Albuminuria after fetal pancreatic islet transplantation: a 10-year follow-up

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Abstract

**Aim of the study.** The prevention of diabetic nephropathy is as yet an unresolved issue. The aim of our study was to assess the effects of transplantation of long-term cultured and cryopreserved fetal pancreas islets on metabolic control and the development of diabetic nephropathy.

**Methods.** Serum C-peptide, glucose, HbA1c, insulin requirements, urinary albumin excretion rate, and blood pressure of 10 insulin-dependent diabetic patients after transplantation were compared with a group of 27 insulin-dependent diabetic controls on insulin therapy only during a 10-year follow-up. Blood glucose and HbA1c were significantly (P < 0.05) lower than in the controls. Mean albumin excretion rates of the transplant and allograft rejection and the side-effects of immunosuppression [3].

**Results.** In the first year after transplantation mean insulin requirement decreased from 53.6 ± 2.2 to 35.8 ± 1.2 units. C-peptide levels appeared (0.55 ± 0.08 ng/ml) and remained detectable throughout the follow-up. Blood glucose and HbA1c were significantly (P < 0.05) lower than in the controls. Mean albumin excretion rates of the transplant and control groups during the follow-up were 18.8 ± 8.5 and 11.7 ± 2.0, 16.6 ± 6.6 and 14.0 ± 2.3, 15.0 ± 5.0 and 15.1 ± 2.7, 15.3 ± 7.5 and 20.4 ± 4.2, 19.8 ± 6.2 and 36.7 ± 11.1, 11.7 ± 3.6 and 51.3 ± 14.6, 14.1 ± 4.2 and 71.4 ± 23.1, 22.7 ± 8.6 and 92.0 ± 28.1, 18.0 ± 5.9 and 107.6 ± 35.6, 21.7 ± 11.0 and 101.5 ± 29.3 µg/min respectively. From the 6th year the difference between the two groups was significant (P < 0.001).

In the transplant group initial mean systolic and diastolic blood pressure values were 132.0 ± 3.3 and 81.5 ± 1.5 mmHg, in the controls 130.4 ± 3.4 and 79.6 ± 1.6 mmHg respectively. Significant changes (P < 0.05) of blood pressure during the follow-up or differences between the two groups were not observed.

**Conclusions.** We conclude that fetal islet transplantation is effective in achieving good long-term diabetes control and in the prevention of diabetic nephropathy.

Key words: albuminuria; diabetic nephropathy; diabetes mellitus; diabetes control; fetal pancreas islet transplantation

Introduction

Despite the recent improvements achieved by intensive insulin therapy, the prevention of late diabetic complications is a largely unresolved issue, since about one-fifth of the patients with ‘good’ glycaemic control develop diabetic nephropathy [1]. One of the promising new methods in the treatment of insulin-dependent diabetes mellitus is the allogenic transplantation of cultured fetal pancreatic islets. Until 1994 less than 200 islet transplantations had been reported to the islet transplant registry, with 1-year graft survival rates (defined as exogenous insulin independence) of less than 10% [2]. Success is related to the number of islets transplanted, the purity and preparation of the islets, allograft rejection and the side-effect of immunosuppression [3]. However, the success of this procedure should not be measured solely in terms of insulin independence. Islet transplantation may result in a significantly improved metabolic control compared to what is achievable with insulin. It almost completely abolishes hypoglycaemia, resulting in an improvement of neuroglycopenic symptoms, and quality of life [4].

As the long-term effect of islet transplantation on late diabetic complications is still unclear, the aim of this study was to assess the effect of this novel treatment on the development and progression of diabetic nephropathy, in relation to metabolic control. We compared the changes of albumin excretion rate and diabetes control in a group of type I diabetic patients after fetal pancreas islets transplantation to a control group of type I diabetics on intensive insulin therapy only, during a 10-year follow-up period.

Subjects and methods

Since 1982 we have performed 30 transplantations of long-term cultured and cryopreserved fetal islets on insulin-
dependent diabetic patients. The aim of the transplantation was improvement of metabolic control, and thus delaying the progression of retinopathy. As the aim of the present study was to access the long-term effect of islet transplantation on the progression of diabetic nephropathy, those patients were selected who had been followed and had functioning grafts after 10 years. Ten of the transplanted patients met the above criteria.

From other insulin-dependent diabetic patients treated at our outpatient department, who also were candidates for islet transplantation, but who had not been transplanted due to the lack of suitable grafts, a control group of 27 insulin-dependent patients with retinopathy, and sufficient follow-up, matched for age, diabetes duration, diabetes onset, incidence of hypertension and degree of albuminuria was selected.

