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Early islets and mesenchyme from an injured adult pancreas improve syngeneic engraftments and islet graft function in diabetic rats

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ABSTRACT

A decrease in mass of isografts and a decline in islet function are major challenges in islet transplantations. Despite this, transplantation of 84 h harvested pancreatic duct ligation (PDL) tissues have been shown to have the same functional ability to foetal pancreata, but there was only 40% success in reverting hyperglycaemia. We tested the potential of early islets with mesenchymal stromal cells (MSCs) to promote isogeneic grafts survival and to restore normoglycemia in diabetic rats, in comparison with late islets. Islets were isolated from injured adult pancreata of donor rats at 24 h post ligation either with MSCs (24 h islet/MSC+) or without MSCs (24 h islet/MSC-), and at 84 h without MSCs (84 h islet/MSC-). These cells were transplanted under the renal capsule of syngeneic STZ-diabetic recipient rats. The islet grafts were monitored using the BGLs of recipients and the immunohistomorphology of the grafts were analysed using anti-insulin and anti-Ki67 antibodies. The mean BGL in 24 h islet/MSC+ recipients was reduced over time toward the control value. The curves of the mean BGLs in the control islet/MSC- and the 24 h islet/MSC- recipients dropped significantly below the control normal glucose group's levels to reach their nadirs on weeks 4 and 6, respectively. Both curves had a peak overshoot on week 9, with no statistical significant difference between them. Engrafted islets were evident in these recipients, lasted for 5 and 6 weeks and correspondingly survived failure. However, insulin+ cells were present in the isografts of all recipients; but, only isografts in the 24 h islet/MSC+ presented with a homogenous subcapsular beta cell mass. In addition, the tendency of 24 h islet/MSC- to restore normoglycaemia with its survival capacity was statistically highly significant compared to the 84 islet/MSC- recipients (80%; 20%; $p = 0.001$). Transplantation of early islets with MSCs from injured adult pancreata prolongs islet graft survival and improves isograft function in diabetic rats. This novel observation requires much further exploration for its clinical application, but this model already provides hope for new sources of donor islets for transplantation.

1. Introduction

Islet transplantation remains a less invasive approach for beta cell replacement therapy to restore normoglycemia in type I diabetes (Ryan et al., 2001; Shapiro et al., 2000). Despite the use of glucocorticoid-free immunosuppressive agents to minimize tissue rejection, patients still experience a decline in islet cell survival following transplantation (Shapiro et al., 2000). To maintain the islet graft for long-term glucose control, more efforts are directed toward transplanting islet cells under a physiological condition similar to their natural environment (Johansson et al., 2005). However, transplantation of late islets harvested from an injured (by pancreatic duct ligation – PDL) adult pancreas has shown the same functional ability with foetal tissues, but with a low rate of graft survival (Page et al., 2004). A morphological study of this injured adult pancreas showed maximum exocrine tissue destruction between 72 and 84 h following PDL, while there were mesenchymal-like cells observed at the early stage of the remodeling of the endocrine component of the pancreas (Tchokonte-Nana, 2011). These findings may suggest that the time for donor tissue harvest has a considerable significance to the graft survival or failure. Furthermore, a study on chronobiology of endocrine developmental genes using statistical modeling of tissue protein expressions (du Toit et al., 2015), suggested an early harvest and islet isolation time periods of 12–24 h post PDL for optimum transplantation outcomes in diabetic animals.

Previous reports from *in vivo* studies (Berman et al., 2010; Ito et al., 2010; Rackham et al., 2013a,b; Scuteri et al., 2014) demonstrated that association of islets with mesenchymal stromal cells (MSCs) improves islet graft survival and function. Meanwhile, a more recent *in vitro* study

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(Manda et al., 2017) using an injured adult pancreas has concluded that MSCs are required for duct-to-beta cell maturation and function. In fact, MSCs are a heterogeneous cell population with a high plasticity (Phinney and Prockop, 2007), and can be isolated in many tissues including bone marrow, adipose tissue (Scuteri et al., 2014; Zuk et al., 2001), skin, amniotic fluid (Scherjon et al., 2003) and umbilical cord (Anker et al., 2003). They are also characterized by their immunomodulatory properties (Ramasamy et al., 2007; Reddi et al., 2010) with the ability to release trophic factors (Satija et al., 2009; Zappia et al., 2005) that can prolong pancreatic islets survival (Crigler et al., 2006; Scuteri et al., 2011). We hypothesized in this study that transplantation of early islets from an injured pancreas will improve the efficacy and function of the islet grafts; and that in association with MSCs, the viability of the islet graft will be maintained.

2. Materials and methods

2.1. The animals

Healthy male Wistar rats (*Rattus norvegicus*) (n = 140) weighing between 200–250 g were obtained from inbred lines at Stellenbosch University Central animal unit of the Faculty of Medicine and Health Sciences. Animals were housed in clean cages, had free access to water and pelleted food throughout the experiments. The study was approved by the Stellenbosch University Animal Care and Use Committee (SU ACUC): ethics number (SU-ACUM13-00036).

2.1.1. Study groups and treatments

Animals were divided into two study groups, the experimental transplant animals (donors, recipients) (n = 120) and control glucose animals (n = 20) (Fig. 1).

