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BRIEF REPORT

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Successful simultaneous transplantation of kidney and fetal pancreatic islet masses

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Abstract This paper reports our experience with the successful simultaneous transplantation of kidney and fetal pancreatic islets in a 46-year-old diabetic man. No detectable C-peptide level was noted and the end-stage nephropathy required hemodialysis. The cadaver kidney and two masses of 8-week-cultured fetal islets were grafted simultaneously. After revascularization of the kidney, the islet masses were placed under the kidney capsule. Following transplantation, islet function was demonstrated by a higher C-peptide level, which subsequently persisted. Twenty-four months after grafting, islet function was provoked by glucagon and glucose, which led to elevations in the C-peptide and insulin levels. The insulin requirement fell from 58 to 24 U/day during the post-transplant period of 24 months. The mean value of HbA_{1c} (5.6 % ± 0.3 %) indicated a constantly normal carbohydrate metabolism. Improvements in retinopathy were also noted. Three periods of kidney rejection were diagnosed, but these proved reversible with high-dose steroid treatment. The serum and urine beta-2-microglobulin levels correlated well with rejection and recovery. More than 2 years after grafting, kidney function is in the normal range. On sonography, the transplanted islet masses were repeatedly clearly visible, and 24 months following transplantation the volume was twice the original one. The results indicate that simultaneous kidney and fetal pancreatic islet grafting is advantageous in end-stage nephropathy secondary to type I diabetes mellitus.

Key words Diabetes mellitus, fetal islets · Kidney transplantation, fetal islets · Fetal islets, kidney transplantation · Islets, fetal, kidney transplantation

Introduction

Microvascular complications of diabetes mellitus may be caused by insufficient glycemic control. Long-term, intensive blood glucose control significantly reduces the risk of diabetic retinopathy and nephropathy progression, but this intensive insulin treatment may cause a more severe hypoglycemic reaction [6, 15]. Diabetic patients who have received renal allografts may suffer new glomerular lesions, manifested structurally by increases in mesangial and glomerular volume [4, 10, 12]. Successful vascularized pancreas transplantation leads to long-term normoglycemia and reduces the likelihood that the disease will recur in the transplanted kidney [3], but no publication has reported the same effect of transplanted adult pancreatic islets. A potential source of islets for human transplantation is the fetal pancreas, and the use of fetal islets would appear more appropriate than that of adult islets. The former may be a better source of islets because they can easily be isolated and cultured, and they may provide the opportunity for growth in vitro. This report describes our experi-

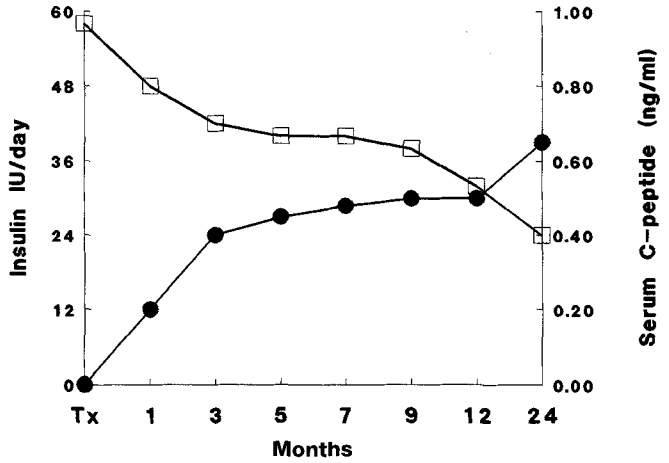

Fig. 1 Islet function and relation between daily insulin requirement (□) and serum C-peptide level (●) for 24 months following transplantation

ence with simultaneous kidney and fetal islet transplantation. The clinical and metabolic effects were determined and the 2-year graft function was followed up.

