Investigating the expression of inflammatory markers in an in vivo islet transplant model

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INTRODUCTION

Currently there are two transplant models used in islet research where islets are either transplanted under the kidney capsule or into the portal vein of the liver. Transplantation under the kidney capsule has the advantage of the kidney capsule being an easily accessible site, allowing the islets to be infused with relatively more ease and also allowing for simple graft retrieval for histologic review. Transplantation under the kidney capsule requires less islets to be infused to achieve normal blood glucose. However the kidney capsule is not an ideal model in that it lacks an adequate blood supply meaning that it is an oxygen poor microenvironment unfavourable for islet function. The portal vein is a more clinically representative model however it carries the disadvantages of being difficult to reach making graft infusion and retrieval more difficult and it is a immune rich microenvironment allowing for allorejection. Improved understanding of the mechanisms behind early graft loss are essential in improving long term insulin independence and this poster will look at early graft function when a marginal islet mass was transplanted in each of the candidate sites

OBJECTIVES

- Identify the functional grafts in the liver tissue using immunohistochemistry
- Phenotype infiltrating immune cells
- Quantify circulating proinflammatory markers by ELISA

METHODS

Diabetes was induced in 12hr fasted male Wistar rats by intra-venous streptozotocin injection (60 mg/kg). Rats with fasted blood glucose ≥20mmol/l were considered diabetic. Absence of pancreas beta-cell regeneration was confirmed by histology at post mortem.

Animals received either 600 islets under the kidney capsule or 2000 rodent islets into the hepatic portal vein. Graft function was monitored by weekly intra-peritoneal glucose tolerance tests (IPGTT) prior to and for up to 3 weeks following surgery. Regular blood samples were taken for plasma insulin (Mercodia) and cytokine analysis (Meso-Scale Discovery).

4µm formalin fixed paraffin embedded liver sections were labelled with insulin. Bound antibody was detected using a DAB peroxidase system.

Data were compared by Student's two tailed t test with $p<0.05$ considered statistically significant unless otherwise stated.

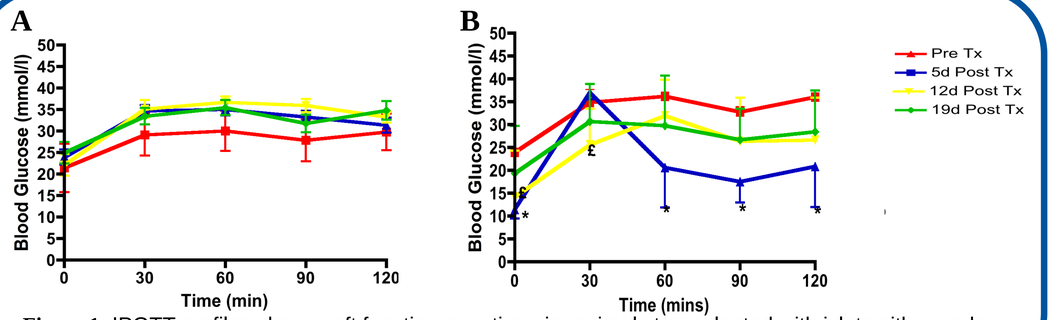

Figure 1: IPGTT profiles show graft function over time in animals transplanted with islets either under the kidney capsule (A) or into the liver (B)

