Effects of Kidney-Pancreas Transplantation on Atherosclerotic Risk Factors and Endothelial Function in Patients With Uremia and Type 1 Diabetes

Paolo Fiorina,[1] Ennio La Rocca,[1] Massimo Venturini,[2] Fabio Minicucci,[3] Isabella Fermo,[4] Rita Paroni,[4] Armando D'Angelo,[4] Marisa Sblendido,[3] Valerio Di Carlo,[5] Marco Cristallo,[5] Alessandro Del Maschio,[2] Guido Pozza,[1] and Antonio Secchi[1]

Cardiovascular disease and the development of coronary artery disease play a pivotal role in increasing mortality in patients with type 1 diabetes. The aim of our study was to evaluate the effects of pancreas transplantation on atherosclerotic risk factors, endothelial-dependent dilation (EDD), and progression of intima media thickness (IMT) in patients with uremia and type 1 diabetes after kidney-alone (KA) or kidney-pancreas (KP) transplantation. A cross-sectional study comparing two groups of patients with type 1 diabetes was performed. Sixty patients underwent KP transplantation and 30 patients underwent KA transplantation. Age and cardiovascular risk profile were comparable in patients before transplantation. In all patients, atherosclerotic risks factors (lipid profile, fasting and post-methionine load plasma homocysteine, von Willebrand factor levels, D-dimer fragments, and fibrinogen) were assessed and Doppler echographic evaluation of IMT and endothelial function with flow-mediated and nitrate dilation of the brachial artery was performed. Twenty healthy subjects were chosen as controls (C) for EDD. Compared with patients undergoing KA transplantation, patients undergoing KP transplantation showed lower values for HbA_{1c} (KP = 6.2 ± 0.1% vs. KA = 8.4 ± 0.5%; $P < 0.01$), fasting homocysteine (KP = 14.0 ± 0.7 μmol/l vs. KA = 19.0 ± 2.0 μmol/l; $P = 0.02$), von Willebrand factor levels (KP = 157.9 ± 8.6% vs. KA = 212.5 ± 16.2%; $P < 0.01$), D-dimer fragments (KP = 0.29 ± 0.02 μg/ml vs. KA = 0.73 ± 0.11 μg/ml; $P < 0.01$), fibrinogen (KP = 363.0 ± 11.1 mg/dl vs. KA = 397.6 ± 19.4 mg/dl; NS), triglycerides (KP = 122.7 ± 8.6 mg/dl vs. KA = 187.0 ± 30.1 mg/dl; $P = 0.01$), and urinary albumin excretion rate (KP = 13.5 ± 1.9 mg/24 h vs. KA = 57.3 ± 26.3 mg/24 h; $P < 0.01$). Patients undergoing KP transplantation showed a normal EDD (KP = 6.21 ± 2.42%, KA = 0.65 ± 2.74%, C = 8.1 ± 2.1%; $P < 0.01$), whereas no differences were observed in nitrate-dependent dilation. Moreover, IMT was lower in patients undergoing KP transplantation than in patients undergoing KA transplantation (KP = 0.74 ± 0.03 mm vs. KA = 0.86 ± 0.09 mm; $P = 0.04$). Our study showed that patients with type 1 diabetes have a lower atherosclerotic risk profile after KP transplantation than after KA transplantation. These differences are tightly correlated with metabolic control, fasting homocysteine levels, lower D-dimer fragments, and lower von Willebrand factor levels. Normal endothelial function and reduction of IMT was observed only in patients undergoing KP transplantation. *Diabetes* 50:496–501, 2001

Increasing evidence shows that patients with type 1 diabetes are at high risk for several cardiovascular disorders, such as coronary artery disease, stroke, peripheral arterial disease, cardiomyopathy, and congestive heart failure (1). These risks are particularly high in patients with uremia and type 1 diabetes, even after kidney transplantation (2–4). A worsening of carotid lesion has been observed in patients undergoing kidney-alone (KA) transplantation (5). Proneness for accelerated atherosclerosis, hypertension, hyperinsulinemia, hyperhomocystinemia, and steroid treatment are the risk factors that could play a major role. Hyperhomocystinemia, high levels of von Willebrand factors (vWFs), a prothrombotic state, and endothelial-dependent dilation (EDD) are commonly involved in development of atherosclerosis, but their role in post-transplant status is not well known. In particular, endothelium modulates platelet adhesion, macrophage migration, lipid transport, and mitogenesis (6). Impairment of dilation ability is an important predictor of atherosclerosis and its complications (7). Diabetes, uremia, and hyperhomocystinemia are conditions with known increased proneness toward EDD (8). Kidney-pancreas (KP) transplantation in patients with type 1 diabetes seems to play a protective role in the progression of cardiovascular disease in these patients: a statistical reduction in mortality (at 7 years, KP = 76.2% vs. KA = 63.5%) is observed in patients undergoing KP transplantation (2,3). Our aim was to evaluate, through a cross-sectional study, the major risk factors for cardiovascular disease in patients with type 1 diabetes undergoing KA or KP transplantation.