Patients and methods

The recipient was a 46-year-old man. The duration of diabetes before grafting was 24 years. There was no detectable intravenous glucose tolerance test (IVGTT)-stimulated C-peptide level and the end-stage nephropathy had required hemodialysis during the last 3 years. In addition, the patient had proliferative retinopathy and pregangrenic lesions on his toes. Two years before transplantation, intensive insulin therapy (multiple daily insulin and frequent blood glucose monitoring) was started; the insulin requirement in the pretransplant period was 52 U/day. The mean value of HbA_{1c} was 7.7 % ± 0.4 %. A cadaver kidney and two masses of cultured fetal islets were grafted simultaneously. The kidney was procured from a 26-year-old cadaveric donor matched for blood group 0 and two HLA antigens (A19 and B13), while the fetal pancreas was obtained from two embryos (18 and 22 weeks of gestation) extracted by transabdominal hysterotomy after medical indications. The fetal tissue matched for blood group 0 and two HLA antigens (A19 and B13 or B17).

Harvesting and culturing of fetal pancreas

Fetal pancreatic islets were isolated by means of a modified collagenase digestion technique [7]. The pancreata were minced into microfragments. The tissue suspension was partially digested with 500 U/g tissue collagenase (Sigma, St. Louis, Mo., USA) for 5 min. Thereafter, an Omnimixer tissue chopper (Sorwall, Norwalk, Conn., USA) was used for 0.5 min. The fetal islet tissue could be readily separated by washing in Hanks' solution and allowing the islets to settle for 0.5 min in the centrifuge tubes. The fetal islet tissues were cultured in Eagle's medium containing 20 % AB human serum at 37 °C in an atmosphere of air containing 5 % CO_2 for 8 weeks. During this period, the insulin production was almost continuous.

Transplantation of islets

After revascularization of the kidney, islet masses from two embryos (about 40000 islets) suspended in Hanks' solution were placed under the kidney capsule via a small cannula inserted into the upper pole of the organ.

Immunosuppressive management

Intraoperatively, an i. v. bolus of 1000 mg methylprednisolone was applied. From day 1 to day 210, the prednisolone dose was reduced progressively from 200 to 5 mg/day, this level subsequently being maintained. Cyclosporin A (CyA) and azathioprine were also administered continuously. CyA (8 mg/kg body weight) was started at the time of simultaneous transplantation and later the dosages were adjusted to keep the serum RIA level in the range of 150–200 ng/ml. The azathioprine dosage was started at 150 mg/day and was reduced progressively. The present doses of CyA and azathioprine are 100 mg/day and 10 mg/day, respectively.

Post-transplant assessment

After kidney and islet transplantation, the plasma glucose, C-peptide (RIA-coat C-Peptid, Byk-Sangtec Diagnostica, Germany; sensitivity 0.03 ng/ml), urine nitrogen (UN), creatinine and glycosylated hemoglobin (HbA_{1c}; REANAL, Hungary; normal range 4.5 %–6.5 %) levels were monitored. The IVGTT (1 g/kg glucose) and i. v. 1 mg glucagon were used as provocative tests of C-peptide and insulin secretion. The glomerular and tubular functions were checked by means of beta-2(β-2)microglobulin and albumin radioimmunoassays (Pharmacia, Sweden).

Retinopathy was investigated by means of color ophthalmography and fluorescence angiography.

Morphological identification of the transplanted islet masses was performed by sonography (ATL Ultramark 4) every 6 months after grafting.

Results

The period that has elapsed since the simultaneous kidney and islet transplantation is now more than 2 years. Islet function was demonstrated by an immediately increased C-peptide level (0.25–0.39 ng/ml), which persisted during the following 24 months. At present, the fasting C-peptide level is in the range of 0.30–0.90 ng/ml. The insulin requirement has fallen from 58 to 24 U/day, i. e., a reduction of 58 %. The relation between the daily insulin requirement and the serum C-peptide level is shown in Fig. 1. The insulin dose reduction closely followed the increased C-peptide production.