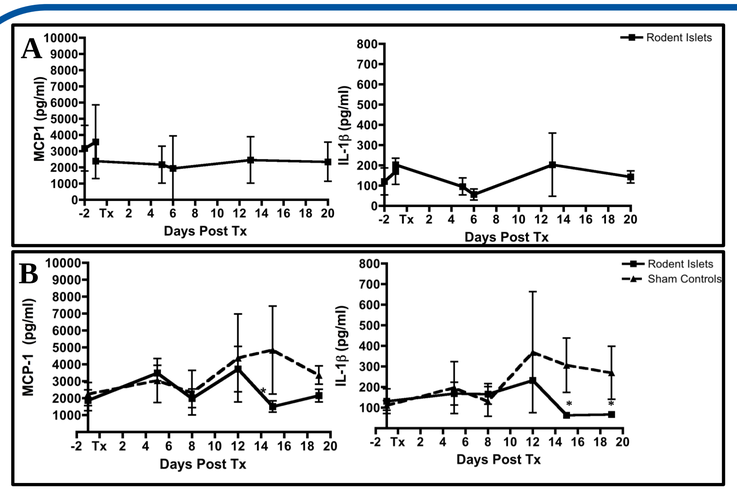

Figure 2: Circulating cytokine profiles for MCP-1 and IL-1β prior to and following transplantation of islets under the kidney capsule (A) or into the liver (B)

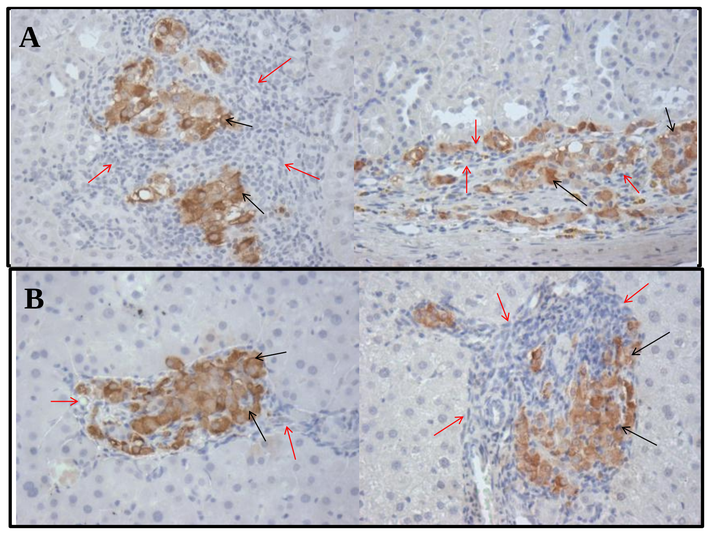

Figure 3: Insulin staining in rat kidney (A) and liver (B) tissue

RESULTS

Figure 1: The IPGTT profiles show graft function in the transplanted animals over time. No significant differences in the IPGTT profile were observed in animals transplanted with islets under the kidney capsule (A). A significant reduction in fasting blood glucose as well as a significantly lower blood glucose ($p<0.001$) following dextrose challenge at 60 ($p<0.001$), 90 ($p<0.001$) and 120 minutes was observed 5d post transplant (B). This was only maintained at fasting and 30 minutes post challenge at 12d (B).

Figure 2: Shows the circulating cytokine profiles for monocyte chemo-attractant protein-1 (MCP-1) and interleukin 1β (IL-1β) prior to and following transplantation of islets under the kidney capsule (A) or into the liver (B). No significant change was seen in MCP-1 in animals receiving islets under the kidney capsule (A). MCP-1 circulating levels were significantly reduced in animals receiving islets into the liver (B) compared to the diabetic sham controls at 19d post transplantation. IL-1β was significantly reduced at 15 and 19d post transplantation in transplanted animals compared to the diabetic sham controls (B).

Figure 3: Shows the brown insulin staining in the rat kidney (A) and liver (B), indicated by the black arrows. The small purple nuclei surrounding the islets are infiltrating immune cells, indicated by the red arrows. These immune cells are responsible for the progressive loss in function cause by allograft rejection.

CONCLUSIONS

- Poorer islet function was observed in the islets transplanted under the kidney capsule
- Better islet function was seen in the animals transplanted into the portal vein
- No significant changes could be observed in the cytokine profiles of the animals transplanted under the kidney capsule while the those of the liver animals were lower by 19d post transplantation
- Further work will be needed to characterise the infiltrating immune cells at the graft sites as well as to assess the condition of the islets and measurement of circulating cytokine biomarkers will facilitate evaluation of novel anti inflammatory and immunomodulatory agents in islet transplantation.