Islet allo-autotransplantation: allogeneic pancreas transplantation followed by transplant pancreatectomy and islet transplantation

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List of abbreviations:

CT scan: computed tomography. **ESRD**: End-stage renal disease. **IEQ**: islet equivalents. **SPK**: simultaneous pancreas-kidney. **T1D**: type 1 diabetes.

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Abstract

Simultaneous pancreas/kidney (SPK) transplantation is an important treatment option for patients with type 1 diabetes (T1D) and end stage renal disease (ESRD). Due to complications, in up to 10% of cases allograft pancreatectomy is necessary shortly after transplantation. Usually the donor pancreas is discarded. Here we report on a novel procedure to rescue endocrine tissue after allograft pancreatectomy. A 39 year old woman with T1D and ESRD who had received an SPK transplantation required emergency allograft pancreatectomy due to bleeding at the vascular anastomosis. Islets were isolated from the removed pancreas allograft and almost 480,000 islet equivalents were infused into the portal vein. The patient recovered fully. After three months near-normal mixed meal test (fasting glucose 7.0 mmol/L, 2-hour glucose 7.5 mmol/L, maximal stimulated C-peptide 3.25 nmol/L, without insulin use in the preceding 36 hours) was achieved. HbA1c while taking a low dose of long-acting insulin was 32.7 mmol/mol Hb (5.3%).

When a donor pancreas is lost after transplantation, rescue beta cell therapy by islet allo-autotransplantation enables optimal use of scarce donor pancreata to optimize glycemic control without additional HLA allo-antigen exposure.

Introduction

Patients with type 1 diabetes (T1D) and end-stage renal disease (ESRD) are potential candidates for a simultaneous pancreas and kidney (SPK) transplantation. This procedure leads to remission of diabetes in up to 75% of patients at 5 years.[1] Benefits include greater patient survival and fewer diabetes-related complications.[2] Unfortunately, pancreas transplantation is associated with considerable complications, and up to 10% of transplanted patients require allograft pancreatectomy, thereby losing the benefits of endogenous insulin secretion.[3,4] Allogeneic islet transplantation is usually performed in patients with severe beta cell failure and unstable glycemic control. This procedure has fewer procedure-related complications compared to pancreas transplantation, but in general long term insulin independence is often not achieved.[5-7] Autologous islet transplantation is performed in patients who require pancreatectomy for benign pancreatic disease.[8]

Optimal use of available donor organ is important to reduce waiting lists. In this respect allograft pancreatectomy, often shortly after transplantation, increases the waiting list burden as patients are often relisted. Here we report on a patient who received an SPK transplantation, but subsequently required emergency pancreatectomy of the allograft due to bleeding from the arterial anastomosis. Islets from this pancreas allograft were immediately isolated and successfully transplanted via the portal vein.

Case description

A 39 year old woman was admitted for an SPK transplantation. She had been diagnosed with type 1 diabetes at the age of three years. She was blind due to diabetic retinopathy, despite laser coagulation treatment and had ESRD due to diabetic nephropathy for which she required hemodialysis since four years. There were no other relevant medical problems. Induction therapy consisted of subcutaneous alemtuzumab 30mg and intravenous methylprednisolone 500mg. Maintenance immunosuppressive therapy consisted of mycophenolate mofetil and tacrolimus. The donor organs were procured from a 32 year old female, with a BMI of 24 kg/m^2, who had died of a subarachnoidal hemorrhage. The donor had no additional relevant medical history. The organs were obtained through a donation after brain-death procedure: time after cross clamping to cold perfusion of the pancreas was 18 minutes, cold ischemia time was 8 hours. The kidney was placed in the left iliac fossa and the pancreas was placed in the right iliac fossa with enteric drainage of exocrine fluids.

The initial postoperative course was uneventful. The patient had rapid normalization of blood glucose concentrations and spontaneous diuresis of over 30 mL/h. Cultures of abdominal drain fluid were positive for *Pseudomonas aeruginosa* (tested sensitive for ceftazidime, ciprofloxacin, and gentamycin), for which ceftazidime was administered four days after transplantation. On day six after transplantation the drains were removed. Seven days after transplantation the patient reported increasing abdominal pain. A computed tomography (CT scan) of the abdomen demonstrated a retropancreatic fluid collection. A drainage catheter was placed in the fluid collection. The next day she went into an acute hypovolemic shock due to a haemorrhage from the arterial anastomosis of the pancreas graft, where an aneurysm had formed. During surgery, leakage near the anastomosis of the mycotic

