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Articles

Effects of pancreas transplantation on glomerular structure in insulin-dependent diabetic patients with their own kidneys

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Summary

Pancreas transplantation prevents or retards development of early diabetic glomerular lesions in renal allografts transplanted to patients with insulin-dependent diabetes mellitus (IDDM), but its effect on established renal lesions in native kidneys of such patients is unknown.

Renal biopsy samples were taken before and 5 years after pancreas transplantation from 13 non-uraemic IDDM patients and compared with baseline and 5-year biopsy samples from 10 persistently hyperglycaemic IDDM patients who did not undergo transplantation. The two groups were similar in age, duration of diabetes, metabolic control, renal function, and blood pressure. Glomerular structures were measured by standard morphometric techniques. Haemoglobin A_1 concentrations fell to within the normal range after pancreas transplantation but did not change in the comparison group. Glomerular basement membrane width did not significantly change in either group. Glomerular volume decreased and mesangial fractional volume increased in the pancreas transplant recipients but there was no significant change in total mesangial volume over 5 years. By contrast, both glomerular volume and mesangial fractional volume increased in the comparison patients, resulting in increased total mesangial volume.

Diabetic glomerular lesions in patients with their own kidneys were not ameliorated by pancreas transplantation, despite 5 years of normoglycaemia. Pancreas transplantation can correct severe metabolic instability and thus improve quality of life, but it cannot yet be recommended for the treatment of established lesions of diabetic nephropathy.

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Introduction

Whole pancreas or islet transplantation can partly reverse early diabetic nephropathic lesions in animals.[1,2] In people with insulin-dependent diabetes mellitus (IDDM) who have received renal allografts, pancreas transplantation prevents or slows progression of the earliest glomerular lesions.[3,4] However, nothing is known about the effects of long-term normoglycaemia on well-established diabetic glomerular lesions. We have studied glomerular structure in 13 IDDM patients with their own kidneys, before and 5 years after successful pancreas transplantation. A comparison group of 10 IDDM patients who did not receive pancreas transplants were assessed over a similar time.

Patients and methods

The study was approved by the committee for the use of human subjects in research of the University of Minnesota. Patients spent a week in the Clinical Research Center at the University of Minnesota for pretransplant assessment. At least three 24 h urine collections were made for measurements of creatinine clearance and urinary albumin excretion rate. Blood pressure was repeatedly measured by nursing staff. Percutaneous kidney biopsy was done. Glycosylated haemoglobin (HbA_1) values were measured. Detailed examinations of the eyes and nervous system were done in all patients and are reported elsewhere.[5,6]

The 13 IDDM patients in the pancreas transplantation group (table) were all on insulin therapy. The patients underwent segmental or whole-organ pancreatic transplantation.[7] 5 patients received cadaveric pancreases, and 8 grafts from living related donors. All patients were given immunosuppression with prednisone, azathioprine, and cyclosporin.[7] Patient 13 received cyclosporin only for 1 year after pancreas transplantation; all others were on this drug for the 5 years of the study. Insulin was discontinued after transplantation in all 13 patients. The patients were followed up for 5 years. 1 patient had rejection of the pancreatic graft and reinstitution of insulin therapy. A second graft was successfully transplanted 3 months later. Renal function tests and metabolic indices were restudied 1, 2, 3·5, and 5 years after pancreas transplantation but only baseline and 5-year data are reported here. Renal biopsies were done a mean of 2·0 (SD 0·4) and 5·0 (0·3) years after transplantation. Patients 10 and 13 underwent kidney transplantation 7·7 and 6·3 years, respectively, after the pancreas operation.

The 10 patients in the comparison group (table) had renal function studies and kidney biopsy as part of assessment for possible pancreas transplantation. 7 never received a pancreatic allograft; 3 received grafts that failed within 6 weeks. These patients, still diabetic and receiving insulin, volunteered for renal function studies and kidney biopsy 5 (mean 5·4 [1·4]) years after the first assessment. The two study groups were similar in age, age at onset, and duration of IDDM (table). Metabolic control at the first assessment was also similar in comparison and transplant groups (HbA_1 11·3 [1·9] *vs* 10·5 [1·4]) as were creatinine clearance, mean blood pressure, and urinary albumin excretion rate (table).

