Natural History of Kidney Graft Survival, Hypertrophy, and Vascular Function in End-Stage Renal Disease Type 1 Diabetic Kidney-Transplanted Patients

Beneficial impact of pancreas and successful islet cotransplantation

OBJECTIVE — Diabetes, hypertension, infections, and nephrotoxicity of certain immunosuppressive drugs (i.e., calcineurin inhibitors) can reduce functional survival of the kidney graft. Our aim was to evaluate survival, hypertrophy, and vascular function of the kidney graft in end-stage renal disease (ESRD) type 1 diabetic patients after transplant.

RESEARCH DESIGN AND METHODS — The study population consisted of 234 ESRD type 1 diabetic patients who underwent kidney-pancreas (KP; 166 patients), successful kidney-islet (KI-s; 24 patients), and kidney (KD; 44 patients) transplant. Kidney size, graft survival, vascular function, and microalbuminuria were evaluated prospectively yearly for 6 years. Sixty-eight protocol kidney biopsies were performed routinely between 1993 and 1998 cross-sectionally (3.2 ± 0.3 years from kidney transplant).

RESULTS — The KP and KI-s groups had better cumulative kidney graft survival at 6 years than did the KD group (KP: 73%; KI-s: 86%; KD: 42%, P < 0.01). The KP group but not the KI-s/KD groups showed a persistent kidney graft hypertrophy up to 6 years of follow-up. A significant increase in creatinine levels from baseline to year 6 was evident in the KD group (1.58 ± 0.08 to 2.78 ± 0.44 mg/dl, P < 0.05) but not in the KP/KI-s groups. The KP/KI-s groups only showed a reduction of renal resistance index from baseline to year 6 (KP at baseline: 0.74 ± 0.01 to 0.68 ± 0.01%, P < 0.01; KI-s at baseline: 0.72 ± 0.02 to 0.69 ± 0.02, P < 0.05). At year 6, an increase from baseline in urinary albumin excretion was observed only in the KD group (31.4 ± 9.0 to 82.9 ± 33.6 mg/l, P < 0.05). Preliminary data suggested that graft nitric oxide (NO) expression was higher in the KP/KI-s groups than in the KD group (data not shown).

CONCLUSIONS — In ESRD type 1 diabetic patients, KP and KI-s compared with KD resulted in enhanced kidney graft survival, hypertrophy, and vascular function.

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Nephropathy is one of the most common and most serious complications in type 1 diabetes (1,2). Glomerular hyperfiltration is the first feature of renal involvement and can be observed soon after diabetes onset, accompanied by a loss of renal functional reserve (3). Microalbuminuria appears later, as do morphological changes such as thickening of the glomerular basement membrane and mesangial expansion (4).

Nephrosclerosis or glomerulosclerosis of the transplanted kidney in end-stage renal disease (ESRD) type 1 diabetic kidney transplant patients may result from the interaction of diabetes, hypertension, obesity, smoking, and dyslipidemia and from nephrotoxicity of certain immunosuppressive drugs (calcineurin inhibitors but not only) (5–6), leading to a reduction in the intrarenal vascular surface area and an increase in vascular resistance (7–10).

Pancreas and islet transplantation can confer insulin independence in type 1 diabetic transplant patients, thus preventing the progression to diabetic nephropathy, improving graft survival, and ameliorating diabetic macro-/microangiopathy (6, 11–17). It is likely that islet transplant could ameliorate renal function through improved glycometabolic control and restoration of C-peptide secretion (14,18–23). The effect of pancreas or islet transplant on kidney vascular function is unknown.

Among the abnormalities observed in diabetes, alteration of NO pathways may have a major role in the development of diabetic nephropathy (24–27). Increased expression of endothelial constitutive NO synthase (NOS) was recently documented in glomerular endothelial cells and preglomerular vessels of diabetic rats in the early phase of the disease, while later in the natural history of the disease, NOS was downregulated (24–27).

To evaluate kidney structure and function, we assessed kidney size, renal
arterial resistance index, microalbuminuria, and kidney NOS expression in three groups of ESRD type 1 diabetic transplant recipients. We compared baseline data with 6-year follow-up data from patients who underwent kidney-pancreas (KP), kidney-islet (KI), or kidney-alone (KD) transplant.