aneurysm of the Y-graft was observed. An emergency allograft pancreatectomy was performed. Pathological examination of the arterial anastomosis revealed a transmural purulent inflammatory and infectious process with gram negative rods. The pancreas was considered to be viable and was immediately perfused with cold UW solution on the back table. The pancreaswas transported on ice to the human islet isolation laboratory for islet isolation according to previously reported protocols.[5,9-12] Macroscopically, the pancreas was oedematous with areas of necrosis. After surgical debridement the pancreas was decontaminated thoroughly with iodine solution (5mg/mL in Ringer's acetate solution) and penicillin 100U/mL / streptomycin 110U/mL solution (in Ringer's acetate solution at pH 7.35). Because of the inflamed nature of the pancreas and young age of the donor, the pancreas was infused with one vial (2533IU) Serva NB-1® collagenase solution (in Ringer's acetate solution with 5mM calcium at pH 7.35) through the pancreatic duct for 28 minutes prior to adding neutral protease solution (200IU in Ringer's acetate solution with 5mM of calcium at pH 7.35) for an additional 20 minutes. Antibiotics (gentamycin (0.5mg/mL), ciprofloxacin (0.02mg/mL) and amphotericin B (2.5ug/mL)) were added to all fluids during the entire isolation procedure. One additional density gradient separation step was necessary due to impurity of the initial isolated islet preparation. The islet preparation contained 478,587 islet equivalents (IEQ) in a volume of 2865µL with a purity of 38%. Gram staining and an endotoxin test were negative. An uncomplicated intraportal islet transplantation was performed following previously reported protocols.[5,12] Before islet transplantation, the patient received intravenous prednisolone 50mg and during the transplantation procedure heparin 50 IU/kg was administered into the portal vein. She was maintained on continuous intravenous insulin with a target glucose range between 4 – 7 mmol/L. After cessation of

intravenous insulin subcutaneous insulin was restarted, targeting glucose concentrations between 5 – 9 mmol/L. Ceftazidime was continued post transplantation for one day, and then switched to ciprofloxacin for ten days. Vancomycin i.v. was added for seven days because the transplant medium culture grew *Enterococcus faecium* (tested sensitive to vancomycin, but resistant to ampicillin). She required five more weeks of clinical inpatient care, mostly due to recurrent intraabdominal fluid collections requiring drainage in the operative bed. She had a full recovery.

Results

After the islet transplantation the patient required intravenous insulin up to a dose of 20 IU per day while aiming for blood glucose concentrations between 4 - 7 mmol/L. C-peptide measurements demonstrated endogenous insulin production during this period (table 1).Twenty days after islet transplantation, she was switched from intravenous insulin to multiple daily injections (long acting insulin glargine and prandial insulin aspart). The insulin requirement increased to a maximum of 40-50 IU per day for adequate glycemic control (glucose 5 – 9 mmol/L, table 1), coinciding with infectious complications in the pancreas transplant bed. After she made a full recovery, insulin was tapered according to fasting and postprandial glucose concentrations.

Three months after the islet transplantation, an extensive evaluation was performed. At this time the patient used only 8 IU of insulin glargine per day (0.13 IU/kg/day) with fasting glucose concentrations between 5 – 6 mmol/L. Her HbA1c had dropped from 56.7 mmol/mol Hb (7.4%) preoperatively to 32.7 mmol/mol Hb (5.3%) (table 1).

However, during this period iron deficiency anemia was present, which could influence HbA1c measurement. A mixed meal test (without administration of exogenous insulin 36 hours before the test) showed a more than three-fold induction of C-peptide and an adequate glucose clearance with 2-hour glucose concentration of 7.5 mmol/L (figure 1).

Nine months after islet infusion her weight had increased from 61.5 to 66.5kg. The hemoglobin had stabilized (7.9 mmol/L) and the HbA1c was 31.9 mmol/mol Hb (5.2%) while the patient was taking 12 IU of insulin glargine per day (0.18 IU/kg/day). No iron deficiency was present anymore. She had not experienced any severe hypoglycemic events since the islet transplantation. At this time, the patient was in excellent general condition. The creatinine clearance averaged 80 mL/min/1.73m^2 (eGFR), indicating satisfactory kidney graft function.

Discussion

Here we report a successful islet transplantation with islets isolated from a pancreas allograft that had been removed in an emergency setting due to life-threatening bleeding from a mycotic aneurysm of the Y-graft. Subsequent islet isolation and transplantation resulted in excellent endogenous insulin production, with low insulin requirement and a near-normal glucose tolerance and HbA1c. This corresponded with a high C-peptide response to a mixed meal tolerance test. We term this procedure islet allo-autotransplantation to indicate the transplantation of islets isolated from an allogeneic donor pancreas that was transplanted previously into the same recipient as the islets.

