

Xenogeneic islet transplantation of microencapsulated porcine islets for therapy of type I diabetes: long-term normoglycemia in STZ-diabetic rats without immunosuppression

Thomas Meyer · Burkhard Höcht · Karin Ulrichs

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Abstract

Purpose Rejection and possible infection with porcine pathogens are obstacles in clinical xenogeneic transplantation of porcine pancreatic islets (PPI) to treat diabetic patients. A solution to this problem could be microencapsulation of the PPI. However, isolation and microencapsulation are highly demanding tasks with considerable risks of damaging the PPI. Thus, it is not surprising that the long-term function (>200 days) of microencapsulated PPI (mPPI), transplanted to diabetic rats, has been observed only in a few cases.

Methods Diabetes was induced in Wistar rats with streptozotocin (STZ 60 mg/kg body weight). Animals with consecutive blood glucose levels >300 mg/dl for more than 2 days were considered diabetic. PPI were isolated from brain-dead hybrid pigs (age 6–7 months or 2–3 years) using the Ricordi-technique and Liberase^{PI}. After in vitro culture PPI were microencapsulated with highly purified barium–alginate and 1,000 mPPI of 300–500 µm Ø were transplanted under the left kidney capsule and/or into the peritoneal cavity of STZ-diabetic rats (*n* = 15) without immunosuppression. Daily, later weekly, blood glucose level and body-weight were measured.

Results mPPI showed normal glucose tolerance in vitro and also in vivo. Normoglycemia occurred between day 1

and 15 after transplantation. Four mPPI grafts functioned for more than 230 days, the longest now for >550 days. Three rats are currently normoglycemic for >40 days. Six rats lost xenograft function after 12–20 days, due to inflammatory reactions at the site of the grafts. Two xenografts failed to induce normoglycemia, because the capsules did not contain enough viable PPI.

Conclusions Microencapsulated xenogeneic islets can induce long term normoglycemia in rats without immunosuppression. However, very often the grafts fail to control the blood glucose level adequately. The reasons for these failures are currently under investigation. Nevertheless, our results are very promising and might lead the way towards preclinical trials in non-human primates.

Keywords Porcine pancreatic islets · Microencapsulation · Xenotransplantation · Non-immunosuppression

Introduction

The long-term complications of insulin-dependent diabetes mellitus (IDDM) have become a very major health care problem in the last years; arise from inadequate homeostatic control of blood glucose by injected replacement insulin. Transplantation of isolated pancreatic islets is arguably the most logical approach to restring metabolic homeostasis in people with diabetes [1], especially in childhood and adolescent. But in the current situation, it is unlikely that the supply of human organs will ever meet the demand. The organ donor pool will therefore, need to extend to include other species as well [2, 3]. The xenogeneic transplantation of porcine islets of Langerhans is regarded as a potential alternative treatment for diabetes

T. Meyer · K. Ulrichs
Experimental Transplantation Immunology,
Department of Surgery, Centre of Operative Medicine,
Julius-Maximilians-University, Würzburg, Germany

T. Meyer (✉) · B. Höcht
Pediatric Surgery Unit, Department of Surgery, Centre
of Operative Medicine, Julius-Maximilians-University,
Oberdürrbacher Strasse 6, 97080 Würzburg, Germany
e-mail: meyer_t@chirurgie.uni-wuerzburg.de

mellitus, but acute rejection [4] and possible infection with PERV [5, 6] are obstacles for the clinical xenogeneic transplantation of porcine pancreatic islets. A solution to these obstacles could be the encapsulation of porcine islets. However, isolation and encapsulation of porcine islets are a demanding task with a considerable risk of damaging the islets. In 1996 Sun et al. [7] published the first successful xenogeneic transplantation of alginate-microencapsulated porcine pancreatic islets into diabetic monkeys. Unfortunately there were no further publications following these promising experiments. On the other hand, Jain et al. [8] favors the immunological protection of porcine pancreatic islets using agarose–collagen macrobeads in the rat model. Based upon these data, we were asking ourselves, whether a highly purified commercial alginate would protect porcine pancreatic islets from being immunologically destroyed and thus we transplanted them without immunosuppression in a Wistar-rat model.