**RESEARCH DESIGN AND METHODS** — The study was conducted from June 1985 to June 2003. All the transplant patients consecutively admitted to San Raffaele Hospital, Milan, for regular check-up were included in the study if they met the inclusion/exclusion criteria. Patients with lymphoproliferative disease or neoplasm were excluded definitively. Those with severe infection, enhanced erythrocyte sedimentation velocity, or C-reactive protein were excluded momentarily from color Doppler ultrasonography (CDU) assessment and reevaluated when the problem was resolved. Twenty-five KP, four KD, and one KI patient were excluded definitively for neoplasm or lymphoproliferative disease. All subjects provided informed consent before study enrollment.

**Kidney and pancreas transplant**

Organs for transplantation were obtained from cadaver donors through Nord Italia Transplant. All patients received a simultaneous kidney-pancreas transplant. The standard technique of organ procurements from brain-dead cadaver donors was used. Selection criteria of the donors were based primarily on the ABO compatibility, the HLA match, the age of the donor, and the anatomy of the pancreas. The kidney was prepared using standard techniques. For pancreas venous drainage, a systemic drainage was used. Systemic venous drainage was obtained by anastomosis of the donor graft portal vein to the recipient iliac vein. Until 1998, bladder exocrine drainage was performed; thereafter, only enteric drainage was used.

**Islet transplant**

Patients underwent islet transplantation based on ABO matching at least 1 year after kidney transplant, except in six patients in whom islet transplant was performed simultaneously with the kidney transplantation. Islets were isolated from the pancreata obtained from multiorgan donors using a previously described method (28). Islets were injected under local anesthesia into the portal vein via a percutaneous approach under ultrasonographic and fluoroscopic guidance.

**Immunosuppression and postoperative management**

Immunosuppression was induced with ATG (thymoglobulin; IMTIX-SangStat) and maintained with cyclosporine (CyA) or tacrolimus (FK 506 from 1998), mycophenolate-mofetil (or azathioprine), and prednisone. Episodes of renal rejection were treated with pulses of 500 mg methylprednisolone or with OKT3/ATG in steroid-resistant cases.

**Clinical follow-up**

All patients were given annual clinical and laboratory assessments. Each of the following parameters was evaluated yearly: kidney-graft cumulative survival, kidney graft size and function, renal arterial resistance index (RI), urinary albumin excretion (UA), and laboratory analysis, and protocol kidney biopsy. The baseline point was considered when patients were discharged from the hospital in the post-transplant period. We decided to use the day of discharge because the kidney graft recovers from cold ischemia and from the tubular necrosis that follows it.

**Kidney graft cumulative survival**

Cumulative survival of renal allografts was estimated in the different groups of transplant patients by the need for dialysis treatment, as previously described (10). Kidney graft survival was evaluated at the time of hospital discharge, according to the quartile values of the most important parameters (RI, UAE, and creatinine levels).

**Kidney diameter and renal RI assessment with CDU**

Experienced operators in all patients performed CDU examinations of the transplanted kidneys yearly during hospital admission, using an ATL-Philips HDI 5000 unit (Bothell, WA) with a 2- to 5-MHz curved array multifrequency transducer. Estimation of kidney diameter was ultrasonographically obtained by measuring its major axis calculated by software as the distance between the upper and lower poles. RI was determined as follows: the peak systolic velocity minus the end diastolic velocity divided by the peak systolic velocity. The usefulness of RI as a noninvasive hemodynamic assessment of the transplanted kidney was previously pointed out (7–10). Our technique for CDU to evaluate RI is reproducible (coefficient of variation [CV], 3–5%) and precise. The results are closely correlated with those of magnetic resonance angiography, as reported by our and other groups (29–32). The intraobserver and interobserver CVs for the measurements of the RI were 3–4 and 4–6%, respectively (29–32).

**Laboratory studies**

UAE was assessed in an early morning spot urine sample. Urine samples with abnormal sediment on routine urinalysis were discarded; the others were assayed for albumin by using reagent strips (Albustix, Ames, Bayer Diagnostic, Bayer, Munich, Germany). In Albustix-positive samples, the urinary albumin concentration was measured by immunonephelometry with N albumin kits (Behring, Somerville, NY). The intra-assay and interassay CVs were <2% and <4%, respectively. A value between 20 and 200 mg/l was defined as indicating microalbuminuria, while higher values were defined as indicating overt macroalbuminuria (1).

**Protocol renal biopsy**

Sixty-eight protocol kidney biopsies were performed routinely between 1993 and 1998. All the patients gave their written and informed consent to undergo kidney biopsies. Biopsy specimens were fixed and analyzed as previously reported (18,33). Sections were incubated with a polyclonal anti-NOS3 antibody (Santa Cruz Biotechnology). Biopsy specimens were blindly evaluated by a pathologist and scored according to the Banff 97 classification (18,32).

