 ± 15 and 109 ± 41 mUmin/L after the first and second transplantations. AIR to arginine demonstrated the same trend as AUC, with peak values of 3.69 ± 2 and 14.9 ± 4.6 mU/L, respectively. In patients with failed grafts (IAK 1 and 2), a sharp decrease in AUC and AIR was observed. The same was observed, to a lesser extent, in the patient who went back on insulin, but retained islet graft function (IAK 7). Mean peak values for AUC and AIR were significantly lower than those of controls (Student’s t-test, p < 0.01 for both AUC and AIR). However, two patients (IAK 4 and IAK 7) had AUC and AIR values within the range observed in controls.

Renal function

Creatinine clearance remained stable in all patients except one (IAK 1). This patient already had a decreasing kidney function while on the waiting list, with a creatinine clearance <50 mL/min at the time of transplantation. After transplantation, his renal function continued to decrease and tacrolimus trough levels were maintained at minimal values (<5 μg/mL). He reached end-stage renal failure 9 months after first islet transplantation, when immunosuppression had to be stopped because of sirolimus-associated interstitial lymphocytic pneumonitis.

Another patient (IAK 5) experienced a humoral rejection and had to be treated with high-dose intravenous immunoglobulin. Posttreatment, renal function went back to baseline level.

In terms of urinary protein excretion, one patient deteriorated preexisting overt proteinuria (IAK 1), one patient deteriorated preexisting microalbuminuria (IAK 4) and one patient developed microalbuminuria (IAK 8). Of the five remaining patients, four had preexisting microalbuminuria, that did not deteriorate further.

Side effects and complications

Procedure-related complications and impairment of renal function have been described above. Infectious complications included lower urinary tract infections (n = 4), upper respiratory tract infections (n = 2), fever of unknown origin (n = 1), vaginal mycosis (n = 1) and anal herpes simplex virus infection (n = 1). There was no case of cytomegalovirus infection or EBV-associated posttransplant lymphoproliferative disease.

Regarding hematological disorders, thrombopenia was observed in 1 patient, anemia in 4 and leucopenia in 7. One case of neutropenia required outpatient treatment with granulocyte colony-stimulating factor (Neupogen, Amgen, Luzern, Switzerland).

Metabolic function

All patients had stable basal C-peptide levels, except for two patients (IAK 1 and 2) who lost their islet graft (Figure 3).

Islet function

All patients achieved insulin independence (Figure 1). Two patients (IAK 1 and IAK 2) resumed insulin therapy 3 and 10 months after the first transplant and went on to lose all islet graft function 5 and 4 months later, as demonstrated by the absence of serum C-peptide. Another patient (IAK 6) had to restart insulin 19.5 months after transplantation, but kept good glucose control thereafter and remained C-peptide positive. The five remaining patients have ongoing insulin independence 11–34 months after transplantation. HbA1c, fructosamine and MAGE decreased after transplantation and remained within normal values, except for the two patients with eventual islet graft failure (Figure 2). HLA matching and cold ischemia time did not correlate with islet transplant outcome. ELISA tests for lymphocytotoxic antibodies remained negative in all patients. Anti-insulin, anti-GAD and anti-IA2 antibodies remained stable compared to pretransplant values, except for an increase of anti-GAD antibodies in patients IAK 4, 7 and 8 (pretransplant values: 0.2, 210 and 4986 U/L; peak values: 989, 446.5 and 15 299 U/L; normal range <9.5 U/L). All three patients are still off insulin. A liver biopsy was taken from patient 8, who had the sharpest increase in anti-GAD titers, and showed islet tissue free of cellular infiltrate.

Figure 1: Islet graft function of 8 recipients of IAK transplants. Gray bars represent partial function (C-peptide >166 pmol/L) after initial islet infusion; white bars full islet function with insulin independence and dashed bars reintroduction of insulin therapy with ongoing islet function. All patients have reached insulin independence. Three patients (IAK 1, 2 and 6) resumed insulin 3, 10 and 19.5 months after first islet infusion. Two of them (IAK 1 and 2) lost all islet function 8 and 14 months after initial islet transplantation (black bars). The five remaining patients have ongoing insulin independence 11–34 months after initial transplantation.

Figure 2: Procedure-related complications. One patient (IAK 4) known for a heterozygous factor V Leiden mutation. In this patient, therapeutic anticoagulation was restarted shortly after infusion, which could explain the bleeding experienced after the second infusion (20).
Other complications included peripheral edema (n = 3), diarrhea (n = 5) and acneiform rash (n = 1). One patient (IAK 5) suffered of finger polyarthritis and had to be put back on 5 mg prednisone po per day. All patients except one experienced mouth ulcerations, which remained superficial and could be treated with topic agents. Prior to transplantation, seven patients were on lipid-lowering medication (statins). In four of them, treatment had to be intensified because of increasing cholesterol levels (n = 1) or switched to atorvastatin, because of increasing cholesterol and triglyceride levels (n = 3). Antihypertensive medication had to be intensified in two patients.