Twenty-four months after grafting, the islet function was provoked with glucagon (Fig. 2), which led to an elevation in C-peptide level from 0.30 to 0.90 ng/ml and an increase in insulin concentration from 14 to 25 µlU/ml. During the IVGTT, an increased serum C-peptide level was noted, but the highest concentration appeared 90 min following glucose stimulation (Fig. 3). The mean HbA1c level was 5.6 % ± 0.3 % and indicated constant-

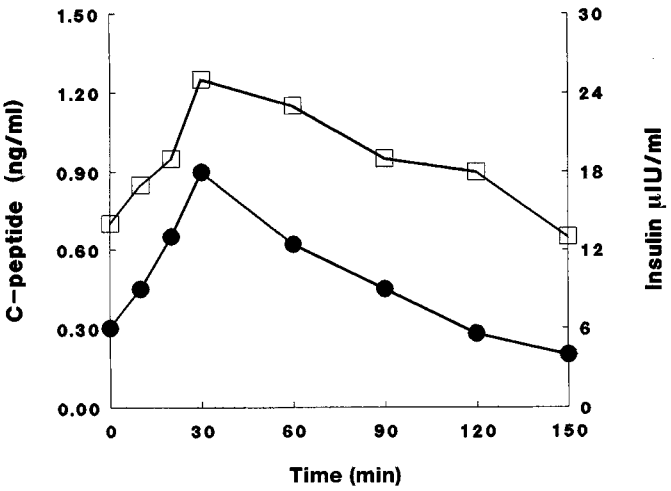

Fig. 2 Serum C-peptide level (●) and insulin production (□) after stimulation with 1 mg glucagon i. v.

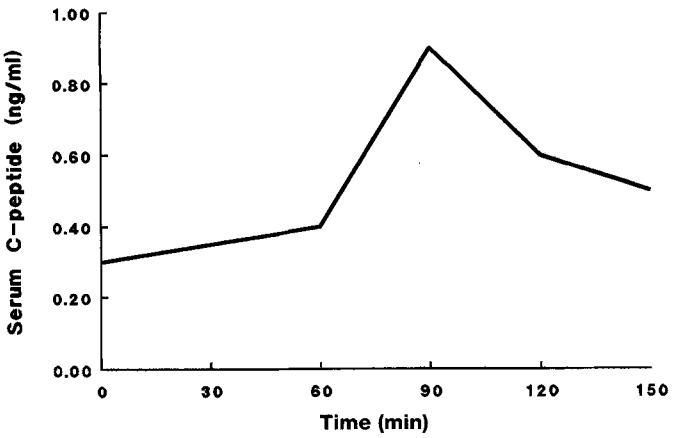

Fig. 3 Variation in serum C-peptide concentration after IVGTT (1 g/kg glucose i. v.)

ly normal glycemia. Similarly, the 24-h glucose and C-peptide profiles revealed normal blood glucose at all test times when the insulin dose of 24 U/day was applied (Fig. 4).

Improvements in retinopathy and peripheral microangiopathy were also noted, followed by a normoglycemic condition. During the 24-month post-transplant period, laser treatment was not required at all and the peripheral pregangrenic lesions disappeared completely.

After grafting, three periods of kidney rejection were diagnosed, but these proved reversible with high-dose steroid treatment. The partially elevated serum and urine β-2 microglobulin and urine albumin levels displayed a good correlation with the rejection episodes and with recovery. More than 2 years after grafting, kidney function is acceptable: UN 7 mmol/l, serum creatinine 92 µmol/l, creatinine clearance 75 ml/min, serum β-2 microglobulin 2100 µg/l, urine β-2 microglobulin 117 µg/l, and urine albumin 9.1 mg/24 h.

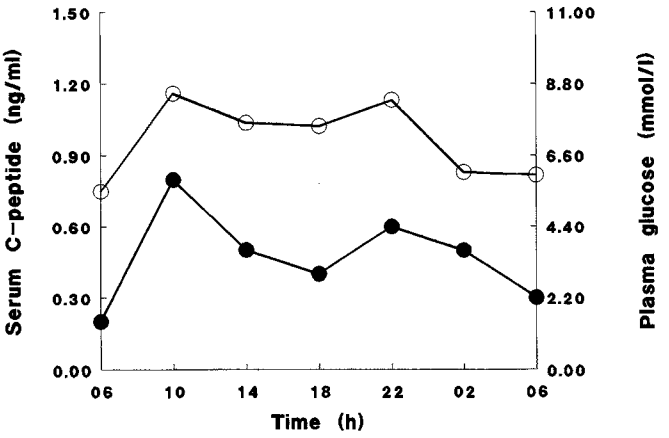

Fig. 4 Changes in plasma glucose (○) and serum C-peptide (●) concentrations in 24-h metabolic profile

On sonography, the transplanted islet masses were repeatedly clearly visible. Figures 5 and 6 show the morphologic pictures 1 and 2 years following transplantation. No change was noted in the grafted kidney, but the volume of the islet masses was more than double (from 4.49 to 11.49 cm^3).