RESEARCH DESIGN AND METHODS

Patients and transplantation. Ninety patients with uremia and type 1 diabetes were enrolled in the study. Sixty patients underwent KP transplantation (9). The group who underwent KA transplantation comprised patients

From the Departments of [1]Internal Medicine, [2]Radiology, [3]Neurophysiology, [4]Laboratory Medicine, and [5]General Surgery, San Raffaele Scientific Institute, Universitâ Vita e Salute, Milan, Italy.

Address correspondence and reprint requests to Antonio Secchi, MD, Medicina I, San Raffaele Scientific Institute, Via Olgettina 60, 20132, Milano, Italy. E-mail: antonio.secchi@hsr.it.

Received for publication 24 July 2000 and accepted in revised form 31 October 2000.

C, control; DDF, D-dimer fragments; EDD, endothelial-dependent dilation; IMT, intima media thickness; KA, kidney alone; KP, kidney-pancreas; tHcy, homocysteine; vWF, von Willebrand factor.

TABLE 1
Pretransplant characteristics of the two groups of patients with uremia and type 1 diabetes undergoing KA or KP transplantation

Parameters	KP (n = 60)	KA (n = 30)	P
Duration of dialysis (months)	31.5 ± 3.1	35.9 ± 3.3	NS
Duration of diabetes (years)	24.0 ± 0.7	24.0 ± 0.9	NS
Sex (M/F)	38/22	20/10	NS
HbA_{1c} (%)	10.9 ± 1.6	10.5 ± 1.9	NS
Creatinine (mg/dl)	8.6 ± 0.4	8.5 ± 0.6	NS
Hypertension (%)	100	100	NS
Triglycerides (mg/dl)	175.1 ± 9.5	180.5 ± 12.5	NS
Cholesterol (mg/dl)	222.6 ± 11.0	210.9 ± 8.8	NS

Data are means ± SE. NS, not significant.

in whom a pancreatic graft was lost early in the postoperative period (10 patients) or patients who underwent renal transplantation only (20 patients) because macroscopic damage of the pancreas at harvesting (30 patients). The clinical characteristics of the two patient populations were similar upon enrollment in the study (Table 1); exclusion criteria included history of stroke, major amputation, and severe dilated cardiomyopathy. Coronary artery disease was defined on the basis of resting electrocardiography, thallium-201 myocardial perfusion scintigraphy, and coronarography in patients with positive results on thallium-201 scintigraphy. None of the patients had a pathological ejection fraction, and none of the patients had characteristics compatible with any of New York Heart Association classifications for heart failure. In particular, cardiovascular conditions were similar among the patients enrolled in the study, and no differences were evident, such as prior myocardial infarction, lipid status, and smoking habit (Table 1). Patients with clear signs of systemic infection, lymphoproliferative disease, urinary infection, enhanced erythrocyte sedimentation velocity, or C-reactive protein were excluded from the study.

Organs for transplantation were obtained from cadaver donors through Nord Italia Transplant. All patients received the following immunosuppressive treatments: anti-thymoglobulins (IMTIX, SANGSTAT), cyclosporine 6 mg · kg^{-1} · day^{-1}, azathioprine 1 mg · kg^{-1} · day^{-1} and prednisone 10 mg/day^{-1}. Renal rejection was treated with 500-mg pulses of methylprednisolone. Cases of "steroid-resistant" rejection were treated with OKT3 or a course of anti-thymoglobulins (IMTIX, SANGSTAT). Patients undergoing KP transplantation were insulin-independent, whereas patients undergoing KA transplantation were on conventional subcutaneous insulin therapy (average 48 IU/day). Patients received long-acting insulin the evening before the test. Cyclosporine levels were within the therapeutic range.