HbA_1 was measured by BioRad column assay until November, 1986, and by high-performance liquid chromatography thereafter

Patient	Sex	Age (yr)*	Age at onset (yr)	IDDM duration (yr)*	Urinary albumin excretion (mg per 24 h)		Mean blood pressure (mm Hg)	
					Baseline	Follow-up	Baseline	Follow-up
Transplant group								
1	M	33	13	20	7	2	82	95
2	M	42	18	24	8	4	87	88
3	F	31	16	15	8	4	85	81†
4	F	44	18	26	9	12	90	80†
5	F	33	9	24	12	21	83	90†
6	F	35	13	22	86	6	84	87†
7	M	30	7	23	120	80	94	91†
8	F	33	16	17	127	155	94†	104†
9	F	31	4	27	278	126	95	102†
10	M	26	14	12	380	2860	100	111†
11	M	17	6	11	804	610	86	87†
12	F	38	9	29	1276	40	90	82†
13	M	27	7	20	2900	1913	111†	103†
Mean (SD)‡		32 (7)	11 (5)	21 (5)	120	40	91 (8)	92 (10)
Comparison group								
1	F	22	12	10		19	84	88
2	F	26	19	7	6	15	78	89
3	F	32	5	27	6	67	83	76
4	F	24	10	14	10	13	79	78
5	F	26	14	12	10	14	78	79†
6	M	48	21	27	12	5	86	82
7	F	25	10	15	22	15	85	88
8	F	22	6	16	68	1200	92	90†
9	F	39	16	23	242	2462	86†	79†
10	F	14	1	13	280	613	95	97†
Mean (SD)‡		28 (10)	11 (6)	16 (7)0	12	19§	85 (6)	85 (7)

* At baseline. † Receiving antihypertensive drugs. ‡ Except for urinary albumin excretion rate, which was logarithmically transformed for analysis; median is given. §p = 0·056, follow-up vs baseline, paired *t* test.

Table: **Demographic and clinical details of study patients**

(BioRad Diamat, Biorad, Hercules, California, USA). All values are expressed as total HbA_1 (normal range 5·4–7·4%). Serum and urine creatinine concentrations were measured by an automated kinetic method that uses the Jaffé reaction; the normal range for creatinine clearance is 90–130 mL/min per 1·73 m². Urinary albumin excretion was measured by nephelometry (Beckman Instruments, Fullerton, California, USA); normal values are below 22 mg per 24 h. Values for systolic and diastolic blood pressure represent the means of many measurements during the hospital stay. Mean blood pressure is the diastolic value plus one third of the pulse pressure.

Renal tissue obtained by percutaneous biopsy was processed for light and electron microscopy morphometry.[8] Measurements were made by a single observer (PF), who was unaware of the patient's identity. A mean of 4 (SD 1) glomeruli per specimen were examined by electron microscopy on all baseline and 5-year samples. Glomeruli were photographed with a JEOL/100 CX electron microscope at a magnification of × 3900 to produce photomontages of the entire glomerular profile, defined as the circumscribed, minimal convex polygon enclosing the glomerular tuft. The montages were used to estimate mesangial fractional volume (mesangial volume as a proportion of glomerular volume).[8,10] Total mesangium per glomerulus was calculated by multiplying the mesangial fractional volume by the mean glomerular volume. Another set of micrographs, photographed at × 12 000 by entering the glomerulus at its lowest segment and systematically sampling about 40% of the glomerular profile, was used to estimate glomerular basement membrane width by the orthogonal intercept method.[11]

As well as the baseline and 5-year samples in both groups, biopsy material taken at 2 years from the transplant recipients was examined by light microscopy after staining with periodic-acid-Schiff. The index of mesangial expansion was determined semiquantitatively. We have previously shown a high correlation ($r = 0·86$, $p < 0·0005$) between this semiquantitative estimate and electron-microscopic measures of mesangial fractional volume.[12] The mean volume of non-sclerosed glomeruli was estimated at a magnification of × 150 by the method of Weibel and Gomez.[13] The normal ranges for the structural measurements were calculated from 66 healthy kidney donors matched for age and sex with the IDDM patients.