Of all side effects, six were considered as severe according to the National Cancer Institute classification, and were observed in four patients: bleeding after percutaneous portal access (n = 2), pneumonitis attributed to sirolimus toxicity (n = 1), kidney graft loss after immunosuppression discontinuation (n = 1), reversible humoral kidney rejection (n = 1) and fever of unknown origin (n = 1).

**Discussion**

This study demonstrates that the Edmonton approach, characterized by high islet mass, using multiple donors if
necessary, and steroid-free immunosuppression, can be reproduced in the islet-after-kidney setting, with a majority of patients achieving stable insulin independence. However, several side effects are associated with the procedure and strict selection criteria should be followed, especially regarding kidney graft function.

Our local policy is to offer simultaneous pancreas/kidney transplantation to patients with type 1 diabetes reaching renal failure, with the aim to improve patient survival (2). Simultaneous islet/kidney transplantation is offered to older patients (usually over 50 years of age), patients with severe comorbidities and to those unwilling to take the risk of a SPK procedure. With such a policy, no patient with type 1 diabetes should be transplanted with a kidney alone since enough pancreas donors are available for both whole pancreas and islet transplant procedures (21). Pancreas-after-kidney transplantation is becoming an increasingly attractive option for patients with type 1 diabetes who either never were offered a pancreas transplant, lost their pancreas transplant for technical reasons or have a live kidney donor available. The procedure is thought to prolong patient survival and delay or prevent the occurrence of secondary diabetic complications thanks to the recovery of glycemic control (22). Along this line, offering islet-after-kidney transplantation to patients who are not candidates for a pancreas transplant seems a logical option for attempting to achieve the same long-term goal. This rationale is supported by the association of successful islet-after-kidney transplantation with long-term improvement of diabetic macro- and microangiopathy (23,24).

Currently, a vast majority of islet transplant procedures is performed in the ITA setting (6,8–11). This series shows that islet transplantation can be done in the IAK setting with equal efficacy and safety. The issues pertaining to the IAK and ITA populations are completely different. In the ITA setting, patients with type 1 diabetes mellitus must have a highly unstable disease on a metabolic standpoint, to warrant the introduction of immunosuppression with its well-known, sometimes poorly tolerated, side effects and risks of infection and malignancy. In the IAK setting, the risks of immunosuppression are less an issue because these patients are already immunosuppressed. On the other hand, they run the genuine risk of jeopardizing the function of their kidney grafts at the time of switching immunosuppression and/or weaning of steroids. This was unfortunately the case in one patient in this series, who lost his kidney graft several months after IAK transplantation.

This complication urges for a very strict selection of recipients. The described patient had a declining creatinine clearance while on the waiting list and was under 50 mL/min at the time of transplantation. It is questionable whether this patient should have been transplanted under this protocol. Cut-off values cannot be determined from this study, but they can be adapted from policies applied for transplantation of pancreas after kidney (22). Creatinine clearance >50 mL/min and proteinuria <0.5 g/day appear as reasonable minimal recipient selection criteria in the IAK setting, and are in accordance with previously published data (7). We would strongly argue against criteria allowing to consider kidney recipients with serum creatinine up to 220 μmol/L (2.5 mg/dL) (25)! However, it seems to us that the absolute value of creatinine clearance is probably of less importance than the stability of kidney graft function. Practically, stability of serum creatinine values over the previous year should be ascertained before listing a patient for IAK transplantation. Although there are only few data about baseline biopsies, they could also help assessing a preexisting nephropathy (22).

Regarding steroid tapering, patient IAK 5 who had been on steroid maintenance for several years, had an episode of humoral kidney rejection after steroid weaning. Fortunately, this episode could be reversed with adequate antirejection therapy. This indicates that steroid tapering should be done cautiously and that keeping small doses of steroids...
might be desirable for patients with long-standing steroid maintenance.

In the present study, patients with functioning islets, demonstrated a stepwise improvement of AUC and AIR of arginine-stimulation tests after each islet transplantation. This improvement was remarkably higher after the second than after the first islet infusion. Both AUC and AIR remained stable afterwards, in accordance with other reports (3,8). However, in most patients, values remained lower than those obtained in control healthy individuals, reflecting a suboptimal endocrine reserve in IAK recipients.

