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BRIEF REPORT

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Oxygen tension in isolated transplanted rat islets and in islets of rat whole-pancreas transplants

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Abstract Despite similar immunosuppressive protocols having been applied, the success rate of pancreatic islet transplantation has been much lower than that of whole-pancreas transplantation. We compared the oxygen tension in syngeneically transplanted isolated rat islets and islets of syngeneic rat whole-pancreatic grafts, with native islets, using Clark-type microelectrodes. An oxygen tension of ~40 mmHg was recorded in both native islets and in islets of the whole-pancreas graft. Isolated islets transplanted under the renal capsule had a markedly lower oxygen tension (~6 mmHg). The exocrine parenchyma of the native and of the transplanted pancreatic gland had an oxygen tension of ~30 mmHg. The lower oxygen tension in transplanted isolated islets may be one explanation for the more frequent failures of transplanted islets compared with the outcome when the whole pancreatic gland is transplanted.

Keywords Angiogenesis · Pancreas transplantation · Cell transplantation · Polarographic technique

Introduction

The Diabetes Control and Complication Trial (DCCT) has clearly demonstrated that the degree of hyperglycemia predicts the risk of subsequent development of microvascular complications [9]. However, intensive insulin therapy did not normalize HbAIC and was associated with a threefold increased risk of severe hypoglycemia [9]. Full restoration of metabolic control may only be achieved by transplantation of the insulin-producing tissue, either by transplanting the whole pancreatic gland, or by transplanting merely the pancreatic islets. Although the results of pancreas transplantations in most centers are good (80%–90% graft survival and insulin independence [11, 30]), islet transplantation has a much lower success rate, < 10% insulin-independent at 1 year [3]. Better results for islet transplantation have been obtained by careful patient selection and adherence to a steroid- and cyclosporine-free immunosuppressive regimen [26, 29]. However when this protocol is applied, islets from two or more pancreata are still needed to cure the diabetic patients [26]. Since whole-pancreas transplantation is associated with a higher surgical risk and is also more expensive (e.g., in terms of postoperative care) than islet transplantation, it seems important to solve the problems associated with islet transplantation. One of the differences between the whole-pancreas and islet transplantation procedures is that transplanted islets are avascular and require revascularization from the surrounding tissues, whereas the transplanted whole pancreatic gland has an intact vascular system that is connected by anastomoses to arteries of the recipient. In the present study, we aimed to record the oxygen tension both in islets of the transplanted whole pancreatic gland and in revascularized transplanted islets, and compare it with that of native pancreas islets.

Materials and methods

Animals

Male, inbred Wistar-Furth rats weighing 300–350 g were purchased from M& B Research and Breeding Center (Ry, Denmark) and used in all experiments. The animals had free access to tap water and pelleted food (R36; Ewos Södertälje, Sweden) and were housed in a room with a 12-h light/dark cycle and humidity of 70% throughout the course of the study. All experiments were performed according to "Principles of laboratory animal care" (NIH, 1985) and approved by the local animal ethics committee at Uppsala University.

Islet transplantation

Islets were isolated by means of collagenase digestion (Roche Molecular Biochemicals, Mannheim, Germany), as described in detail elsewhere [28]. Groups of ~150 islets were cultured free-floating for 4–6 days in RPMI 1640 medium (Sigma-Aldrich, Irvine, UK) supplemented with 10% (v/v) fetal calf serum (Sigma-Aldrich). The medium was changed every 2nd day. At transplantation, ~250 islets were packed in a braking pipette and implanted beneath the renal capsule on the dorsal side of the left kidney in pentobarbital-anesthetized rats (60 mg/kg i.p.; Apoteket, Umeå, Sweden). For this purpose, a left subcostal flank incision was performed, followed by a small incision in the renal capsule, allowing the braking pipette to be introduced. The pipette was then inserted 0.5–1 cm between the capsule and the organ parenchyma before the islets were gently ejected. This was done to ensure that none of the islets was lost through the incision hole. The subcostal flank incision was closed, and the animals were observed until they fully recovered from anesthesia.