At the start of the follow up, the mean age of the transplant group was 33.6 ± 2.7 that of the controls was 35.3 ± 1.5 years. The mean age at the onset of diabetes was 13.5 ± 2.3 years in the transplant and 16.2 ± 2.0 years in the control group, mean diabetes duration was 20.1 ± 1.2 and 18.5 ± 1.5 years respectively. The mean body mass index of the transplant and the control groups were 24.76 ± 0.96 and 23.41 ± 0.5 kg/m². There were two light smokers (less than 15 cigarettes/day) in the transplant group and four in the control group. C-peptide was absent from the serum of all patients before transplantation or the start of the follow up, even after glucagon stimulation. The male:female ratio of the two groups was 7.3 and 16.11. None of the above parameters showed a significant difference between the two groups (P < 0.05). All patients had retinopathy. In the transplant group six patients had background retinopathy and four had proliferative retinopathy, in the control group 21 had background and six had proliferative retinopathy at the start of the study. In the transplant group one patient had hypertension before transplantation. In the control group three patients had hypertension at the start of the study. Hypertension was defined as systolic and/or diastolic blood pressure above 140/90 mmHg [5]. Blood pressure was measured according to the recommendation of Joint National Committee V with sphygmomanometry [6].

All patients were receiving calcium dobesilate for retinopathy. Hypertensive patients received captopril and furosemide. In the transplant group cyclosporin (8 mg/kg body-weight per day cyclosporin after transplantation, with subsequent dosage adjustments to keep the serum RIA level in the range of 40–60 ng/ml) was given for 1–2 years. All patients received rapid-acting insulin (Actrapid MC, Novo) three time daily before meals and an intermediate insulin (Monotard MC, Novo) at bedtime as basal insulin (25–40% of the total daily insulin dose). After human insulin became available, patients were gradually switched to these (Actrapid HMge and Insulatard HMge, Novo or Humulin R and Humulin N, Lilly). Apart from these no other medication was given.

At the start, two of the transplant group and five of the controls had microalbuminuria, the others were normoalbuminuric. Normoalbuminuria was defined as urinary albumin excretion rate <20 μg/min, microalbuminuria as 20–200 μg/min, macroalbuminuria as >200 μg/min [7]. The fetal pancreatic islets were isolated from human embryos aged 15–32 weeks, by a modified collagenase digestion technique. Fetal islet tissue was cultured in Eagle’s medium at 37°C in an atmosphere of 5% CO₂ for 10 weeks [8]. Islet masses were also cryopreserved at −196°C for 6–10 months. After tissue typing, (blood group and 1–3 HLA antigens were compatible), the transplantation was performed into the liver through the umbilical vein [9]. Each patient was given 2–3 cultured and cryopreserved embryonic tissue masses (about 40 000–60 000 islets).

Blood glucose was measured with the enzymatic (glucose oxidase) colorometric test (Diagnosticum test, Diagnosticum Rt., Budapest, Hungary) on a Hitachi 717 autoanalyzer, 2 h after breakfast. C-peptide levels were measured with radioimmunoassay (RIA-coat C-Peptide kit, Byk-Sangtec Diagnostika GmbH, Germany). The lower limit of sensitivity, as given by the manufacturer, is 0.03 ng/ml. Before transplantation or at the start of the study, C-peptide was measured 6 min after glucagon provocation (1 mg Glucagon Novo i.v.). In the transplant group fasting C-peptide was monitored during the follow-up. Plasma creatinine was measured with the kinetic Jaffe reaction (HitCo creatinin CREA Boehringer Mannheim GmbH, Germany). The urinary albumin excretion rate was measured with radioimmunoassay (Pharmacia RIA 100, Pharmacia Diagnostics AB, Uppsala, Sweden; normal range 25 μg/l; total coefficient of variation 7.1%; recovery 99–103%) from collected 24-h samples. Two parallel measurements were done of every specimen, the mean value of which was taken. As a marker of diabetes control HbA₁c levels were measured with colorimetry (Reanal-kit, Reanal Rt., Hungary; normal range 4.5–6.5% coefficient of variation 2.5%). β-2-Microglobulin was measured with Pharmacia β-2-micro RIA (Pharmacia Diagnostika GmbH, Germany). The lower limit of sensitivity for β-2-micro was 1 μmol/l. Serum cholesterol was measured with Diagnosticum test (Diagnosticum, Budapest; normal range 0.6–2.4 mmol/l). Serum triglyceride was measured with Diagnosticum test (Diagnosticum, Budapest; normal range 1.3–5.2 mmol/l). The alterations of retinopathy were checked by means of colour stereo-ophthalmography and fluorescein angiography.