**Statistical analysis**

Data were expressed as means ± SE. Data were tested for normal distribution with the Kolmogorov-Smirnov test and for homogeneity of variances with Levene’s test. Two-sided paired Student’s t test (for parametric data) and Wilcoxon test (for nonparametric data) were used to compare baseline parameters with follow-up data. ANOVA (for parametric data) or Kruskal-Wallis (for nonparametric data)
Table 1—Pre- and peritransplant characteristics of ESRD type 1 diabetic patients who underwent KI-s, KP, or KD transplant

<table>
<thead>
<tr>
<th>Pretransplant data</th>
<th>KI-s</th>
<th>KP</th>
<th>KD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>41.1 ± 1.7</td>
<td>37.9 ± 0.9</td>
<td>39.9 ± 2.2</td>
</tr>
<tr>
<td>C-peptide (ng/ml)</td>
<td>0.15 ± 0.02</td>
<td>0.11 ± 0.02</td>
<td>0.13 ± 0.03</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>24.9 ± 2.1</td>
<td>26.4 ± 0.9</td>
<td>22.7 ± 1.4</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>8.3 ± 0.3*</td>
<td>11.2 ± 1.7</td>
<td>11.1 ± 2.3</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>59.5 ± 1.9</td>
<td>58.7 ± 2.7</td>
<td>62.0 ± 2.0</td>
</tr>
<tr>
<td>Hypertension rate (%)</td>
<td>90</td>
<td>78</td>
<td>93</td>
</tr>
<tr>
<td>Dialysis duration (months)</td>
<td>37.2 ± 3.9</td>
<td>30.5 ± 2.1</td>
<td>26.1 ± 4.1</td>
</tr>
</tbody>
</table>

Transplant-related data

| Cold ischemia time (minutes) | 791 ± 61 | 836 ± 35 | 875 ± 62 |
| Warm ischemia time (minutes) | 44 ± 2 | 41 ± 1 | 43 ± 3 |
| Donor age (years) | 32.4 ± 2.8 | 28.5 ± 1.3 | 32.2 ± 4.8 |
| Cytomegalovirus infection episodes per patients | 0.33 ± 0.11 | 0.47 ± 0.04 | 0.32 ± 0.07 |
| Rejection episodes per patients† | 0.2 ± 0.1 | 0.7 ± 0.1 | 0.4 ± 0.1 |

Data are means ± SE, unless otherwise indicated. *Before islet but after kidney transplant; †P < 0.05.

RESULTS

Patient demographics

We evaluated 234 ESRD type 1 diabetic patients enrolled on our waiting list for KP transplant who underwent KP (166 patients), KI-s (24 patients with basal C-peptide secretion >0.5 ng/ml for >1 year [corresponding fasting glycemia = 205.8 ± 25.8 mg/dl]) (16-18), or KD (4 patients) transplant. Patients in the KD group were transplant recipients enrolled in the KP waiting list who had either lost their pancreatic graft early in the postoperative period (n = 15 patients), who only received a renal transplant due to macroscopic damage of the donor pancreas at harvesting, or who received KI transplant with an early failure of the transplant islet (KI-u: kidney-islet unsuccessful, with basal C-peptide secretion <0.5 ng/ml, n = 11 patients [corresponding fasting glycemia = 198.1 ± 27.5 mg/dl]). The numbers of evaluable patients in each group at the follow-up assessments were as follows: 166, 156, and 141 at 2, 4, and 6 years, respectively, in the KP group; 24, 21, and 12 at 2, 4, and 6 years, respectively, in the KI-s group; and 44, 41, and 38 at 2, 4, and 6 years, respectively, in the KD group.

The three transplant groups were similar in age, body weight, C-peptide levels, duration of diabetes and dialysis, cold ischemia time, number of episodes of posttransplant cytomegalovirus infection but not for number of episodes of kidney rejection (Table 1). Glycated hemoglobin was lower in KI-s patients, who were not uremic at the time of islet transplantation (Table 1).

Kidney graft survival, hypertrophy, and function

As shown in Fig. 1A, a higher kidney graft actuarial survival was evident in the KP and KI-s groups compared with the KD group. Poor graft survival was predicted by high levels of UAE (P < 0.05, relative risk 7.3 [1.8–60.2]) but not by high levels of RI (Fig. 1B) and creatinine (data not shown). Even by considering the three groups separately, higher UAE levels still predict a poor survival, except in the KI group (data not shown).