Study design and laboratory assessment. For this cross-sectional study, only patients with transplantation follow-up longer than 1 year were selected. In all patients, the following data were evaluated in the fasting condition: creatinine, cyclosporine, blood glucose, GHb, serum C-peptide, serum free insulin, total cholesterol, triglycerides, folate, vitamin B_{12}, urinary albumin excretion rate, fasting plasmatic homocysteine (tHcy), post–methionine load tHcy, vWF levels, D-dimer fragments (DDF), and fibrinogen. Plasma vitamin B_{12} and folate levels were determined by chemiluminometric immunoassay (Ciba Corning ACS, Medfield, MA). Fasting tHcy levels were obtained after cleavage and reduction with sodium borohydride followed by derivatization with SBD-F (ammonium-7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate) (10). Levels of vWF were determined by an immunoelectrophoresis method based on the use of polyclonal goat IgG anti-human vWF (American Diagnostic, Greenwich, CT) (11). The other parameters were assessed with common laboratory kit assay. Blood pressure was measured three times with a sphygmomanometer with patients in the sitting position; the average of the last two measurements was recorded. Hypertension was considered systolic blood pressure of 140 mmHg or higher, diastolic blood pressure of 90 mmHg or higher, or use of an antihypertensive medication. The categories of medication assessed in this study were ACE inhibitors, vasodilatory agents, beta blockers, and calcium channel blockers (12). No lipid-lowering agents were administered. Blood pressure was recorded at each outpatient control (every ~3–6 months). Pancreatic function and renal function (GHb and serum creatinine) were tested during enrollment and every 6 months thereafter. Only patients with good graft function were included in the study.

Endothelial function evaluation. All patients underwent endothelial function assessment, and 20 healthy subjects were chosen as control subjects (mean age = 44.5 ± 1.5 years, sex (M/F) = 12/8, HbA_{1c} = 4.7 ± 0.2%, smokers = 25%, BMI = 24.5 ± 0.4 kg/m^2). EDD (e.g., produced by nitric oxide release) was assessed by evaluation of flow increase after hyperemia and studied by vasodilation mediated by substances that release exogenously nitric oxide (e.g., nitrates). Anteroposterior diameter and peak systolic velocity changes of the right brachial artery were evaluated as parameters of endothelial function. The vessel was studied with a color Doppler unit (ATL HDI 3,000, Bothell, WA) using a 7.5 linear probe in a longitudinal section above the antecubital fossa at the same location (at rest) after local hyperemia and after systemic vasodilation. The diameter of the artery was preferentially evaluated in B mode and flow velocity measurements were recorded (gate width = 1 mm) adjusting the color Doppler unit for medium or high flow settings: pulse-repetition frequency was variable from 3,000 to 4,000 Hz. An occluding forearm cuff placed 5 cm below the antecubital fossa was inflated to 50 mmHg above systolic pressure for 5 min and then released to induce reactive local hyperemia. Recordings were made 5, 60, and 120 s after onset of reactive hyperemia. Brachial artery diameter and flow velocity were measured before and 3 min after sublingual administration of nitroglycerin spray (400 mg). Blood pressure was recorded with an automated sphygmomanometer (LifeStat 200, Physio Control). All examinations were performed by the same operator (M.V.) at 8:00 A.M. with patients in fasting condition and in the supine position; care was taken to avoid artifacts by exerting minimal pressure, particularly on the brachial artery, which courses superficially. Angle correction was essential to obtain reproductive flow velocity measurements. In all patients, the arm without the shunt was used for testing. Our reproducibility for EDD performed in control subjects was similar to that reported in the literature, ranging from 2.5 to 3.0% (13), whereas the variability was lower for nitrate dilation.

Intima media thickness assessment. Ultrasonographic analysis of the carotid artery was performed with a high-resolution ultrasound scanner (Acuson 128 Xp/10) equipped with a linear array 3.5- to 5-MHz transducer. Optimal axial resolution was ~0.20 mm. The angle of examination was antero-oblique and lateral; we collected longitudinal images from both arteries and stored them with a color video printer. Subsequently, we transformed them in a digital format with an image scanner and calculated the distances. The analysis limited to the "far wall" of a restricted area of common carotid artery was at least 1 cm below the bifurcation; the start of the carotid bifurcation was evaluated as the point of loss of parallel configuration of the far and near wall.

All images were recorded by a single trained technician. All intima media thickness (IMT) data were analyzed in the same session by a single trained, blinded physician. IMT was defined as the distance from the leading edge of the lumen-intima interface and the leading edge of the media-adventitia interface of the far wall. The mean of the right and the left longitudinal common carotid artery IMT measurements was used in the analysis. Intraobserver variability varied between a mean ± SD difference of 0.02 ± 0.02 mm in selected and trained groups (14); this is quite similar to our reproducibility.

Statistical analyses. Student's t test for unpaired data and χ^2 test for categorical variables were used to compare data from the two groups of patients. If normal distribution was not present, the Mann-Whitney U test was used. The Spearman rank test was used to assess correlation between parameters. Furthermore, stepwise regression was used to evaluate the relationship among multiple variables. Data are expressed as means ± SE.