Discussion

Transplantation of a whole pancreas or of isolated pancreatic islets prevents and reverses the development of renal glomerular lesions in diabetic animals [11, 13]. Studies reporting simultaneous pancreas and kidney transplantation have suggested that the risk of recurrence of diabetic glomerulopathy in the renal allograft may be decreased by successful pancreatic transplantation. This may be due to the effects of long-term normoglycemia. Intensive insulin therapy has been shown to prevent the occurrence and recurrence of diabetic complications [6, 17]. However, intensive insulin therapy does not allow physiologic control of the glucose metabolism. The Diabetes Control and Complications Trial Research Group has reported [6] that improved metabolic control is easier in patients who retain endogenous insulin secretion, as determined by connecting peptide levels. Thus, islet transplantation in conjunction with intensive insulin therapy may increase the likelihood of achieving normoglycemia [8]. The present report supports this notion since long-term normoglycemia was attained in our patient.

In the present case, simultaneous kidney and long-term-cultured fetal pancreatic islets were grafted and the duration of follow-up was 24 months. During this per-

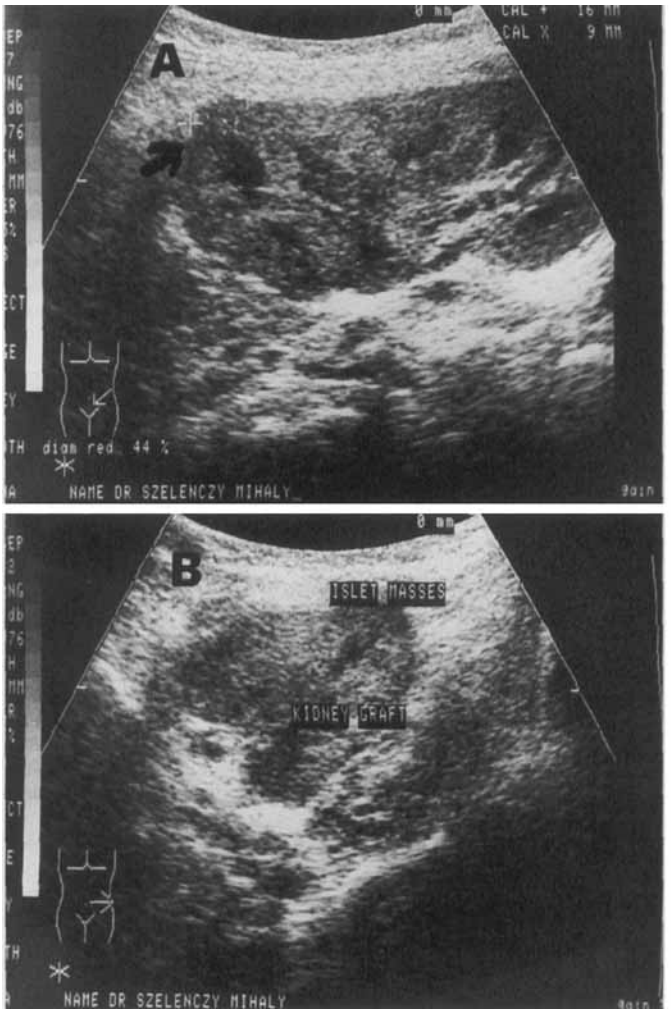

Fig. 5 A, B Sonographic pictures of simultaneous kidney and fetal islet transplants 1 year after grafting in different positions