Urinary albumin excretion rates were not normally distributed so were logarithmically transformed before analysis. Most comparisons used a two-factor analysis of variance (ANOVA) with treatment (pancreas transplant *vs* untreated) and time (baseline and 5-year biopsy samples) as the factors. When a significant interaction between treatment and comparison group was shown by ANOVA, paired *t* tests were done. At baseline the two groups were similar in terms of age, duration of IDDM, and HbA_1. The only significant structural difference between groups at baseline was in glomerular volume; however, there was a trend in the pancreas transplant group for more advanced renal disease, as suggested by measures of renal function and some of the morphometric variables. We therefore repeated the statistical analyses of each structural variable with covariance analysis, factoring for either the baseline value of that variable or for the baseline factors age, duration of IDDM, HbA_1, mesangial fractional volume, and glomerular volume. These additional analyses did not affect group comparisons; thus only the two-factor ANOVAs are reported. Statistical significance was set at $p < 0·02$ because we did so many comparisons; p values between 0·05 and 0·02 were considered to approach significance. Data are expressed as mean (SD), except for urinary albumin excretion rates (median).

Results

In the transplant group HbA_1 was significantly lower at 5 years than at the baseline pretransplant evaluation (10·5 [1·4] *vs* 6·6 [0·7]%, $p < 0·0001$). By contrast, there was no change in HbA_1 in the comparison group (11·3 [1·9] *vs* 11·8 [2·2]%). Creatinine clearance fell in the transplant recipients (from 102 [21] to 68 [24] mL/min per 1·73 m², $p < 0·0001$) but was virtually unchanged in the comparison group (102 [20] *vs* 91 [26] mL/min per 1·73 m²). Serum creatinine increased from 88·4 (26·5) to 123·8 (53·0) µmol/L ($p < 0·004$) after pancreas transplantation but did not change in the comparison group (79·6 [8·8] *vs* 79·6 [17·7] µmol/L). Neither urinary albumin excretion nor mean blood pressure changed significantly in either group (table). However, urinary albumin excretion increased in the 3 comparison patients (8, 9, and 10) whose rates were abnormal at baseline, and patient 3 became microalbuminuric.

There was no significant change between baseline and 5-year follow-up in glomerular basement membrane width in either the pancreas transplant group (603 [139] *vs* 565 [111] nm, $p = 0·07$) or the comparison group (594 [151] *vs* 609 [160] nm, $p > 0·1$). Furthermore, there were no differences between the groups. Mesangial fractional volume increased in both the pancreas transplant (from 0·33 [0·08] to 0·38 [0·11], $p = 0·008$), and the comparison groups (from 0·29 [0·05] to 0·35 [0·07], $p = 0·004$; ANOVA, $p = 0·03$; figure, A), and there were no differences between the groups. ANOVA revealed an interaction for glomerular volume ($p = 0·002$). Further analysis showed that baseline glomerular volume was greater in the pancreas transplant group than in the comparison group ($p = 0·01$). Mean glomerular volume decreased after pancreas transplantation (from 2·13 [0·63] to 1·74 [0·32] × 10^6 µm³, $p = 0·04$), but increased in the comparison group (from 1·52 [0·40] to 2·06 [0·57] × 10^6 µm³, $p = 0·002$; figure, B). Mean glomerular volume was smaller 2 years after transplantation than at baseline (1·82 [0·48] *vs* 2·13 [0·63] × 10^6 µm³, $p = 0·02$), and similar to the 5 year value. ANOVA also revealed an interaction for the total volume of mesangium per glomerulus ($p = 0·04$), which increased in the comparison patients (from 0·45 [0·17] to 0·75 [0·40] × 10^6