RESULTS

Metabolic and clinical characteristics during the study. No differences between the groups were evident with regard to age, number of rejection, episodes of infection, creatinine, cyclosporine, folate levels, vitamin B_{12} levels, and hematocrit or immunosuppresive treatment. All cardiovascular risks factors (duration of diabetes, duration of dialysis, total cholesterol, creatinine, age, hypertension, and smoking habits), except triglycerides, were similar in the two groups. Triglyceride levels were statistically higher in the patients undergoing KA transplantation (Table 2). Patients undergoing KP transplantation showed better GHb (KP = 6.2 ± 0.1% vs. KA = 8.4 ± 0.5%; $P < 0.01$) (Table 2). Insulin and blood glucose levels were significantly higher in the KA transplantation group (Table 2).

Atherothrombotic risk factors. A lower rate of hypertension was observed in patients undergoing KP transplantation than in those undergoing KA transplantation (NS), even if blood pressure was quite well controlled by anti-

TABLE 2
Clinical characteristics of the two groups of patients with uremia and type 1 diabetes undergoing KA or KP transplantation at the moment of the study

Parameters	KP (n = 60)	KA (n = 30)	P value
Age (years)	42.5 ± 1.0	45.3 ± 1.4	NS
Duration of treatment (years [range])	3.5 ± 0.3 (1.3–6.0)	3.7 ± 0.2 (1.5–6.1)	NS
Smoking habit (n)	21/60	12/30	NS
Hypertension (n)	38/60	20/30	NS
Systolic blood pressure (mmHg)	135.3 ± 4.5	138.2 ± 3.6	NS
Diastolic blood pressure (mmHg)	81.1 ± 2.0	85.2 ± 3.1	NS
Antihypertensive drugs assumed (n)	0.9 ± 0.1	1.3 ± 0.2	NS
Creatinine (mg/dl)	1.10 ± 0.04	1.2 ± 0.07	NS
Cyclosporine (ng/ml)	190.2 ± 7.8	187.2 ± 6.9	NS
Blood glucose (mg/dl)	90.2 ± 2.7	193.8 ± 14.2	<0.01
GHb (%)	6.2 ± 0.1	8.4 ± 0.5	<0.01
Serum C-peptide (ng/ml)	1.43 ± 0.08	0.02 ± 0.01	<0.01
Serum free insulin (μU/ml)	16.9 ± 2.2	26.0 ± 3.5	<0.01
Cholesterol (mg/dl)	214.0 ± 8.23	231.0 ± 11.7	NS
Triglycerides (mg/dl)	122.7 ± 8.6	187.0 ± 30.1	0.01
Folate (ng/dl)	6.8 ± 0.5	5.4 ± 0.5	NS
Vitamin B_{12} (pg/ml)	525.2 ± 41.3	626.4 ± 107.2	NS
Urinary albumin excretion rate (mg/24 h)	13.5 ± 1.9	57.3 ± 26.3	<0.01
Fasting tHcy (μmol/l)	14.0 ± 0.7	19.0 ± 2.0	0.02
Postload tHcy (μmol/l)	33.5 ± 2.6	33.8 ± 2.7	NS
vWF (%)	157.9 ± 8.6	212.5 ± 16.2	<0.01
D-dimer fragments (μg/ml)	0.29 ± 0.02	0.73 ± 0.11	<0.01
Fibrinogen (mg/dl)	363.0 ± 11.1	397.6 ± 19.4	NS

Data are means ± SE. NS, not significant.

hypertensive drugs (as shown by systolic and diastolic blood pressures in the normal ranges) (Table 2). Globally, patients in the KA transplantation group had been treated with more antihypertensive drugs than those in the KP transplantation group (NS). Patients in the KA transplantation group showed higher values for fasting but not post–methionine load of tHcy, vWF levels, DDF, and fibrinogen levels (Table 2). No differences between the two groups were evident regarding folate or vitamin B_{12} status, and only six patients in the KP transplantation group and four patients in the KA transplantation group (NS) received folate supplementation. The urinary albumin excretion rate was higher in the KA transplantation group than in the KP transplantation group (KA = 57.3 ± 26.3 mg/24 h vs. KP = 13.5 ± 1.9 mg/24 h; $P < 0.01$) (Table 2). Finally, tHcy levels were positively related to creatinine levels ($P < 0.01$).

