CHAPTER 7: SUMMARY

7.1. INTRODUCTION

Obesity induced metabolic dysregulation results in cluster of chronic conditions mainly hyperglycemia, hyperinsulinemia, dyslipidemia, diabetes, cardiovascular complications and insulin resistance (Hursting and Dunlap, 2012). Metabolic pathways in organisms have developed to deliver energy to tissues in times of physical threat and survival, or to efficiently save energy in times of food deprivation. Today, the combination of abundance of food and lack of physical activity has led to excessive nutrient storage, introducing major stress on our metabolic pathways, resulting in an increase in the occurrence of disease originating from metabolic dysfunction (Collins et al., 2018).

Stem cells have a great potential for basic research and also hold the future for clinical applications. The characteristic features of these cells are quite unique from somatic cells (Ranganath et al., 2012). The area of mesenchymal stem cell (MSC) research has bloomed recently. MSCs are known to be multipotent and have the ability of self-renewal and differentiation into several mesodermal cell lineages and certainly have the capacity to repair several damaged tissues (Pileggi, 2012). Adipose-derived mesenchymal stem cells (ADSCs) is emerging as a source of stem cells that can be obtained by a less invasive method and in larger quantities than from BM. These cells can be isolated from human lipoaspirates and, like MSCs, can differentiate toward osteogenic, adipogenic, myogenic, chondrogenic, and especially neurogenic lineages 3. ADSCs, can be readily isolated from fat tissue after liposuction and easily expanded in culture in large numbers, have developed an striking source for cell therapy (Badimon et al., 2015; Cao et al., 2015).
7.2. DESCRIPTION OF THE WORK

Objective 1: Examining the role of human adipose tissue derived mesenchymal stem cells (hADSCs) in the reversal of insulin resistance in vitro

Here, we established an insulin resistant model of 3T3L1 and C2C12 cells and treated with ADSCs-CM. 2-NBDG (2-(N-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)Amino)-2-Deoxyglucose) uptake was performed to assess improvement in glucose uptake. Genes involved in glucose transport and in inflammation were also analysed. Western blot for glucose transporter-4 and Akt was performed to evaluate translocation of Glut4 and insulin signalling respectively. We found that the ADSCs-CM treated cells restored insulin stimulated glucose uptake as compared to the untreated control indicating the insulin sensitizing effect of the CM. The treated cells also showed inhibition adipogenesis in 3T3L1 cells and significant reduction of intramuscular triglyceride accumulation in C2C12 cells. Gene expressions studies revealed the drastic upregulation of GLUT4 gene and significant reduction in IL6 and PAI1 gene in both 3T3L1 and C2C12 cells indicating possible mechanism of glucose uptake with concomitant decrease in inflammation. Enhancement of GLUT4 and phosphoAkt protein expression seems to be responsible for the increment in glucose uptake and enhanced insulin signalling respectively. Our study revealed for the first time that ADSCs-CM acts as an alternative insulin sensitizer providing stem cell solution to IR.

Objective 2: Evaluation of the role of hADSCs and its conditioned media on normalising acute inflammation induced by carrageenan in db/db mice

Since, inflammation is the root cause of all the metabolic dysregulation, we aimed to evaluate the effect of human adipose derived mesenchymal stem cells (ADSCs) and its conditioned media (CM) on carrageenan induced acute inflammation in db/db mice. We injected $5 \times 10^5$ ADSCs or the CM in the inflamed paw. We assessed the paw volume, serum
IL6 levels and histopathology of the paw to reveal the anti inflammatory effect. We observed a single injection of ADSCs or CM could reverse the inflammation within 24h as evidenced by reduction in paw volume, IL6 levels and histological examination. Our result equivocally demonstrates the role of CM in normalising the inflammation better than ADSCs.

**Objective 3: Assessment of the effect of intramuscular injection of hADSCs in three different forms in diet induced obese model of mice**

The earlier objective proved the beneficial effect ADSCs and its CM in inhibiting inflammation in acute inflammatory animal model. Further, the role of adipose tissue derived MSCs in controlling hyperglycemia, muscle wasting and deranged lipid profile caught our attention. We injected high fat diet (HFD) induced C57BL/6 mice with human adipose tissue derived mesenchymal stem cells (ADSCs) as cell suspension (CS), conditioned medium (CM) and the cell lysate (CL) intramuscularly. Metformin was used as a positive control. ADSCs treated mice exhibited remarkable decrease in IR as quantified by HOMA-IR and Triglyceride Glucose index with concomitant decrease in Oxd LDL and IL6 as compared to the untreated HFD control. MSC injection showed improvement in glucose tolerance and reduction of fatty infiltration in the liver, reduction of macrophage infiltration in adipose and reduction in hypertrophied pancreatic islets due to HFD feeding. Upregulation of miRNA-206, MyoD and increase in protein content of the skeletal muscle in CS treated mice indicates that ADSCs treatment in increase the muscle mass and improving lipid profile in HFD mice. Thus, we conclude that autologous or allogeneic ADSCs could be a novel intervention strategy to control high fat diet induced obesity probably by targeting inflammation.

**Objective 4: Examination of the role of hADSCs transplantation preconditioned with metformin in the management of obesity in mice**

In our previous study, we have found that metformin significantly reduces body weight whereas ADSCs alone fail to do that and hence we were interested in knowing the
effect of the combination of ADSCs and metformin treatment in Diet Induced Obese model of mice. We examined whether preconditioning of adipose derived mesenchymal stem cells (ADSCs) with metformin could have a better therapeutic value for the reversal of type 2 diabetes along with reduction in body weight. We compared the effect of metformin, ADSCs and metformin preconditioned ADSCs (MetADSCs) in high fat diet induced C57BL/6 mice by injecting the cells intramuscularly only once whereas metformin was given at a concentration of 300 mg per kg body weight orally daily. Fasting glucose was measured every week for 4 weeks. At the end of the study insulin, triglycerides, IL6 and oxidised LDL were evaluated from the serum. Gene expression studies were performed for muscle (GLUT4) and liver tissues (IL6 and PAI1). There was a remarkable decrease in hyperglycemia within two weeks of injection by MetADSCs as compared to metformin and ADSCs alone. A significant decrement of hyperinsulinemia, triglyceridemia, serum IL6 and oxidised LDL were observed at the end of the study. Gene expression studies for muscle tissue revealed the drastic upregulation of GLUT4 gene levels in the MetADSCs group indicating enhanced glucose uptake in muscle. Liver tissue analyzed for the genes involved in inflammation viz. IL6 and PAI1 showed significant downregulation in the MetADSCs group as compared to the other groups. This is a first report demonstrating the synergistic effect of metformin preconditioning of ADSCs leading to reduction in obesity and obesity induced metabolic dysregulation like hyperglycemia, hyperinsulinemia and triglyceridemia.

7.3. LIMITATIONS

There are few limitations of the study which is mentioned below.

CM could have been injected more frequently for better results and to confirm the insulin resistant state of the mice, hyperglycemic-euglycemic clamp study could have been performed.
Despite the mentioned limitations, a set of data has been validated for the first time and a few of them can be added to the existing knowledge of the subject.

7.4. FUTURE DIRECTIONS

It would be worthwhile to trace the path of intramuscular injection in DIO animals to understand the bio distribution of ADSCs and to predict the possible mechanism of action in metabolic dysregulation.