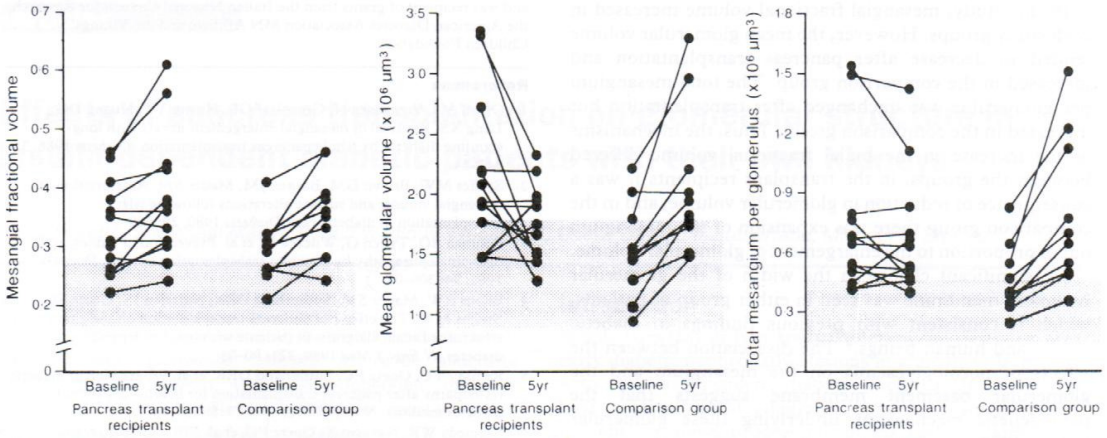

Figure: **Mesangial fractional volume (A), mean glomerular volume (B), and total mesangium per glomerulus (C) in pancreas transplant recipients and comparison patients at baseline and 5-year follow-up**

Shaded area represents normal range (mean ± 2 SD) in our laboratory.

µm³, p = 0·003), but did not change in the transplant group (0·72 [0·37] *vs* 0·67 [0·30] × 10⁶ µm³, p > 0·1; figure, C). Moreover, we found no change in the index of mesangial expansion by light microscopy between 2 and 5 years after pancreas transplantation (1·8 [0·9] *vs* 1·7 [0·9]).

Discussion

5 years of normoglycaemia after successful pancreas transplantation did not reverse established diabetic glomerular lesions in patients with IDDM of long duration with their own kidneys. Further, there was no clear benefit of euglycaemia on glomerular structure when the transplant recipients were compared with similar patients who had not undergone transplantation. This finding contrasts with the rapid reversal of early mesangial expansion in diabetic rats rendered normoglycaemic by islet transplantation.[1,2] Perhaps the earlier stages of diabetic nephropathy are reversible, and later lesions are not. Alternatively, the nature of mesangial expansion in human beings with IDDM and rats with streptozotocin-induced diabetes may differ. It is possible that normal and, perhaps especially, glycosylated matrix glycoproteins have a very slow turnover in diabetes,[14] and that a longer follow-up study might detect a beneficial effect of normoglycaemia. It is also possible that glucose induces long-lasting changes in renal cells, resulting in continued overproduction[15] or decreased degradation of matrix glycoproteins, despite the new euglycaemic state. Our studies do not allow further investigation of these possibilities.

It is unlikely that immunosuppressant drugs concealed beneficial effects of euglycaemia. Bohman et al[3] reported that patients with simultaneous pancreas and kidney transplantation, some of whom received cyclosporin, had no lesions of diabetic glomerulopathy 2 years after transplantation. Furthermore pancreas transplantation in IDDM patients, an average of 4·2 years after kidney transplantation, prevented or slowed progression of early diabetic mesangial lesions whether or not the patients received cyclosporin.[4] These patients also had smaller glomeruli than IDDM patients who received only renal allografts. Similarly, pancreas or islet transplantation without immunosuppression reversed mesangial lesions[1,2] and produced normal glomerular volume[2] in diabetic rats. Thus, reversal of the diabetic state could in itself result in a decrease in glomerular volume. Preliminary comparative data from diabetic renal transplant recipients who did or did not receive cyclosporin show that the drug has no effect on the rate of mesangial expansion[16] or glomerular hypertrophy (unpublished). Moreover, although the distribution of the volumes of individual glomeruli was wider in heart transplant recipients on cyclosporin, mean glomerular volume was no different from that in healthy subjects.[17,18]