Endothelial function. Patients in the KP transplantation group, but not the KA group, showed a normal EDD, whereas no differences in nitrate-dependent dilation were noted between the two groups (Fig. 1). EDD in the KA group was statistically lower than in the KP and control groups. In particular, 16 of 30 patients in the KA transplantation group and 9 of 60 patients in the KP transplantation group ($P = 0.01$, $\chi^2 = 6.4$, df = 1) showed a paradoxical vasoconstrictor response during the hyperemia test, a phenomenon described in the presence of severe EDD and diffuse atherosclerosis. EDD, in all patients, was negatively associated with tHcy ($P < 0.01$), vWF ($P < 0.01$), and total cholesterol ($P < 0.01$) levels; this was confirmed in both groups. Even considering the two groups separately, tHcy and vWF still seemed to be the major determinants for EDD, particularly in the KP transplantation group. No correlations were evident between EDD and blood glucose or free insulin levels.

IMT. IMT was lower in the KP transplantation group than in the KA transplantation group (KP = 0.74 ± 0.03 mm vs. KA = 0.86 ± 0.09 mm, $P = 0.04$), with a difference of more than 0.1 mm (the cutoff for an increased risk for myocardial infarction was 11%). Moreover, IMT was positively related to vWF ($P < 0.01$).

Subanalysis of the KA transplantation group. The KA transplantation group (30 patients) comprised patients in whom the pancreas graft was lost within a few days after transplantation (10 patients) and patients who in whom only the kidney was transplanted due to macroscopic damage of the pancreas at harvesting. Results observed in these two subgroups were similar (Table 3).

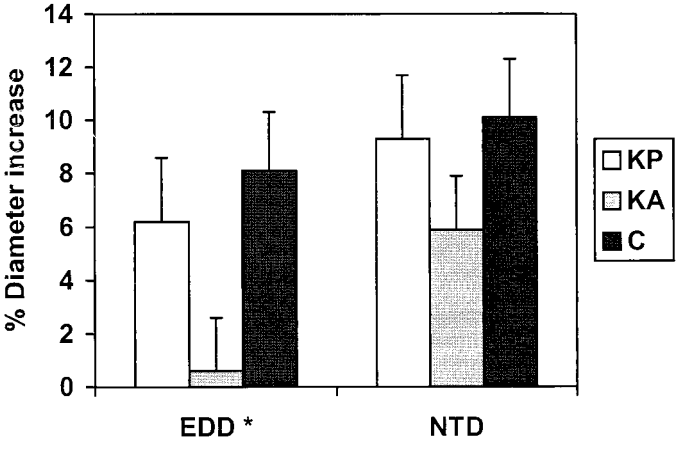

FIG. 1. EDD (%) and nitrate-dependent dilation (NTD; %) in patients with uremia and type 1 diabetes undergoing KP transplantation (n = 60, □), patients with uremia and type 1 diabetes undergoing KA transplantation (n = 30, ▨), and control subjects (C; n = 20, ■). * P < 0.01.

TABLE 3
Clinical characteristics of patients with uremia and type 1 diabetes undergoing kidney transplantation: comparison between KA due to macroscopic damage of pancreas at harvesting and KP failed in which the pancreas was lost within few days after transplantation

Parameters	KA (n = 20)	KP failed (n = 10)	P value
Age (years)	45.0 ± 1.5	45.7 ± 1.3	NS
Duration of treatment (years [range])	3.8 ± 0.4 (1.4–6.5)	3.6 ± 0.3	NS
Creatinine (mg/dl)	1.3 ± 0.1	1.1 ± 0.2	NS
Blood glucose (mg/dl)	180.0 ± 14.3	200.0 ± 12.1	NS
GHb (%)	8.7 ± 0.5	8.2 ± 0.4	NS
Cyclosporine (ng/ml)	198.0 ± 15.9	180.0 ± 9.6	NS
Fasting tHcy (μmol/l)	19.3 ± 2.1	18.8 ± 2.1	NS
vWF (%)	228.7 ± 20.5	200.1 ± 12.5	NS
Cholesterol (mg/dl)	215.0 ± 13.9	245.1 ± 12.0	NS
Triglycerides (mg/dl)	196.8 ± 21.9	180.7 ± 25.3	NS
IMT (mm)	0.85 ± 0.05	0.86 ± 0.07	NS
EDD (%)	0.16 ± 1.06	0.80 ± 2.01	NS

Data are means ± SE. NS, not significant.

DISCUSSION

Our study shows that markers of prothrombotic state (vWF, DDF, tHcy, and fibrinogen) are lower in patients with type 1 diabetes after KP transplantation than in patients with type 1 diabetes after KA transplantation. Normal EDD and reduction of IMT was observed in patients with type 1 diabetes who had undergone KP transplantation, but not in those who had undergone KA transplantation. KP transplantation seems to play a protective role against atherosclerotic risk factors and progression of macroangiopathy when compared with KA transplantation.

