Chapter 6

Effect of sitagliptin and melatonin combination treatment on β-cell proliferation in humanized euglycemic mouse model of islet transplantation

6.1 Introduction

Chronic hyperglycemia, the hallmark feature of type 1 diabetes (T1D) and type 2 diabetes (T2D) is due to limited proliferation and increased apoptosis of pancreatic β-cells (Jin and Weng 2016; Pettus et al., 2013). The current therapeutic strategy for T1D patients is insulin injections to maintain normal blood glucose levels. Islet transplantation is also an effective therapeutic strategy for T1D which modulates blood glucose levels to achieve its normal levels. Over the past few years, considerable progress has been made in the advancement of protocols for human pancreatic islet transplantation in T1D patients (Shapiro et al., 2000). However, studies show only 10% of transplant recipients were insulin independent at the end 5 years (Ryan et al., 2005). Moreover, more than one donor pancreas is generally needed per transplantation (Boker et al., 2001) because of the reduction in islet viability both during isolation and after transplantation. There are multiple causes of posttransplant islet loss and probably include deficiencies in survival factors (Robertson, 2002); inflammation, and immune-mediated destruction; altered islet vasculature resulting in deficient nutrient and oxygen delivery (Davalli et al., 1996; Narang and Mahato, 2006) and the toxic effects of immunosuppressive agents (Narang and Mahato, 2006). Henceforth, there is an urgent need to develop methods for prolonging the survival of islets. We hypothesized that combination therapy with the existing drugs, and potential novel drugs, could induce β-cell proliferation in human islet transplant model and provide improved treatments for T1D patients.

There are limited studies on the potential for dipeptidyl peptidase- IV (DPP-IV) inhibitors in T1D management. In preclinical studies, the DPP-IV inhibitor, sitagliptin prolonged islet graft retention in streptozotocin (STZ)-induced T1D mice by increasing the endogenous levels of incretin hormones (Kim et al., 2008). Melatonin, a pineal hormone is secreted during the night hours and exhibits multiple functions. It has immunomodulatory effects besides an effective antioxidant and scavenger of free radicals. Lin et al., (2009) showed that exogenous melatonin treatment suppresses autoimmune recurrence by inhibiting the proliferation of Th1 cells in non-obese diabetic (NOD) mice and thus prolongs the survival of syngeneic islet grafts.

In chapter 4, we have shown that combination therapy of sitagliptin and melatonin promotes β-cell proliferation and reduces apoptosis in T1D mice. However, there are no such studies reported on human islets. Hence, we carried out a pilot study to assess the effect of *in vivo* daily treatment of combination of sitagliptin and melatonin on β-cell proliferation in human

islets transplanted under the kidney capsules of euglycemic immunodeficient NOD-severe combined immunodeficient (scid) mice.

6.2 Materials and Methods

All the experimental procedures were performed with prior approval of, and in compliance with, the Icahn School of Medicine Institutional Animal Care and Use Committee, Icahn School of Medicine at Mount Sinai, New York, NY, USA.

6.2.1 Human Islets

Human islets were obtained from Prodo Laboratories Inc. (Aliso Viejo, CA, USA). Human islet donors were all male, the mean age of the donors was 42 ± 12 years, the purity of the islet preparations was 90-95%, and the viability was 95%. Islets were cultured as described in Chapter 3.

6.2.2 Islet Transplantation

Islet transplantation was carried out as described by Garcia-Ocana et al., (2001) with a few modifications. Male (8 - 12 weeks of age) NOD-scid mice (stock number 001303- NOD.Cg-*Prkdc*^{scid}/J -The Jackson Laboratory, Bar Harbor, ME, USA) were anaesthetized with 100 mg/kg BW ketamine and 10 mg/kg BW xylazine, i.p. (Sigma, St. Louis, MO, USA). The skin of the left and right lateral side was shaved and cleaned with Fisherbrand® sterile cotton gauze pad (Fisher Scientific, Waltham, MA, USA). A lumbar incision of 1 to 1.5 cm perpendicular to the axis of the kidney across left and right side was made. The kidneys were carefully pushed out through a lumbar incision by using a Q-tip. Using two small forceps, a small hole in the lower half of the kidney capsules was opened. PE 50 polyethylene tubing (Becton Dickinson, Sparks, MD, USA) adapted to a 1 mL syringe containing human islets was inserted beneath the kidney capsules and gently pushed from the lower end to the upper end. The hole of the kidney capsules was closed by cautery loop. The incision was closed by using 3.0-nonabsorbable, sterile, surgical suture (PERMA-HAND; Ethicon, INC., Somerville, NJ, USA). Animals were then returned to their cages for recovery.