All three groups showed an increase in graft size after the transplant, but only the KP group showed a persistent kidney graft hypertrophy up to 6 years of follow-up (Fig. 1C). This increase is statistically significant in the KP groups at 2 and 4 years (P < 0.05 vs. baseline) (Fig. 1C). On the contrary, a nonsignificant reduction of kidney graft diameter was evident in the KD and KI-s groups after 6 years from transplantation (Fig. 1C). By analyzing the KI-u group alone, kidney graft survival was similar to the KD group.

Mean creatinine levels increased from baseline to year 6 in the KD group (from 1.58 ± 0.08 to 2.78 ± 0.44 mg/dl, P < 0.05) and in the KI-s group (from 1.38 ± 0.08 to 1.91 ± 0.36 mg/dl, NS) but not in the KP group (from 1.48 ± 0.03 to 1.46 ± 0.06 mg/dl) (Fig. 2A1–A3). Patients in the KP group also showed a slight but significant reduction from baseline in mean creatinine levels at 2 and 4 years (P < 0.05 baseline vs. 2 and 4 years). Mean creatinine levels were significantly higher in the KD group than in the KP group at 2 years (P < 0.05) and at 4 and 6 years (P < 0.01 for both comparisons) (Fig. 2A1 and A3).

Mean UAE was significantly worse at year 6 in the KD group compared with baseline (baseline 31.4 ± 9.0 to 82.9 ± 33.6 mg/dl at year 6, P < 0.05) but not in the KP group (baseline 22.3 ± 3.7 to 12.0 ± 1.2 mg/dl at year 6) or the KI-s group (baseline 76.9 ± 26.0 to 46.9 ± 21.2 mg/dl at year 6) (Fig. 2B1–B3). Patients in the KP group had significantly lower UAE levels at 6 years compared with patients in the KD group (P < 0.01) and at baseline, 2, and 4 years compared with patients in the KI-s group (P < 0.01 for each comparison).

Progressive improvements from baseline of mean RI values were observed for patients in the KP group (0.74 ± 0.01 to 0.68 ± 0.01%, P < 0.01) and in the KI-s group (0.72 ± 0.02 to 0.69 ± 0.02%, P < 0.05) but not for patients in the KD group (0.76 ± 0.02 to 0.76 ± 0.04%, NS) (Fig. 2C1–C3). Mean RI values were significantly lower for patients in the KP group at 4 years (P < 0.05) compared with patients in the KI-s group and at 2 years (P < 0.01), 4 years (P < 0.01), and 6 years (P < 0.05) compared with patients in the KD group (Fig. 2C1–C3). The majority of KD patients displayed higher RI values compared with the normal value reported in literature (0.8), even after 2 and 4 years of follow-up (11,12).
Glycometabolic control, islet function, and lipid levels

All KP patients were insulin independent during the 6 years of follow-up, whereas the KD patients were on intensive subcutaneous insulin therapy. In the KI-s group, 12 patients maintained insulin independence for a period longer than 3 months, with a mean duration of insulin independence of 21.0 ± 4.2 months. Patients in the KP group had near-normal HbA1c levels, significantly lower than those in the other groups (from 5.7 ± 0.1 at baseline to 5.8 ± 0.2% at 6 years). High HbA1c levels persisted in the KD group (from 8.0 ± 0.4 at baseline to 7.8 ± 0.2% at 6 years), whereas patients in the KI-s group showed a slight increase from baseline in HbA1c levels at 2 years (from 7.4 ± 0.2 at baseline to 8.1 ± 0.3% at 6 years, P < 0.05).

C-peptide secretion was sustained in the KI-s group for up to 6 years of follow-up (mean C-peptide levels of 1.6 ± 0.2 at 2 years, 1.1 ± 0.1 at 4 years, and 1.1 ± 0.4 ng/ml at 6 years). Insulin requirement was significantly reduced in the KI-s group, as previously reported (16–18), to ~50% from baseline at 2, 4, and 6 years.

Over the entire follow-up, patients in the KP group showed improved triglyceride levels (Table 2, P < 0.01). Mean triglyceride levels appeared lower in the KP and KI-s groups compared with the KD group at 2 and 4 years (P < 0.01 vs. both groups). At 6 years, the KP group showed lower triglyceride levels compared with the KD and KI-s groups (Table 2, P < 0.05 vs. both groups). Mean total cholesterol levels showed slight but significant increases from baseline in the KP group at
A trend toward a reduction in blood cyclosporine levels was evident in the three groups of patients during the follow-up, due to an adjustment of immunosuppressive treatment to less nephrotoxic levels (Table 2). Stable UAE levels were evident in the KP and KI-s groups (B1 and B2). A worsening of UAE was evident in KD group (B3). The KP group showed lower UAE levels compared with the KI-s group at baseline, 2, and 4 years (*P < 0.05) and compared with KD at 6 years (**P < 0.01). The KP and KI-s groups (C1 and C2), but not the KD group (C3), showed a progressive improvement of RI. The KP group showed lower RI at 2, 4, (#P < 0.01), and 6 years (##P < 0.05) compared with KD group and at 4 years (†P < 0.05) compared with the KI-s group.