Atherothrombotic risk factors.

tHcy. Hyperhomocystinemia is common in patients who have undergone kidney transplantation, patients undergoing dialysis, and patients with type 1 diabetes (15,16), conditions that were or had been present in the patients in our study. tHcy, which is a well-established risk factor for atherosclerosis in patients with uremia or diabetic microalbuminuria (16), can be an important factor in worsening of coronary artery disease in patients who have undergone kidney transplantation (17). There is increasing evidence that tHcy may affect the coagulation system and the resistance of the endothelium to thrombosis and that it may interfere with the vasodilator and antithrombotic functions of nitric oxide (18). Hyperhomocystinemia impairs EDD in human resistance and conduit vessels (8). It is interesting to note that tHcy levels >20 μmol/l are associated with a fourfold increase in total mortality in patients with coronary artery disease (18). Lower tHcy levels were observed in the KP transplantation group than in the KA transplantation group, and the reason is unclear. This could be because of initial impairment of renal function, as shown by the urinary albumin excretion rate in the KA transplantation group, by the worst glycometabolic control in the KA transplantation group (as shown by high HbA_{1c} levels), or by these two variables, which are known to be interdependent (19). Another hypothesis is that the hepatic tHcy metabolic pathway could act differently in both the KA and KP transplantation patients. In particular, it has been observed that insulin-treated diabetic rats showed a reduction in hepatic trans-sulfuration enzymes (cystathionine β-synthase and cystathionine γ-lyase) with reduction of tHcy catabolism (20), suggesting that tHcy could be linked to insulin levels. Lower serum insulin levels and improved insulin sensitivity was shown in KP transplantation patients, compared with patients in the KA transplantation group with type 1 diabetes or those undergoing dialysis, although these parameters were not fully in the normal range (21). Our data confirmed the presence of higher fasting insulin levels in the KA transplantation group than in the KP transplantation group.

vWF levels and prothrombotic state. Regarding higher levels of vWF found in the KA transplantation group, it is interesting to note that the formation of platelet thrombi is needed as a defense mechanism, but may induce conditions such as myocardial infarction, coronary artery disease, and ischemic stroke (22). vWF is a glycoprotein that is synthesized in endothelial cells and megakaryocytes as a precursor and stored as a multimer in specific organelles (Weibel-Palade bodies in endothelial cells and α-granules in megakaryocytes). The function of vWF is the promotion of thrombus formation by mediating adhesion of platelets to the injured vessel wall (23). vWF is involved in the development of atherosclerotic lesions. High plasma levels of the protein have been found to be an independent risk factor for recurrent myocardial infarction and death (24). Endothelial cells are the origin of all circulating vWF, and increased plasma levels may reflect the extent of vascular damage. The presence of enhanced levels of DDF and fibrinogen in patients in the KA transplantation group, without signs of infections (e.g., enhanced white blood counts, C-reactive protein, erythrocyte sedimentation velocity), confirmed the presence of a prothrombotic state. KP transplantation could present lower values of vWF, probably for the concomitant lower tHcy levels, for the better glycometabolic and lipid profile; all of these factors could induce endothelial damage with vWF release.

Endothelial function. Endothelial function is impaired in KA transplantation versus KP transplantation. It is well known that endothelial function reflects the ability of endothelium to produce nitric oxide; this ability is profoundly impaired in patients with uremia (25). Brachial artery atherosclerosis correlates with coronary and carotid atherosclerosis (26). Forearm endothelial function is impaired in patients with established coronary atherosclerosis (26). Therefore, the brachial circulation seems to be

a reasonable surrogate for the study of coronary circulation (26–28). In addition to vascular tone, the endothelium regulates cell adhesion, platelet aggregation, coagulation, lipid transport and oxidation, inflammation, and mitogenesis (6). Changes in EDD precede structural changes during experimental induction or regression of atherosclerosis in monkeys (7). Endothelial function in patients who have undergone KP transplantation is normal; this could be the result of the reduction of the atherothrombotic state. Moreover, it seems likely that hyperglycemia per se could only partially account for the differences in endothelial function between patients who have undergone either KP or KA transplantation. A recent study showed that patients with type 1 diabetes of recent onset without complications (except hyperglycemia) did not have endothelial dysfunction (29). Finally, in our study, no correlations regarding blood glucose levels and EDD were evident between the KA and KP transplantation groups.