6.2.3 Euglycemic Human Islet Transplant Model

300 human islet equivalents (IEQ, 1IEQ= 125µm diameter islet) from three different human cadaveric donors were transplanted under both the kidney capsules of six euglycemic NOD-scid mice (n=6). Animals were allowed to recover for seven days and for the islets to be

engrafted, and were then randomly selected to be given daily sitagliptin (S) (20 mg/kg BW, p.o.) and melatonin (M) (0.5 mg/kg BW, i.p.) combination (n=3) or vehicle (saline) (n=3) for seven days. On the day eight, mice were sacrificed, kidneys containing the grafts were harvested, fixed in 4% formaldehyde, paraffin embedded, sectioned and immunostained for insulin and Ki67 as described in Chapter 3.

6.2.4 Statistical Analysis

Statistical analysis of β-cell proliferation was performed by unpaired t-test (GraphPad Sofware, San Diego, CA, USA). The significance level was set as $p<0.05$. Results are expressed as mean±SEM.

6.3 Results

6.3.1 Assessment of β-cell Proliferation in the Human Islet Grafts

Immunofluorescence analysis of human islet grafts in mice revealed that S+M daily treatment for one week significantly increased human β-cell proliferation ($p<0.001$) as compared to vehicle (Fig. 6.1).

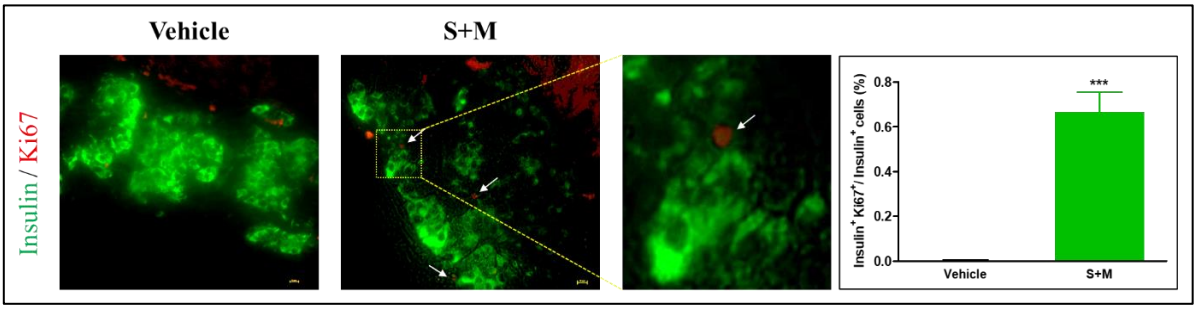

Figure 6.1 Immunofluorescence analysis for human β-cell proliferation in human islet graft as shown by Ki67$^+$ insulin$^+$ cells. S+M treated group showed a significant increase in β-cell proliferation as compared to vehicle. Arrows illustrate Ki67$^+$ β-cells. Scale: 10μm, Magnification: 40X. (***$p<0.001$, n=3/group).

6.4 Discussion

Several approaches have been developed to induce islet β-cell regeneration to increase β-cell mass in rodents, however, a few of these therapies showed similar effects on human β-cells**.** Although in the past, human islet β-cells were defined as terminally differentiated cells with limited proliferative capacity (Baeyens et al., 2018), recent studies suggest that human islet β-cells exhibit proliferative plasticity in the case of increased metabolic demands and in euglycemic humanised model of islet transplantation (Dai et al., 2017; Liu et al., 2021; Wang et al., 2015).

In chapter 4, we showed that monotherapies had a stimulatory effect on β-cell proliferation and a protective effect against apoptosis in STZ-induced T1D mice. This was accompanied by increased insulin levels, as well as improved glucose homeostasis. Moreover, the combination therapy showed greater therapeutic benefits in increasing β-cell proliferation, plasma insulin levels, glucose tolerance, and decreasing apoptosis in T1D mice. Unfortunately, in view of current COVID-19 pandemic situation, there was a limitation of the availability of human islets for research to determine whether the effects seen in mice could be recapitulated in human islets as well. Thus, we aimed to study the effect of only combination therapy on β-cell proliferation in human islet grafts. Our results suggest that combination therapy significantly increases human β-cell proliferation as compared to vehicle.