**Preliminary data on NOS kidney expression**

Patients underwent a protocol biopsy of the transplanted kidney 3.2 ± 0.3 years after transplant (KI-s = 4.5 ± 0.9, KP = 2.5 ± 0.3, KD = 3.6 ± 0.4 years, P < 0.05). Thirty-three specimens were evaluable. The remaining biopsy specimens were not evaluable due to absence of glomeruli, severe chronic rejection, advanced fibrosis, or severe lymphocytes infiltrates. Of the 33 evaluable specimens, 20 were from the KP group, 7 from the KI-s group, and 6 from the KD group. They had similar creatinine levels at the moment of biopsies (data not shown).

No major differences among the three groups, including differences in the Banff 97 scores, were evident based on the kidney biopsy specimens. All biopsies were classified as normal. In two KP, one KI-s, and one KD patient, we observed a mild tubular injury. Biopsy specimens from a single patient in the KI-s group and a single patient in the KD group showed a scanty cellular infiltrate. Our very preliminary data on kidney graft NOS expression showed that it was higher in the KP group overall and in some patients in the KI-s group compared with patients in the KD group (data not shown). But these data need to be carefully reevaluated in the view of more appropriate controls.
CONCLUSIONS — Here, we report that kidney-pancreas and kidney-islet transplants, when successful, prevent the worsening of graft survival and vascular function of the kidney graft that may occur in ESRD diabetic patients receiving kidney transplants. Noninvasive assessments of graft vascular function using RI and UAE evaluations showed that the KP and KI-s groups had better cumulative kidney graft survival at 2, 4, and 6 years than did the KD group. Expression of NOS in the kidney graft correlated well with the functional data, showing an early improvement in RI and UAE that was more suggestive of a role for both RI and UAE in the early detection of renal vascular dysfunction. In addition to these functional studies, we performed an immunocytochemical and molecular evaluation of the expression of NOS in the renal microcirculation. Even if at a very preliminary stage and without the appropriate control, it was possible to observe a slightly reduced expression of endothelial NOS in kidneys in the KD group at 3 years after transplantation, even if not statistically significant. Interestingly, in KD subjects the kidney biopsy appears substantially normal except for NO expression. It is possible that a shorter follow-up that did not allow a morphological difference can appear. The downregulation of endothelial NOS in the KD group could probably contribute, in the long term, not only to the worsening of kidney graft vascular function but to the reduction of compensatory hypertrophy of the kidney graft, given that NO has a regulatory effect on cell survival and apoptosis, in the kidney too (42). However, prospective studies are needed in this field. An alternative explanation could be the presence of an increased alloreactivity to donor antigens in patients who experienced an early failure of the pancreas or islet grafts, leading these latter two groups to an earlier deterioration of the kidney graft, too. An increased number of rejection episodes was evident in the KP group only compared with KI group, probably due to the double induction who underwent the KI group.

Pancreas and islet transplantations confer adjunctive benefits for kidney graft survival in ESRD type 1 diabetic patients (6,12–14). It is possible that restoration of full endocrine function in the pancreas, or at least partial B-cell function, could lead to improved NOS expression in the kidney endocrine (14) and thereby ameliorate kidney vascular function. It is likely that simply having more stable glucose levels could be helpful for kidney graft, or, more suggestively, the partial restoration of C-peptide secretion by the transplanted islet can protect vascular function.
All the patients in the KI-s group had a long-term C-peptide secretion (>0.5 ng/ml for >1 year [12]). Insulin requirement is reduced by 50% in this group of patients, thus reducing hyperinsulinemia and ensuing insulin resistance, possibly also at the level of the vasculature. Insulin independence was achieved in 19 of 24 patients, with a mean duration of 2.15 ± 4.2 months. It is likely that this secretion is associated with improved global glycometabolic control. However, it is difficult to judge the distinct role on vascular function of C-peptide secretion per se and of the improved glycometabolic control.

In conclusion, KP and KI-s transplants, when successful, improved kidney graft survival and prevented the worsening of graft vascular function in our study population.

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References
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