IMT and cardiovascular risk. Higher values of IMT present in the KA transplantation group could be an important index of future coronary and cerebral ischemic diseases. It is known that intimal thickening precedes atherosclerotic lesions: for each 0.1 mm of IMT, the risk of acute myocardial infarction increases by 11% (30). Therefore, IMT could be used as an intermediate point for cardiovascular disease (31). This suggests an important proneness toward cardiovascular events in patients who have undergone KP transplantation. Increased IMT could be the final step of the cascade induced by the enhanced prothrombotic state. Our study confirmed the importance of hyperglycemia and related conditions in inducing impaired microvascular and macrovascular reactivity and blood/plasma viscosity (32,33).

Limitations of the study. The major limitation of this study is the use of a cross-sectional approach. Although the two populations were homogeneous before transplantation, as far as cardiovascular status is concerned, prothrombotic status and endothelial function were not specifically studied before transplantation.

Conclusions. This study shows a lower prothrombotic state in patients with type 1 diabetes who undergo KP transplantation than in patients with type 1 diabetes who undergo KA transplantation. This has direct consequences on endothelial function and IMT. From these data, it seems that KP transplantation is protective (compared with KA transplantation) against atherosclerosis progression. It is noteworthy that these results were achieved after a relatively short follow-up, confirming the importance of pancreas transplantation on macroangiopathy. This protection is reached through an amelioration of glycometabolic profile, a lower prothrombotic state, normal endothelial function, and reduced IMT.

ACKNOWLEDGMENTS

This work was partially supported by a Ministero per l'Università a la Ricerca Scientifica grant.

We thank Dr. Tara Zoll-Folli for English editing and Dr. Franco Folli for suggestions.

REFERENCES

1. Diabetes mellitus: a major risk factor for cardiovascular disease. A joint editorial statement by the American Diabetes Association; the National Heart, Lung, and Blood Institute; the Juvenile Diabetes Foundation International; the National Institute of Diabetes and Digestive and Kidney Diseases; and the American Heart Association. *Circulation* 100:1132–1133, 1999
2. Secchi A, Caldara R, La Rocca E, Fiorina P, Di Carlo V: Cardiovascular disease and neoplasm after pancreas transplantation. *Lancet* 352:65, 1998
3. Tyden G, Bolinder J, Solders G, Brattstrom C, Tibell A, Groth CG: Improved survival in patients with insulin-dependent diabetes mellitus and end-stage diabetic nephropathy 10 years after combined pancreas and kidney transplantation. *Transplantation* 5:645–648, 1999
4. Ibels LS, Stewart JH, Mahony JF, Sheil AG: Deaths from occlusive arterial disease in renal allograft recipients. *BMJ* 3:552–554, 1974
5. La Rocca E, Minicucci F, Secchi A, Ciurlino D, Bonfatti D, Ferrari G, Castoldi R, Di Carlo V, Pozza G: Evolution of carotid vascular lesions in kidney-pancreas and kidney-alone transplanted insulin-dependent diabetic patients. *Transplant Proc* 6:3072, 1995
6. Ross R: The pathogenesis of atherosclerosis: a perspective for the 1990's. *Nature* 362:801–809, 1993
7. Benzuly KH, Padgett RC, Kaul S, Piegors DJ, Armstrong ML, Heistad DD: Functional improvement precedes structural regression of atherosclerosis. *Circulation* 89:1810–1818, 1994
8. Fiorina P, Lanfredini M, Montanari A, Pega MG, Veronelli A, Mello A, Astorri E, Craveri A: Plasma homocysteine and folate are related to arterial blood pressure in type 2 diabetes mellitus. *Am J Hypertens* 11:1100–1107, 1998
9. Prieta M, Sutherland DER, Goete FC, Rosenberg ME, Najarian JS: Pancreas transplant results according to the technique of duct management: bladder versus enteric drainage. *Surgery* 84:633–639, 1987
10. Fermo I, Arcelloni C, Mazzola G, D'Angelo A, Paroni R: High-performance liquid chromatographic method for measuring total plasma homocysteine levels. *J Chromatogr Biomed Sci Appl* 719:31–36, 1998
11. Silveira AM, Yamamoto T, Adamson L, Hessel B, Blombäck B: Application of an enzyme-linked immunosorbent assay (ELISA) to von Willebrand factor (vWF) and its derivatives. *Thromb Res* 43:91–102, 1986
12. Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the sixth report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. *Arch Intern Med* 157:2431–2446, 1997
13. Uehata A, Lieberman EH, Gerhard MD, Anderson TJ, Ganz P, Polak JF, Creager MA, Yeung AC: Noninvasive assessment of endothelium-dependent flow-mediated dilation of the brachial artery. *Vasc Med* 2:87–92, 1997
14. Kanters SDJM, Algra A, van Leeuwen MS, Banga JD: Reproducibility of in vivo carotid intima-media thickness measurements: a review. *Stroke* 28:665–671, 1997
15. Arnadottir M, Hultberg B, Vladov V, Nilsson-Ehle P, Thysell H: Hyperhomocysteinemia in cyclosporine-treated renal transplant recipients. *Transplantation* 61:509–512, 1996
16. Lanfredini M, Fiorina P, Peca MG, Veronelli A, Mello A, Astorri E, Dall'Aglio P, Craveri A: Fasting and post-methionine load homocyst(e)ine values are correlated with microalbuminuria and could contribute to worsening vascular damage in non-insulin-dependent diabetes mellitus. *Metabolism* 47:915–921, 1998
17. Lindholm A, Albrechtsen D, Frödin L, Tufveson G, Persson NH, Lundgren G: Ischemic heart disease: major cause of death and graft loss after renal transplantation in Scandinavia. *Transplantation* 60:451–457, 1995
18. Nygard O, Nordrehaug JE, Refsum H, Ueland PM, Farstad M, Vollset E: Plasma homocysteine levels and mortality in patients with coronary artery disease. *N Engl J Med* 337:230–236, 1997
19. Fioretto P, Steffes MW, Sutherland DER, Goetz FC, Mauer M: Reversal of lesions of diabetic nephropathy after pancreas transplantation. *N Engl J Med* 339:69–75, 1998
20. Jacobs RL, House JD, Brosnan ME, Brosnan JT: Effects of streptozotocin-induced diabetes and of insulin treatment on homocysteine metabolism in the rat. *Diabetes* 47:1967–1970, 1998
21. Luzi L, Secchi A, Facchini F, Battezzati A, Staudacher C, Spotti D, Castoldi R, Ferrari G, Di Carlo V: Reduction of insulin resistance by combined kidney-pancreas transplantation in type 1 (insulin-dependent) diabetic patients. *Diabetologia* 33:549–556, 1990
22. Fuster V, Badimon L, Badimon JJ, Chesebro JH: The pathogenesis of coronary artery disease and the acute coronary syndromes (1). *N Engl J Med* 326:242–250, 1992
23. Ruggeri ZM: von Willebrand factor. *J Clin Invest* 100 (Suppl.):S41–S46, 1997
24. Jansson JH, Nilsson TK, Johnson O: Von Willebrand factor in plasma: a novel risk factor for recurrent myocardial infarction and death. *Br Heart J* 66:351–355, 1991
25. Wever R, Boer P, Hijmering M, Stroes E, Verhaar M, Kastelein J, Versluis