2.1.1.1. Experimental transplant animals. The experimental transplant animals were grouped into donor (n = 80) and recipient (n = 40) animals. Sixty donor animals were exposed to the surgical ligation of the pancreatic duct (PDL) as described by Tchokonte-Nana (Tchokonte-Nana, 2011), and were considered PDL donor animals, while the other 20 animals were sham-operated, did not undergo duct ligation and were considered sham-operated control donor animals. The recipient animals (n = 40) were rendered diabetic by intraperitoneal injection of a single dose of 65 mg/kg STZ-Streptozotocin (Sigma) freshly dissolved in citrate buffer.

Blood glucose levels (BGL) were measured using a blood glucose meter and strips (Gluco-plus INC, 2323 Halper, Quebec, Canada); and animals with a fasting BGLs > 20 mMol/L for three consecutive days were considered diabetic and used as recipients.

2.1.1.2. Control glucose animals. Animals in this group (n = 20) were divided into 2 equal halves. One half of animals were rendered diabetic as described above and were considered control diabetic glucose group, while the other half were given an intraperitoneal injection of saline solution at equal volume to the

citrate buffer and were considered control normal glucose group. The fasting BGLs in these groups were used as controls for the study.

2.2. Islet isolation and MSCs separation from donor animals

Twenty-four hours following PDL, islets were harvested with or without MSCs from the splenic portion of the pancreas of equal halves from 40 PDL donor animals and were considered 24 h islet/MSC+ donor islets and 24 h islet/MSC- donor islets, respectively. Meanwhile at 84 h post PDL, islets were harvested without MSCs from the remaining 20 PDL donor animals and were considered 84 h islet/ MSC^{-} donor islets. Additionally, islets or MSCs were harvested from the sham-operated donor animals and were considered control donor islets (Fig. 1).

For islet isolation, 2 ml of 1.5 mg/mL collagenase P (Sigma-Aldrich) was injected into each lobe of the pancreas. Pancreatic tissues were allowed to digest at 37 °C in the collagenase solution for 30 min followed by Ficoll (Sigma) density gradient separation. Islets were washed in HBSS twice before re-suspension in RPMI-1640 (GIBCO), and were further separated from ductal, vascular, and lymphoid tissue by hand-picking (using a pipette) under a dissecting Microscope (Scuteri et al., 2014). Each preparation gave a yield of 300 islets that were assessed for purity using Dithizone (Sigma-Aldrich) staining and kept in complete media (RPMI 1640) at 37 °C in a humidified atmosphere of 95% air and 5% CO2 ready for transplantation.

On the other hand, MSCs were separated and characterized

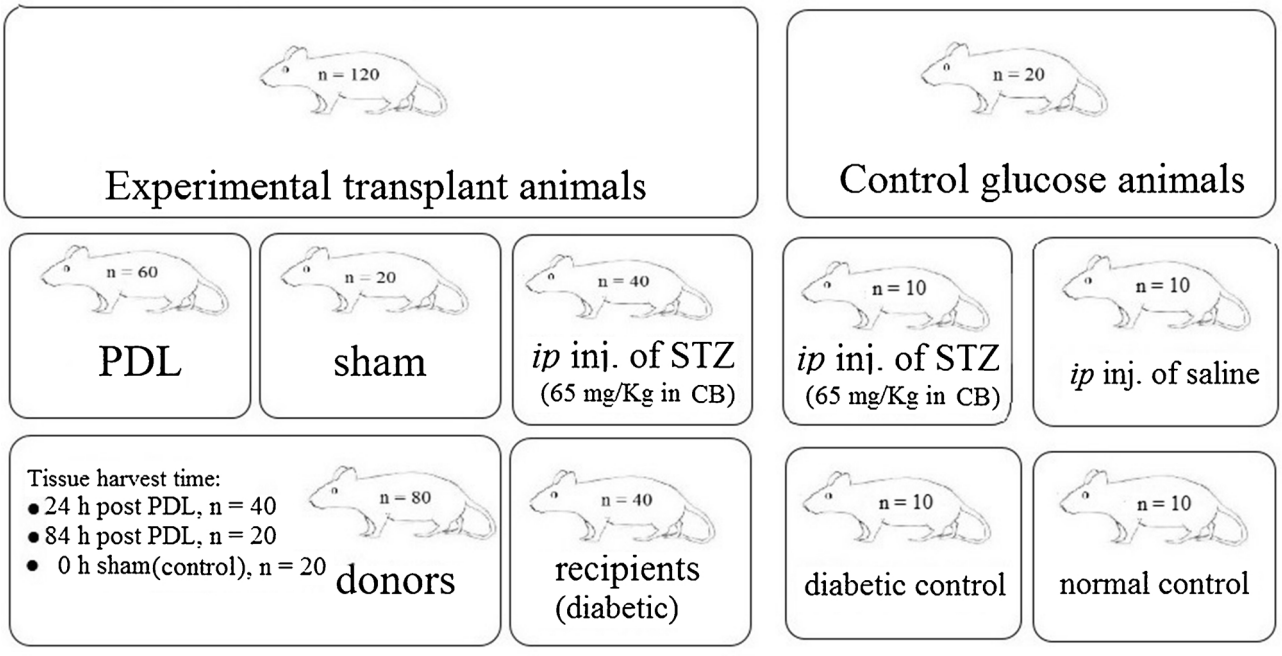

Fig. 1. A schematic description of the experimental design of the study.