Pancreatico–duodenal transplantations

This procedure has been described in detail elsewhere [15]. Briefly, the donor animal was anesthetized with an i.p. injection of ekviticin (a combination of pentobarbital and chloralhydrate) and placed on a heated (38 °C) operating pad. The whole pancreas, together with 3–4 cm of the small intestine to secure adequate exocrine drainage, was carefully dissected from surrounding tissues and blood vessels. The aorta was then cannulated with a polyethylene catheter, and the graft was perfused with 4–6 ml of a chilled (4 °C) University of Wisconsin solution (ViaSpan; DuPont Pharmaceuticals, Wilmington, Del., USA) at a pressure of approximately 35 mmHg. The warm ischemia time was less than 2 min in all animals. The graft was then removed, together with 1 cm of the aorta containing the celiac and superior mesenteric arteries (i.e., the two arterial blood vessels to the pancreas and duodenum) and stored at 4 °C for ~1 h (cold ischemia time) until required for implantation. The syngeneic recipients were anesthetized with ekviticin (cf. above) and placed on the heated operating pad. The left kidney was exposed through a midline incision and removed. Using a cuff technique [20] we then anastomosed the aortic piece of the pancreaticoduodenal graft to the renal artery, while the portal vein was connected to the renal vein. The proximal end of the grafted duodenum was closed with sutures, whereas the distal end was anastomosed to the recipient's proximal colon with approximately ten sutures (with 7–0 silk). The abdominal wound was then closed. After surgery, the animals were given an injection of doxycycline (Leo, Malmö, Sweden; 10 mg/kg s.c.) and observed until fully recovered from anesthesia.

Oxygen tension measurements

One month after islet or whole-pancreas transplantation, the animals were anesthetized with thiobutabarbital sodium (Inactin; Research Biochemicals International, Natick, Mass., USA; 120 mg/kg i.p.) and placed on a heated (38 °C) operating pad. They were tracheostomized, and polyethylene catheters were inserted into the right femoral artery and vein. The former catheter was connected to a pressure transducer (P23dB; Statham Laboratories, Los Angeles, Calif., USA) to monitor mean arterial blood pressure, whereas the latter catheter was used for continuous infusion (5 ml/kg/h) of Ringer solution to substitute for loss of body fluid. In animals with islets transplanted beneath the left renal capsule, a left subcostal flank incision was made. The left kidney was thereafter immobilized in a plastic cup attached to the operating table and embedded in pieces of cotton wool soaked in Ringer solution. The renal surface was covered with mineral oil (Apoteket) to prevent evaporation and keep the tissue moist and at body temperature. During the course of the experiment, the temperature of the tissue surface was monitored with a thermocouple probe (CT D85, Ellab, Copenhagen, Denmark). The animals were then allowed to rest for 30 min to minimize the influence of surgical stress on the measurements. In whole-pancreas implanted animals, the abdominal cavity was opened with a midline incision, and the native and transplanted pancreata were, one at a time, carefully immobilized over a cylindrical plastic block attached to the operating table, then superfused with mineral oil. An i.v. injection of 0.8 ml sterile-filtered 2% (w/v) neutral red (Kebo Grave, Stockholm, Sweden) dissolved in saline was given to visualize the islets [5]. We had previously evaluated this dye without noticing any adverse effects on islet function, blood flow or tissue oxygen tension in the intact pancreas [4, 5]. Oxygen tension was measured in the islet grafts, whole-pancreas transplants and native pancreas with modified Clark-type microelectrodes. (Unisense, Aarhus, Denmark) [6, 24]. The microelectrodes were polarized at −0.8 V, which gives a linear response between the oxygen tension and the electrode current. The latter was measured with a picoammeter (University of Aarhus, Aarhus, Denmark). The electrodes (outer tip diameters 2–6 μm) were inserted into the tissues with a micromanipulator under a stereoscopic microscope. The electrodes were calibrated in water saturated with $Na_2S_2O_5$ or air at 38 °C. The drift of the microelectrode recordings was < 0.5%/h. In the transplanted islets and surrounding renal cortex, ten or more measurements of oxygen tension were performed in each animal. In whole-pancreas transplanted animals, measurements were performed in three to five superficial pancreatic islets and in surrounding exocrine parenchyma of both the transplanted and native pancreata. All measurements began with the transplanted gland, since the mobilization of this was a prerequisite for access to the native pancreas. Multiple measurements were usually performed within the same islet; the mean was calculated to obtain the oxygen tension value for one islet. The mean of all measurements in each tissue and animal was calculated and considered to be one experiment. During the oxygen tension measurements, blood pressure, body, and tissue temperatures were continuously recorded with a MacLab Instrument (AD Instruments, Hastings, UK) connected to a Power MacIntosh 6100 computer. Blood glucose concentrations were determined with test reagent strips (Medisense, Baxter Travenol, Deerfield, Ill., USA) from samples obtained from the cut tip of the tail. At the end of the experiments, arterial blood samples for analysis of hematocrit (hcr) and blood gases were obtained from the catheter in the femoral artery and collected in microhematocrit tubes (Kebo Grave). A mean arterial blood pressure < 80 mmHg, pH < 7.30, pO_2 < 10 kPa, pCO_2 > 6.8 kPa, or hcr < 40, were used as exclusion criteria.