K, Lagerwerfl F, van Rijn H, Koomans H, Rabelink T: Nitric oxide is reduced in patients with chronic renal failure. *Arterioscler Thromb Vasc Biol* 19:1168–1172, 1999
26. Kanani P, Sinkey CA, Browning RL, Allaman M, Knapp HR, Haynes WG: Role of oxidant stress in endothelial dysfunction produced by experimental hyperomocysteinemia in humans. *Circulation* 100:1161–1168, 1999
27. Sorensen KE, Kristensen IB, Celermaier DS: Atherosclerosis in the human brachial artery. *J Am Coll Cardiol* 29:318–322, 1997
28. Anderson TJ, Uehata A, Gerhard MD, Meredith IT, Knab S, Delagrange D, Lieberman EH, Ganz P, Creager MA, Yeung AC: Close relation of endothelial function in the human coronary and peripheral circulations. *J Am Coll Cardiol* 26:1235–1241, 1995
29. Enderle MD, Benda N, Schmuelling RM, Haering HU, Pfohl M: Preserved endothelial function in IDDM patients, but not in NIDDM patients, compared with healthy subjects. *Diabetes Care* 21:271–277, 1998
30. Salonen JT, Salonen R: Ultrasound B-mode imaging in observational studies of atherosclerotic progression. *Circulation* 87(Suppl. II):56–65, 1993
31. Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobbee DE: Common carotid intima-media thickness and risk of stroke and myocardial infarction. *Circulation* 96:1432–1437, 1997
32. Caballero AE, Arora S, Saouaf R, Lim SC, Smakowski P, Park JY, King GL, LoGerfo FW, Horton ES, Veves A: Microvascular and macrovascular reactivity is reduced in subjects at risk for type 2 diabetes. *Diabetes* 48:1856–1862, 1999
33. Mellinghoff AC, Reininger AJ, Wurzinger LJ, Landgraf R, Hepp KD: Impact of pancreas and kidney transplantation on determinants of blood and plasma viscosity. *Clin Hemorheol Microcirc* 18:175–184, 1998