All patients and all parameters were checked 2–4 times annually, and the geometric means of the various measurements in 1 year was calculated. The usual method of assessing albuminuria from three urine specimens collected during a short time period was not used, as sufficient patient compliance with such a large number of urine collections for a decade could not be expected; however, the yearly 2–4 measurements (at least 3 in the year preceding transplantation), the long follow-up period, and the number of patients was expected to counteract the effect of the biological variability of albuminuria sufficiently to make the two groups comparable.

A subgroup of the controls who did not have hypertension was examined separately, and also compared to the transplant group.

Statistical analysis was performed using the SAS software package version 6.12. Repeated measures analysis of variance was used to study the effects of time and treatment on the glucose level, HbA₁c, insulin requirement, and albuminuria. Since the distribution of all these variables were skewed to the right, logarithmic transformation was applied prior to the analysis, to achieve a normal distribution. The ANOVA was performed using the GLM procedure of SAS. The Huynh–Feldt adjusted F test was used in all cases of the multivariate analysis. The significance level of significance was P < 0.05 unless stated otherwise.

Results

After islet transplantation C-peptide levels appeared (0.55 ± 0.07 ng/ml), and despite their gradual
decrease they were still detectable after 10 years (0.25 ± 0.02 ng/ml) (Table 1). The mean daily insulin requirement of the transplant group decreased from 53.6 ± 2.2 to 35.8 ± 1.2 units (32.01 ± 3.9%) in the first year. Their insulin requirements were significantly (P<0.01) lower than in the control group throughout the follow-up. Although insulin requirements increased gradually during the study period, they were 28.56 ± 3.2% less than before the transplantation even after 10 years of follow up (Figure 1). The mean HbA1c of the transplant group changed from 8.4 ± 0.3 to 6.2 ± 0.2% in the first year and remained stable during the follow-up (Table 1). HbA1c levels of this group were significantly lower (P<0.01) than in the controls throughout the follow up period.

In the transplant group postprandial blood glucose levels decreased from 9.6 ± 0.6 to 6.2 ± 0.2 mmol/l, stayed relatively stable for 10 years, and were significantly (P<0.05) lower than in the control group throughout (Table 1). In the transplant patients the frequency of hypoglycaemic episodes causing clinical symptoms was one episode per year, while in the control group it was seven episodes per year.

The mean albumin excretion rate of the two groups are summarized in Figure 2. Albumin excretion rates were found to be dependent on time, with a significant increase (P<0.01) from the 5th year. From the 6th year a significant difference (P<0.05) was seen between the two groups. In the transplant group there were eight patients with normal albuminuria and two with microalbuminuria, which did not change during the follow up. In the control group, of the 22 previously normalalbuminurics, 11 remained normalalbuminuric, six progressed to microalbuminuria and five to macroalbuminuria; one of the latter developed azotaemia (creatinine >180 (μmol/l)). Of the five microalbuminurics, two progressed to macroalbuminuria, both of them developing azotaemia, while the other three remained unchanged. During the follow up of the transplant group one new case of hypertension was seen beside the one previously hypertensive patient. In the control group, besides the three previously hypertensive patients, newly diagnosed hypertension developed in eight cases. In the subgroup of the 16 controls without hypertension, out of the 15 previously normoalbuminurics, four progressed to microalbuminuria. One patient with pre-existing microalbuminuria remained at the same stage. In the transplant group mean urinary β2-microglobulin levels were 80.1 ± 17.8 μg/l prior to transplantation, with no significant changes during cyclosporin administration (81.0 ± 14.9 μg/l in the first and 82.6 ± 15.7 μg/l in the second year). Mean systolic and diastolic blood pressure before the transplantation was 132.0 ± 3.3 and 81.5 ± 1.5 mmHg. In the control group initial mean systolic and diastolic blood pressure values were 130.4 ± 3.4 and 79.6 ± 1.6 mmHg. Neither significant changes of blood pressure during the follow-up in either group, nor significant differences between the two groups were seen. (Table 2) Mean serum cholesterol was 4.8 ± 0.1 mmol/l in the transplant and 4.8 ± 0.1 mmol/l in the control group initially, and no

<table>
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<tr>
<th>Years</th>
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<td>Blood glucose</td>
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<td>9.0 ± 0.6</td>
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<td>HbA1c</td>
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<td>6.4 ± 0.4*</td>
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<td>C-peptide</td>
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<td>0.28 ± 0.04</td>
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*The difference between the transplant and the control group is significant (P<0.05).
significant subsequent changes were seen. Mean serum triglyceride was $2.0 \pm 0.1$ mmol/l in the transplant and $1.7 \pm 0.1$ in the control group. Serum creatinin and creatinine clearance values are given in Table 3. Significant differences between the two groups or changes in either group were not observed.