Allogeneic pancreas transplantation combined with kidney transplantation is considered standard of care for patients with type 1 diabetes and end-stage renal disease, but it is still a procedure that is characterised by considerable complications. These complications include abdominal infections, thrombosis, bleeding and transplant pancreatitis. Up to 10% of transplanted patients require allograft pancreatectomy.[3,4] In light of the shortage of donor organs,[13,14] discarding a donor pancreas after transplantectomy could be considered a waste of resources if islets from this pancreas are still viable and thus useful for the patient. Therefore, utilising this otherwise discarded pancreas provides an opportunity to rescue the endocrine tissue. Islet transplantation has important beneficial effects. By partial or sometimes even full restoration of endogenous insulin production, it alleviates hypoglycemia burden, and improves quality of life.[15-17] Endogenous insulin production has also been shown to be associated with prevention or stabilisation of diabetes-related complications, even if insulin independence is not achieved.[18,19] Therefore, restoration of endogenous insulin production is an important goal of treatment. With islet allo-autotransplantation, the advantages of endogenous insulin production are achieved shortly after allograft pancreatectomy, avoiding additional donor HLA exposure, in case the patient would have been waitlisted and transplanted again, and making optimal use of donor organ resources. Therefore, this procedure has important implications both for the individual patient with diabetes and for transplantation health care in general.

The procedure differs in several aspects from regular islet auto- or allotransplantation. Allograft pancreatectomy is often performed in an acute clinical setting with more challenging logistics for back table flushing of the allograft with preservation solution and subsequent islet isolation compared to a planned

autologous pancreatectomy and islet isolation. Therefore, optimal coordination between the surgical team and islet isolation team are critical to the success of this complicated procedure. In addition, the donor pancreas is often damaged (i.e. by infection, thrombosis, fibrosis, or inflammation) and the presence of peripancreatic fluid collections, that are often present after pancreas transplantation,[1] is an important risk for contamination of the islet preparation after isolation. In order to reduce the risk of infection after islet infusion, a strategy to use antibiotics in all isolation fluids, to infuse islets as quickly as possible and to apply antibiotic coverage against enteric microorganisms (preferably based on the antibiogram of previous cultures) is appropriate. Furthermore, the pro-inflammatory state of the patient can increase the risk of both bleeding and thrombosis.[20] Therefore we used a lower dose of intraportal heparin during the islet transplantation procedure. The transplantation team should weigh the benefits of restoration of endogenous insulin production against the risks of infection and thrombotic or bleeding complications due to the islet infusion.

In conclusion, islet allo-autotransplantation is a novel treatment option that offers patients who have undergone pancreas transplantation but require transplant pancreatectomy a second chance at endogenous insulin production with its associated benefits. With this procedure, a repeated pancreas or islet transplantation and exposure to additional allogeneic HLA antigens may be avoided.

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Time	Exogenous longacting insulin dose (IU/day)	Exogenous shortacting insulin dose (IU/day)	Fasting glucose (mmol/L)	Fasting C-peptide (nmol/L)	HbA1c (mmol/mol)	Weight (kg)
Day -9	17	37	8.1	<0.03	56.7	69.1
Day 0	0	0	5.4	1.71		
Day 3	0	20 (i.v.)	6.4	0.43		
Day 7	0	30 (i.v.)	7.8	0.74		
Day 28	16	28	7.6	0.55	37.1*	63.6
Day 90	8	0	5.6	0.72	32.7*	61.5
Day 270	12	0	5.5	0.87	31.9	66.5

Table 1: HbA1c, exogenous insulin requirement, fasting glucose and C-peptide of the patient. Day -9 is 1 day before the simultaneous pancreas-kidney transplantation. Day 0 is the day of allograft pancreatectomy and islet infusion. Exogenous insulin is subcutaneous unless otherwise indicated. *indicates situation of altered red blood cell turnover (anemia, iron deficiency), which may influence HbA1c measurement.

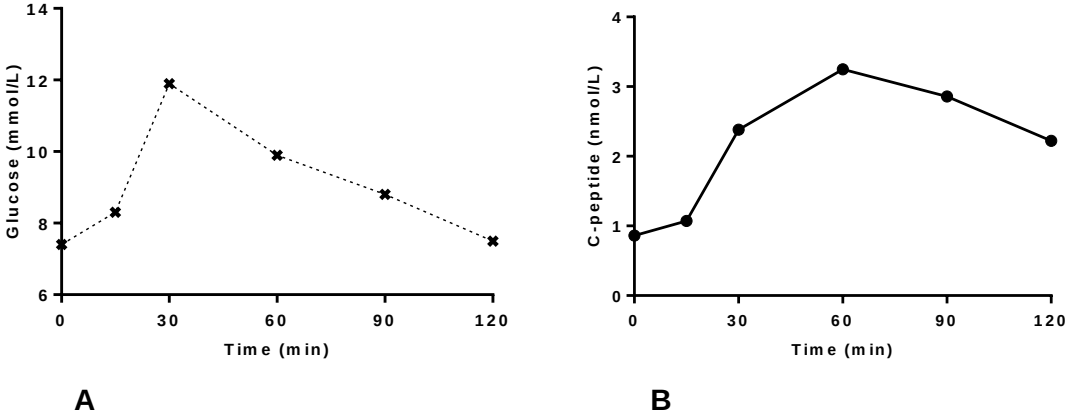

Figure 1: mixed meal test of the patient without previous insulin administration at three months after islet transplantation. A) Glucose concentrations, B) C-peptide concentrations