Melatonin treatment increased insulin secretion and promoted β-cell survival via decreased c-JUN N-terminal kinase (JNK) activation in human pancreatic islets (Chan et al., 2002). Lin et al., (2009) have reported that melatonin supresses autoimmune recurrence in NOD mice by inhibiting proliferation of T helper type 1 (Th1) cells, and melatonin signalling promotes β-cell survival and function (Costes et al., 2015). Further, the effect of DPP-IV inhibitors on β-cells is mediated by glucagon like peptide 1 (GLP-1) (Matveyenko et al., 2009). Previous studies have shown that GLP-1 increases rodent β-cell proliferation and reduces apoptosis by binding to its receptor (De Leon et al., 2003) via activation of P13K/Akt and CREB-IRS2 signalling pathways (Wang and Brubaker, 2002; Wang et al., 2004; Whalley et al., 2011). The effect of sitagliptin was evaluated in T1D patients who underwent human stem cells (HSCs) transplantation, and in NOD mice with islet graft survival. Both these studies showed increased C-peptide levels, and the T1D patients were also not in need of insulin therapy for six months, confirming sitagliptin's immunoregulatory role in T1D. However, there was no beneficial effect on hyperglycemic state (Couri et al., 2009; Kim et al., 2009). In pre-clinical studies, sitagliptin treatment phosphorylated CREB and through VEGF-A/VEGFR-2 signalling pathway, induced islet vascularization (Samikannu et al., 2013). In addition, a recent study revealed that administration of either GABA or sitagliptin normalized blood glucose levels, increased transplanted human β-cell counts and plasma human insulin levels in human islet transplant model (Liu et al., 2021). With respect to β-cell proliferation in a euglycemic humanised mouse model of islet transplantation, there are a few studies which showed that harmine (DYRK1A inhibitor) alone or in combination with exendin-4 (GLP-1 analog) treatment induces human β-cell proliferation by 2 to 3- fold in NOD-scid mouse as

compared to vehicle (Ackeifi et al., 2020; Rosselot et al., 2020; Wang et al., 2015). Thus, our results on the effect of combination therapy on human β-cell proliferation are novel and complement previous studies in rodents.

In conclusion, our study indicates that combination therapy of sitagliptin and melatonin induces human β-cell proliferation (Fig. 6.2), and it could be used as future therapy for β-cell regeneration in diabetes patients.

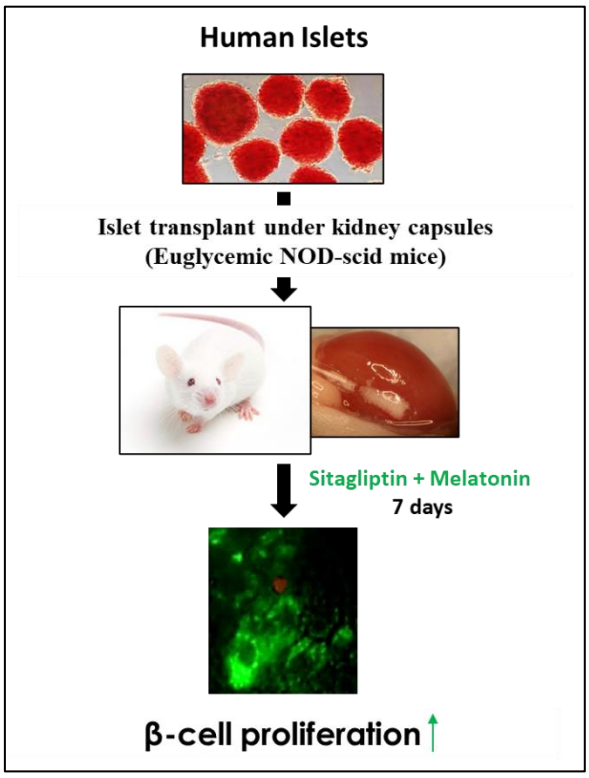

Figure 6.2 Combination therapy of sitagliptin and melatonin induces human β-cell proliferation in humanized euglycemic mouse model of islet transplantation.

6.5 References

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