Statistical analysis

All values are given as mean ± SEM. Probabilities of chance differences between the experimental groups were calculated with analysis of variance (ANOVA; Statview, Abacus Concepts, Berkeley, Calif., USA) and the Bonferroni post-hoc test [31] For all comparisons, $P < 0.05$ was considered statistically significant.

Results

Mean arterial blood pressure and blood glucose concentrations

The mean arterial blood pressure in animals transplanted with isolated islets under the renal capsule was116±3 mmHg ($n=8$). Whole-pancreas transplanted animals had a mean arterial blood pressure of 92±3 mmHg ($n=6$). In animals transplanted with isolated islets, the blood glucose values were 5.9±0.1 mmol/l ($n=8$). Similar blood glucose concentrations were recorded in whole-pancreas transplanted animals (5.6±0.1 mmol/l; $n=6$).

Oxygen tension measurements

In native pancreatic islets, tissue oxygen tension was ~40 mmHg (Fig. 1). Similar values for oxygen tension was obtained in islets of the whole-pancreas graft. In contrast, pancreatic islets transplanted and revascularized under the renal capsule had a markedly lower tissue oxygen tension. The exocrine parenchyma of the native and transplanted pancreatic gland both had an oxygen tension of ~30 mmHg (Fig. 2). Tissue oxygen tension in the renal cortex adjacent to the islet graft was somewhat lower (Fig. 2).

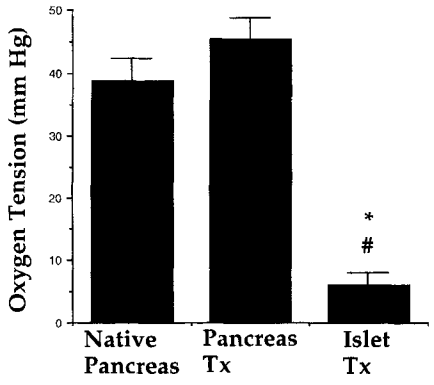

Fig. 1 Oxygen tension in native pancreatic islets, in islets of whole pancreas transplants and in islets transplanted beneath the renal capsule. Measurements were performed 1 month after syngeneic transplantation in male Wistar-Furth rats. *$P<0.05$ when compared with native islets, #$P<0.05$ when compared with islets of the whole-pancreas transplant. The values represent mean ± SEM for 6–8 animals