We have previously argued that increased mesangial fractional volume is a crucial structural abnormality in diabetic glomerulopathy.[9,12] Most patients in this study had mesangial expansion at baseline, and mesangial fractional volume continued to increase during follow-up in both study groups. The increase was detectable not only in patients with severe glomerulopathy and clinical features of overt nephropathy at baseline, but also in those with normal renal function and normal or near-normal baseline values. Mesangial expansion can be expressed as mesangial fractional volume (percent of the glomerulus occupied by the mesangium) or as calculated total mesangium per glomerulus (mesangial fractional volume × mean glomerular volume). Mesangial fractional volume provides important information on the architecture of the glomerulus and correlates well with peripheral capillary filtration surface per glomerulus,[19] because glomerular hypertrophy occurring in parallel with mesangial expansion can obviate the effect of mesangial expansion on the contiguous glomerular capillaries, thus sparing the filtration surface and glomerular filtration rate. We have found previously that in patients who develop clinical diabetic nephropathy, those with a longer duration of IDDM before development of nephropathy have larger glomeruli than those with shorter durations of IDDM.[20] In that study, both groups had similarly increased mesangial fractional volume, but the total mesangium was greater in the longer-duration group.[20] Thus, expansion of the mesangium out of proportion to glomerular size is more closely related to the development of clinical diabetic nephropathy than is absolute volume of the mesangium per glomerulus.

In this study, mesangial fractional volume increased in both study groups. However, the mean glomerular volume tended to decrease after pancreas transplantation and increased in the comparison group. The total mesangium per glomerulus was unchanged after transplantation but increased in the comparison group. Thus, the mechanisms of the increase in mesangial fractional volume differed between the groups; in the transplant recipients it was a consequence of reduction in glomerular volume, and in the comparison group there was expansion of the mesangium out of proportion to the enlargement of glomerular volume.

No significant change in the width of the glomerular basement membrane was seen in either group of patients, which is consistent with previous findings in diabetic rats[2,21] and human beings.[4] The dissociation between the effects of normoglycaemia on the mesangium and the glomerular basement membrane suggests that the pathogenetic mechanisms underlying these glomerular lesions differ.

It is difficult to assess the importance of the glomerular structural alterations over 5 years in the pancreas transplant recipients. The findings on both glomerular volume and mesangial expansion showed little change between 2 and 5 years. These data could be describing an early decrease in glomerular volume leading to relative mesangial expansion by 2 years, followed by constant glomerular structure thereafter. They could be interpreted as showing a benefit of pancreas transplantation, in leading to stability of diabetic glomerular lesions, rather than progressive diabetic nephropathology despite reversal of the diabetic state. This issue will require longer follow-up of both groups of patients.

Renal functional measures were not helpful in showing whether pancreas transplantation had an effect on the clinical progression of diabetic nephropathy, because of the confounding renal effects of cyclosporin given only to the transplant recipients. These patients had a significant decline in glomerular filtration rate and an increase in serum creatinine. The reduction in glomerular filtration rate occurred during the first year after transplantation and it did not change thereafter, except in 2 patients who had low rates before transplantation and in whom there was a progressive decline.[22] Thus, functional outcomes cannot be compared in the two groups of patients, and we have focused on the structural data.

Given the equivocal benefits of euglycaemia on the established lesions of diabetic nephropathy, as well as the potential nephrotoxic effects of long-term cyclosporin therapy,[23] pancreas transplantation cannot be recommended as a means of treating diabetic nephropathy in the native kidneys of long-term IDDM patients. Development of non-nephrotoxic immunosuppressive agents and studies with longer follow-up could alter this conclusion. Our present policy is to select non-uraemic IDDM patients with their own kidneys for pancreas transplantation primarily because of severe metabolic instability seriously affecting the patient's quality of life and well being,[24,25] rather than on the potential for the operation to arrest or reverse renal or eye[5] complications.

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