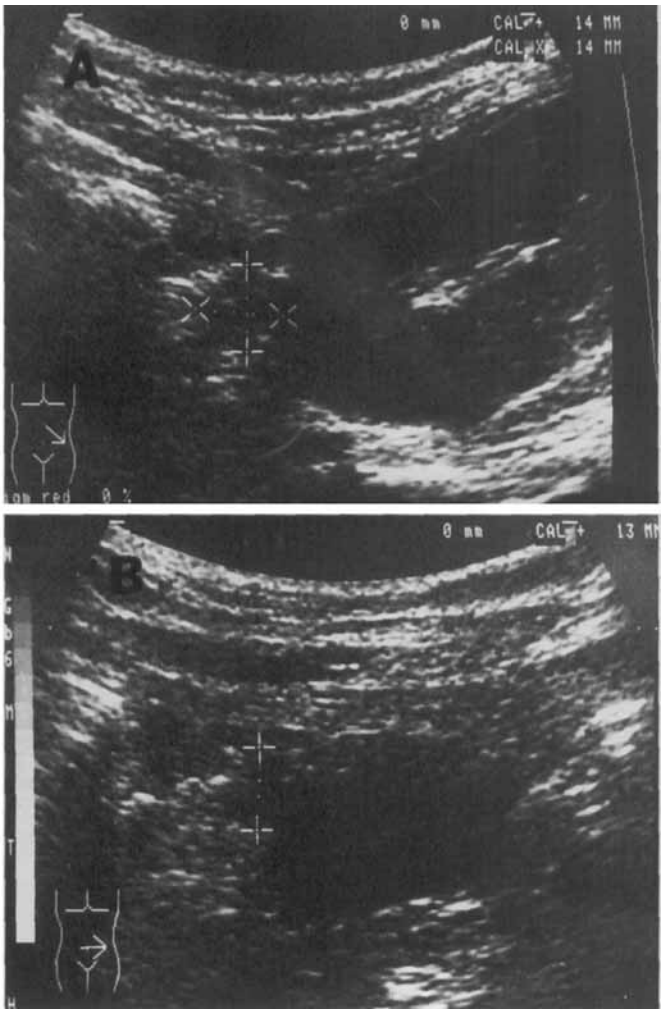

Fig. 6 A, B Sonographic pictures of kidney and fetal islet masses 2 years after transplantation in different positions

iod the patient became normoglycemic (but not insulin-independent), providing a beneficial condition for the improvement or prevention of diabetic complications.

It seems important to stress some factors influencing successful transplantation. The primary one is the use of matched [5] long-term-cultured fetal islets, grafted to the subcapsular site of the revascularized kidney. The long-term culturing of fetal islets provides an opportunity to produce an increased islet mass and to reduce immunogenicity [7]. Then again, the renal subcapsular space is an immunologically privileged region [1] and provides an opportunity for permanent growth [9, 14]. This was the reason why subcapsular transplantation was applied. After grafting, a higher C-peptide level (0.25–0.39 ng/ml) was measured, demonstrating immediate islet function. The reason for this observation would appear to be the use of double, long-term-cultured islet masses and the appropriate site for transplantation. During the 2-year post-transplant period, the volume of the grafted islet mass more than doubled and the C-peptide secretion increased continuously. The reduction in daily insulin requirement showed an inverse correlation with this secretion, but the corticosteroid medication, as part of the immunosuppression, did not allow total elimination of the insulin dose. Following transplantation, kidney graft rejections were diagnosed on three occasions and the levels of serum and urine β-2 microglobulin correlated well with both rejection and recovery. β-2 microglobulin determination therefore appears to be a clinically useful method of checking on the function of the transplanted kidney [2, 16]. No rejection episode was noted for the islet grafts, but biopsy from the renal subcapsular site was not performed. However, the unreduced C-peptide levels (basal) and stable carbohydrate metabolism suggest that the kidney subcapsular area is a suitable site for islet transplantation.

In conclusion, our results provide evidence that simultaneous fetal islet grafting may have a beneficial effect on kidney grafting and secondary complications in cases of diabetes mellitus. The space under the kidney capsule seems a reasonable site for islet grafting because this is a privileged place against immunological reactions and provides an opportunity for islet grafts to grow. Although the present patient is not insulin-independent 2 years post-transplantation, the well-functioning islet grafts provide a normoglycemic condition and a good quality for work and life.

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