Methods

Islet isolation

Porcine pancreata were obtained from young hybrid pigs (HY, 4–6 months old, approx. 110 kg body weight) and old retired breeders (RB, 2–3 years old, 200–300 kg) from two local slaughterhouses, each with a warm ischemic time of approx. 20 min. The splenic lobe was dissected and shipped to the laboratory in cold HBSS with 25 mM Hepes buffer. Fat, vessels and lymph nodes were removed from the donor organ. The common duct was cannulated with a 18G catheter and the organ was distended through the common duct/catheter with cold UW solution containing 1.2 mg/ml Liberase^{PI} (Roche Diagnostics, Germany). Porcine pancreatic islets of Langerhans were isolated using a modification of the half automated digestion-filtration method previously described by Ricordi et al. [9]. Dithizone (Diphenylthiocarbazone) staining was used throughout this study to identify endocrine pancreatic tissue and monitor the degree of the tissue dissociation. When the first free islets were detected in the biopsy, the isolation process was stopped by flushing the system with ice-cold HBSS containing 25 mM Hepes buffer and 5% heat-inactivated fetal calf serum (FCS). The digest was collected, and was washed twice and stored on ice. After 1 h, islets were purified using the discontinuous OptiPrepTM density gradient (Nycomed Pharma AS Diagnostics, Norway) in the COBE 2991 cell processor (COBE Inc., USA) described by the group of van der Burg [10]. Islets were then cultured (5% CO₂, 24°C) for 48 h in Ham's F12 medium supplemented with 10% FCS.

Quantitative and qualitative assessment

Purity of the isolation was determined on an aliquot of the islet suspension under a stereo microscope (Axiovert 25, Zeiss, Germany, magnification: 2.5). The total of the actual islet number was converted into 150 µm diameter islet equivalents (IEQ) and counted by the same person, as described in detail elsewhere [11].

Islet encapsulation

Porcine pancreatic islets were suspended in a 2.2% (w/v) highly purified sodium alginate (Pronova Biomedical, Norway), at a concentration of ~3.000 IEQ/ml. Microcapsules were generated using air-jet-technique and the alginate was complexed with a 20 mM bariumchloride solution, as described elsewhere [12]. The microcapsules were washed three times with 0.9% sodiumchloride and cultured (5% CO₂, 24°C) over night in Ham's F12 medium supplemented with 10% FCS. Microcapsules contained either numerous viable intact, fragmented islets or single islet cells and had a mainly diameter of 350–500 µm (Fig. 1).

Islet xenotransplantation

The transplant recipients ($n = 15$) were male Wistar–Unilever-rats (Harlan Winkelmann GmbH, Germany) weighing approximately 250–300 g at the time of transplantation. Diabetes was induced with Streptozotocin (STZ; *N*-[Methylnitrosocarbamoyl]-D-Glucosamine, Sigma–Aldrich Chemie, Germany), using 60 mg STZ per kg bodyweight. Only animals with consecutive blood glucose levels higher than 300 mg/dl were considered diabetic. Islet transplantation was performed through a midline laparotomy under isofluran (1-Chloro-2,2,

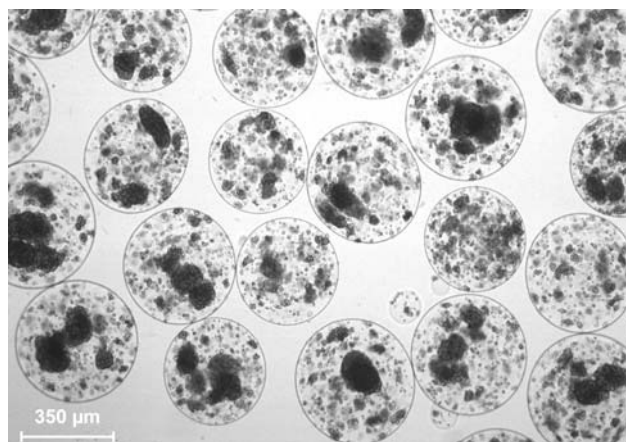


Fig. 1 Barium–alginate microencapsulated porcine pancreatic islets: microcapsules contained either numerous viable intact, fragmented islets, or single islet cells and had a mainly diameter of 350–500 µm

2-trifluorethyldifluoromethylether; Abbott, Germany) anesthesia. Approx. 1000 microcapsules were transplanted under one kidney capsule using a G 22 needle and/or into the peritoneal cavity of STZ-diabetic Wistar–Unilever-rats. No immunosuppression was applied before or after xenotransplantation.

Daily, later weekly, the blood glucose level was monitored. Glucofilm[®] as well as Glucometer[®] (Bayer Diagnostics GmbH, Germany) was used for blood glucose determination. Oral glucose tolerance tests (oGTT) were performed, using 1 g glucose. Blood glucose levels were measured before and at 10, 30, 50, 70, 90, 110 and 130 min after oral glucose stimulus.