**Discussion**

It is generally accepted that among other factors, insufficient metabolic control has a major role in the development of late diabetic complications. Effective long-term intensive insulin therapy significantly reduces the risk of diabetic nephropathy and retinopathy, but unfortunately it is accompanied by a higher frequency of hypoglycaemic episodes [1]. Transplantation of the whole pancreas or of isolated pancreas islets prevents and reverses the development of glomerular lesions in diabetic animals [10]. The Diabetes Control and Complications Trial Research Group has reported that
improved metabolic control is easier to achieve in patients, who retain endogenous insulin secretion, as determined by connecting peptide levels [1]. Islet transplantation together with insulin therapy may increase the chances of achieving normoglycaemia, perhaps with a lower risk of hypoglycaemia [11].

In our patients the appearance of C-peptide levels proved the insulin production of the allografts, which persisted throughout the follow up period. Our results are similar to the experience of others [4], and can explain the improved diabetes control and smaller exogenous insulin requirement of the transplanted patients. In the present study insulin independence, occasionally experienced after islet transplantation [4], was not achieved, but recipients had lower exogenous insulin requirements. Although the difference decreased during the course of the follow up, it was still significant after 10 years. The reduction of insulin doses, however, should not in itself be a goal, as hyperglycaemia resulting from the overzealous reduction of exogenous insulin can have an adverse effect on the survival of the transplanted islets [12].

Transplant patients had significantly lower blood glucose and HbA1c levels throughout the follow-up period, and this can be one of the factors, that contribute to the nephroprotective effect of pancreatic islet transplantation. Furthermore the ratio between portal and peripheral insulin concentrations is likely to be more favourable in islet transplantation patients than in insulin-dependent diabetics treated with subcutaneous insulin only. This ratio in the transplant group is likely to be closer to the 3:1 ratio seen in non-diabetic individuals [13]. This may be beneficial, as systemic hyperinsulinaemia is considered to be atherogenic [14].

Islet transplantation seems to have delayed the development of diabetic nephropathy. In the transplant group no major changes of albuminuria were seen. Although some increase of the albumin excretion rates was experienced, all patients remained in the same stage of albuminuria in which they were before the transplantation. In the control group mean albuminuria increased, and after the fifth year significantly higher mean albumin excretion rates were seen than in the transplant group. Progression to micro- and macroalbuminuria was seen in nearly half of the control patients, with azotaemia developing in some cases. If we compare the transplant recipients to the subgroup of 16 controls who were without hypertension throughout the follow up, the above tendency can still be observed, as in about one-third of the normotensive controls a progression of albuminuria was found. Four of the 15 normoalbuminuric normotensive controls became microalbuminuric. Hypertensive patients received angiotensin-converting enzyme inhibitors, which may have slowed the development of nephropathy [15]. Since the control group had a larger percentage of hypertensive patients, it is unlikely, that the administration of angiotensin-converting enzyme inhibitors could have contributed to the more favourable outcome of the transplant group in terms of albuminuria.

Table 3. Changes of mean ± SEM serum creatinine (μmol/l) and creatinine clearance (ml/min) in transplant and non-transplant (control) patients before and during the 10 years follow-up

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<tr>
<th>Years</th>
<th>Serum creatinine (μmol/l)</th>
<th>Creatinine clearance (ml/min)</th>
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<td>10</td>
<td>92.0 ± 2.2</td>
<td>95.6 ± 1.9</td>
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Albuminuria after fetal pancreatic islet transplantation 2903
Glycaemic control seems to show a correlation with albuminuria; however, other factors may contribute to the nephroprotective effect. With the described method the whole pancreas islet is transplanted, not the β-cells alone. In a previous study we demonstrated the presence of somatostatin in pancreas islet cultures in vitro, signalling the activity of β-cells [16], which can have a beneficial effect in vivo on diabetic microangiopathy [17]. The glucagon secretion of the transplanted islets has not been examined in the present study, but a lower frequency of hypoglycaemic episodes was seen in the transplant group. One of the factors contributing to the improved counterregulation of hypoglycaemia may be the possible activity of β-cells, which has been previously demonstrated in whole pancreas transplantations [18].

Despite the low dose and short duration of immunosuppressive therapy used, its effect may have been supported by the small quantity of implanted tissue, and the cryopreservation used during the preparation. This method can weaken the immunogenic properties of the implanted tissue through immune modulation [19]. With the relatively small dose of cyclosporin-A, tubular damage was not seen; β₂-microglobulin levels remained in the normal range (<250 μg/min) throughout the follow up.

We conclude that the transplantation of long-term cultured fetal pancreatic islets is a promising possibility in the treatment of insulin-dependent diabetes, which despite the as yet limited number of cases seems to have a potential for preventing or delaying the development of diabetic nephropathy.

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