ABSTRACT

Abstract

Diabetes Mellitus is one of the leading metabolic syndromes which has caused a burden on the healthcare system worldwide. The current hypoglycaemic drugs that are commercially available in the market for the treatment of diabetes can only manage the condition and possess side effects. Hence alternative and complementary medicines focus our attention on the herbal repertoire which is cost-effective and safe to use. Hence exploring novel bioactive is one of the major areas being elucidated for effective diabetes therapy in the present thesis. One such medicinal plant is *Enicostemma littorale (EL)* whose various anti-diabetic properties have been well explored in our lab. We have systemically elucidated that Swertisin, a C glucosyl flavone from *EL* is a potent differentiating agent in islet differentiation and facilitates islet neogenesis from Pancreatic Stem/Progenitor Cells. Bioactive swertisin has also shown a promising glucose-lowering effect. Hence, here we had aimed to mechanistically dissect the glucose lowering property of swertisin. A systematic *in silico, in vitro,* and *in vivo* approach was directed for target analysis of swertisin. Molecular docking and simulation were performed with Swertisn. Sodium glucose cotransporter 2 (SGLT2) was identified as a potential target, responsible for glucose reabsorption from the kidney and reducing blood glucose levels. SGLT2 inhibitors are amongst the various therapeutic candidates available commercially. Swertisin-hSGLT2 molecularly docked complex showed similar binding energy compared to the Canagliflozin-hSGLT2 complex. Glucose uptake assay and protein expression for SGLT2 and regulatory proteins were performed under the swertisin effect. Swertisin inhibited glucose uptake and decreased expression of SGLT2 in HEK293 cells. Swertisin does not affect GLUT mediated glucose transport. Various physiological and metabolic parameters were evaluated in STZ induced BALB/c mice using swertisin treatment. SGLT2 expression was evaluated in the kidney tissue of mice. Swertisin treated diabetic mice demonstrated remarkable improvement in overall glucose homeostasis. Reduced expression of SGLT2 was found in kidney tissue along with reduced PKC expression which is one of the key regulators of SGLT2. Our study explored SGLT2 as a selective target of swertisin for its swift glucose-lowering action which not only inhibits SGLT2 but also reduces its expression in diabetic conditions. Thus, the potential property of swertisin as a glucose-lowering agent is remarkable which points toward a wider avenue of diabetes therapy.

Abstract

On the other hand, cell replacement and regenerative therapies have become a beacon of hope in the paradigm of diabetes therapeutics. As the supply of islets for cell replacement therapy is very limited we optimized our understanding of organ crosstalk and secretome therapeutics and generated functional insulin producing islet clusters from pancreatic progenitors which is another major area of investigation in the present thesis. Insulin sensitive tissues like skeletal muscle and adipose derived stem cells from adipose tissues were explored for their trophic effects in the form of secretomes on islet functionality as well as islet differentiation. The secretory nature of skeletal muscle has made it an integral part of the inter organ crosstalk network. The communication by secretory products to various organs contributes towards a harmonic and maintained metabolic homeostasis. But a pathological condition like insulin resistance (IR), alters the secretome including myokine panel. These altered panel detrimentally affects the pancreatic islet β cells functionality. In the present study, differential myokine and protein profile of control and insulin resistant secretome were monitored. PREPs were differentiated into Islet like cell clusters (ILCCs) under the influence of control and altered skeletal muscle secretome and various islet differentiation and functionality parameters were studied. Expression of key transcription factors like HNF-3B, NGN-3, PAX4, Nkx 6.1, Nestin, NeuroD, PDX- 1, Maf-A, and GLUT-2 involved in development were differentially regulated in IR muscle secretome as compared to control. ILCCs functionality and viability were critically hampered under IR muscle secretome. ILCCs had compromised yield and morphometry, lower expression of C-peptide and Glucagon with increased ROS activity and cell death parameters.

Islet differentiation is also affected by crosstalk with Adipose derived stem cells (ADSC) which secrete a large array of factors in the extracellular milieu and exhibit regulatory effects. The microenvironment of metabolically compromised human ADSCs (hADSCs) has a detrimental impact on islet functionality. In the present study, the role of hADSC secretome was studied on the differentiation of islets. Expression of key transcription factors like HNF-3B, NGN-3, NeuroD, PDX- 1, Maf-A, and GLUT-2 involved in development was differentially regulated in obese hADSC secretome as compared to control hADSC secretome. Islet like cell clusters (ILCCs) functionality and viability were critically hampered under obese hADSC secretome with compromised yield, morphometry, lower expression of C-peptide and Glucagon as well as higher ROS activity and cell death parameters. This study demonstrates the basis of loss of islet function

Abstract

and differentiation due to compromised secretome of skeletal muscle and ADSC from adipose tissue. Further, it provides considerable insight into exploring the careful use of skeletal muscle and ADSC secretome in islet differentiation for better diabetes therapeutics.

Thus, the present thesis provides a considerable insights on three major findings which are (1) Swertisin as a potent SGLT2 inhibitor (2) exploring the use of muscle and hADSC secretome in islet differentiation and (ii) understanding the regulating effect of altered muscle and hADSC secretome under a metabolically compromised condition and their cautious therapeutic use.