Histology

After the reappearance of diabetes, the microencapsulated xenografts were removed from the animals and fixed frozen tissue sections (5 μ m) were evaluated microscopically using H&E staining.

Results

STZ-diabetic rats displayed clinical features of diabetes mellitus including polyuria, polydipsia, weight loss and fatigue. All these rats showed a significant less concentration of rat-c-peptide levels, compared to normal rats (data not shown).

Diabetes was reversed in most animals (>85%) after xenotransplantation of barium alginate microencapsulated porcine pancreatic islets. Of 15 animals transplanted so far, two showed a primary xenograft non-function (13.3%). This was particularly so at the beginning of our experiments, when we still lacked some experience. Seven xenografts (46.6%) showed only a short term function between day 6 and day 20 and six xenografts (40.0%) showed a long-term function beyond day 20. One of these xenografts functions now for more than 550 days (Fig. 2). No immunosuppressive or antiinflammatory drugs were applied before or after xenotransplantation.

In addition, oral glucose tolerance test (oGTT) was performed 100 days after xenotransplantation and the blood glucose level of these animals were measured. All transplanted rats showed a normal physiological, however, individually differing decrease of blood glucose after the initial increase.

Histological staining of the short-term compared to long-term surviving xenografts showed varying degrees of fibrosis around the capsules, depending on the site of transplantation, a distinct infiltration of leukocytes in the intercapsular space, and intensive neovascularization of microencapsulated islet-grafts (data not shown).

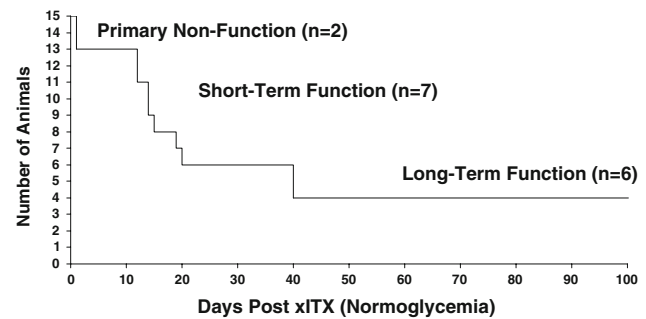


Fig. 2 Grafts survival/normoglycemia in STZ-diabetic Wistar-rats after xenogeneic islet transplantation (xITx) without immunosuppression

Discussion

Islet transplantation offers the most physiological possibility to substitute lost islet functions and control the blood glucose level continuously, but it is limited to (1) supply of human islet tissue, (2) adverse effects of current immunosuppressive protocols and (3) the rejection of the islet grafts [1]. Clinical transplantation of human islets from cadavers proved that transplantation can normalize hyperglycemia in diabetic recipients [13]. However the limited supply of human islets and the difficulties with harvesting pure islets, make the use of human islets impractical. The xenogeneic transplantation of porcine islets of Langerhans is regarded as a potential alternative treatment for diabetes mellitus, but acute rejection [3, 4] and possible infection with PERV [5, 6] are obstacles for the clinical xenogeneic transplantation of porcine pancreatic islets. A solution to these obstacles could be the encapsulation of porcine islets. Immunoisolation prevents rejection by separation of the transplanted cells from the host immune system and therefore makes immunosuppression unnecessary. We demonstrated in 40% of the cases, diabetes can be reversed for long periods of time (in one case over 550 days) by immunoprotected porcine xenografts without resorting to exogenous insulin therapy or any pre- or postoperative immunosuppression. The long survival of microencapsulated xenografts in these experiments can probably be attributed to two factors: (1) the purity of the porcine islet tissue and (2) the purity of the barium–alginate.

We suppose that the fragments of exocrine cells emerge from encapsulated porcine pancreatic islets. Such fragments could be phagocyted by macrophages and this could result in direct cytotoxic tissue destruction via the inflammatory cytokines TNF- α or IL-1. On the other hand, these cytokines are known to activate the proliferation of fibroblasts which could be seen around the microcapsules. This could be the reason for the varying degrees of fibrosis around the capsules. The purity of the alginate seems to be

much more important for the biocompatibility of the barium–alginate microencapsulated porcine pancreatic islets [14].

These results show that microencapsulated xenogeneic islets can induce long-term normoglycemia in rats without any immunosuppressive drug therapy. However, in 40% the grafts fail to control the blood glucose level adequately for more than 20 days. The reasons for these failures are currently under closer investigation. Nevertheless, our results are very promising and might lead the way toward preclinical trials in non-human primates.

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