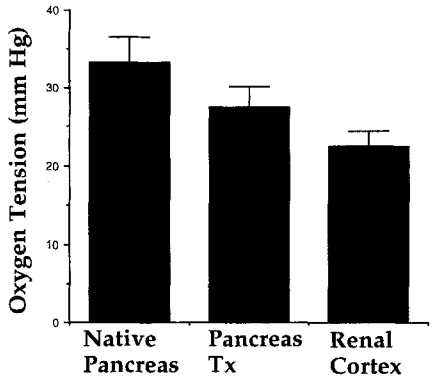

Fig. 2 Oxygen tension in the exocrine parenchyma of native and transplanted pancreata, and in the renal cortex adjacent to transplanted isolated islets. Measurements were performed 1 month after syngeneic transplantation in male Wistar-Furth rats. The values represent mean ± SEM for 6–8 animals

Discussion

Clinical islet transplantation has so far had a poor outcome, resulting in fewer than 10% of insulin-independent patients 1 year after transplantation [3] Several factors have been suggested as contributing to this, most commonly, chronic rejection and recurrence of disease [25, 32]. However, the same immunosuppressive drugs as are used in islet transplantation are also used in whole-pancreas transplantation, where a markedly better outcome is observed [11, 30]. Other factors are therefore likely to contribute and be of importance, to explain the frequent failures in islet transplantation. In the present study, we compared the tissue oxygen tension in transplanted islets and islets of whole-pancreas transplants, with native islets. Although a markedly decreased oxygen tension was recorded in the islet transplants, compared with both islets of whole-pancreas transplants and native islets, the islets of whole-pancreas transplants had a similar oxygen tension to native islets. Despite the low tissue oxygen tension, functional activity remains in transplanted islets, and when transplanted in sufficient numbers they may reverse hyperglycemia in streptozotocin-diabetic recipients [8]. However, it is uncertain whether the attained function after islet transplantation is optimal. In clinical islet transplantation, a surprisingly large number of islets (900,000 islet equivalents) are still needed to obtain insulin independence, and also when the Edmonton Protocol is applied [26, 29]. Pancreatic β-cells normally have a very high metabolic activity and oxygen consumption to meet the varying needs for insulin secretion [12, 13]. Indeed, oxygen tension levels similar to those recorded in the present study (5–6 mmHg) have previously been shown to affect islet function. In βTC3 cells, an insulinoma cell line, pO_2 levels <25 mmHg gradually shift these cells from aerobic to anaerobic metabolism with a concomitant increased lactate production [21, 22] Reduced insulin secretion from the βTC3 cells was observed at pO_2 <7 mmHg. Similarly, glucose-stimulated insulin secretion from rat single islet-cell aggregates (5–10 cells) was affected below 12 mmHg [10]. Transplanted whole

pancreata maintain their internal vascular system, which is anastomosed to arteries in the recipient. The complex islet angioarchitecture that ensures that no portion of an islet is more than a cell away from arterial blood [2] is therefore intact. Due to denervation of the transplanted gland, an islet blood flow even higher than in the native gland is established [14, 15]. In the present study, oxygen tension in whole-pancreatic grafts was measured for the first time. Consistent with the re-establishment of the pancreatic vascular system, oxygen tension values similar to those in the native pancreas were recorded in both the endocrine and exocrine parenchyma. This suggests optimal oxygenation of the transplanted tissue. In contrast to the intact vascular system in whole-pancreas transplants, the isolation of pancreatic islets, and subsequent in-vitro culture preceding islet transplantation, causes the islet vasculature to disrupt and de-differentiate or degenerate [23]. The transplanted islets are generally thought to be fully revascularized within 7–14 days, and certainly before 1 month post-transplantation [1, 17, 18, 19, 27]. However, recent data suggest an inappropriate vascular distribution within such islet grafts [16]. This is also supported by the low tissue oxygen tension recorded in the islet transplants in the present and previous studies [6, 7, 8]. In conclusion, the present study compared the oxygen tension in isolated transplanted islets and islets of whole-pancreatic grafts, with native islets. Although the islets of the transplanted whole pancreas had a tissue oxygen tension similar to native islets, the oxygen tension in transplanted islets was also markedly lower after revascularization. This may be one of the mechanisms for the frequent failure of transplanted islets, compared with the outcome when the whole pancreatic gland is transplanted.

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