Figure 4: Arginine stimulation tests were performed by measuring blood insulin and glucose – 10, 0, 2, 3, 4, 5, 7 and 10 min from i.v. injection of 5 g arginine. A, B: area under the curve for insulin (AUC). C, D: acute insulin response (AIR). Individual values are represented for each patient at various time points after first (A, C) and second (B, D) islet transplantation.
Loss of graft function, even partial, was always associated with or preceded by a decrease of responses to arginine stimulation.

On the immunosuppression standpoint, steroid avoidance appears as a key determinant for the success of the Edmonton protocol and was made possible by associating low-dose tacrolimus with high-dose sirolimus (26,27). In this regard, our results (71% insulin independence at 1 year) compare favorably with a former large series of IAK transplantation available, performed by the Milan group with a steroid-containing IS regimen and reporting 1-year insulin independence in 6 of 15 patients (40%) (28).

In accordance with other reports, the present study shows that the sirolimus/low-dose tacrolimus combination is linked to a high incidence of side effects, including nephrotoxicity (29, 30). The complication rate is similar to that reported in the ITA setting under the same IS regimen (31). Another approach, that has met good preliminary results in a similar IAK patient population was recently reported by the Philadelphia group and consisted in continuing the kidney graft maintenance immunosuppression, only adding daclizumab at the time of islet transplantation (32). New emerging drugs would probably allow to design novel protocols, which could ideally be steroid, calcineurin inhibitor and mTOR inhibitor free (27), in order to minimize side effects, and most of all nephrotoxicity.

In the IAK setting, the main priority is not to be harmful to the established kidney graft. Our policy was to avoid the repeat of HLA determinants with respect to the kidney graft. The rationale for this is based on the theoretical concern that a rechallenge with an HLA antigen borne by the kidney might be harmful. On the other hand, some patients have remained on the waiting list for very long time expecting donors, who did not bear the HLA antigens to avoid. In other centers, several patients with repeat mismatches between kidney and islet donors demonstrated good outcomes, without any kidney rejection (33). In animal models, improved islet survivals have been achieved with MHC class I repeats (34). While avoiding repeats was intended as the safest way to prevent harm to the kidney, this rule could probably be softened, allowing easier matching between donors and recipients.

Regarding complications, two patients suffered of postinjection bleedings (on a total of 15 injections), one requiring an emergency laparotomy. These cases were recorded while 6 French catheters were used. In the last cases, 4.5 French catheters were used and no more bleeding occurred. One of the patients with bleeding had a factor V Leiden mutation and had required prompt anticoagulation after transplantation. In order to reduce bleeding risks after islet transplantation, patients with coagulopathy should be excluded, only careful postinjection anticoagulation prophylaxis should be given, only small catheter should be used (≤4.5 French) and the radiologist should be experienced to prevent repeated punctures (35,36). We did not record any thrombosis, although some islet preparations had low purity and some patients had significant but transient increases in postinfusion portal pressure.

Altogether, the Edmonton results could be reproduced in the IAK setting, but this procedure can be associated with severe side effects: one kidney graft loss, one reversible humoral kidney rejection, one pneumonitis presumably sirolimus associated, two procedure-associated intraabdominal bleedings, and one fever of unknown origin occurred in four patients. Only selected patients, with stable kidney graft function before transplantation should be considered for such a procedure. Optimal cutoff values for creatinine clearance and proteinuria have not yet been determined, but 50 ml/min creatinine clearance and 0.5 g/day proteinuria appear as reasonable thresholds.

Overall, the net harm versus benefit of the IAK procedure with the present immunosuppression regimen has not yet been established and will require further studies with larger populations of enrolled subjects. Improved immunosuppression regimens with less side effects and nephrotoxicity must be designed.

Acknowledgments

The authors thank the staff and former fellows of the Cell Isolation and Transplantation Center (Axel Andres, MD, Elisabeth Bernouilli, Pascal Bucher, MD, Solange Charvier, Raymond Mage, Zolten Mathe, MD, David Matthey, Nadine Pernin, Corinne Singaliala), the transplant coordinators (Nadine de Carpenter, Monica Perruchoud, Marie-Claude Kempf, Florence Roch Barrena), the radiology team (Christophe Constantin, MD, Sylvain Terraz, MD, the immunology team (Sylvie Ferrari-Lacraz, MD, Jean Villard, MD), the diabetology team (Michel Procopiou, MD), the nephrology team (Nicola Marangon, MD, Pierre-Yves Martin, MD) and all Swiss and French surgical harvesting teams.

This study was supported by a grant from the Swiss National Foundation for Scientific Research (3200 BO-102134, to TB, DB, PM).

References

Toso et al.


