EXAMINATION OF THE ROLE OF hADSCs TRANSPLANTATION PRECONDITIONED WITH METFORMIN IN THE MANAGEMENT OF OBESITY IN MICE

CHAPTER 6: OBJECTIVE 4

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6.1. INTRODUCTION

One of the principle pathogenic defects of type 2 diabetes is insulin resistance which is the inability of insulin to exert a biological action at the level of its target tissue (Giannarelli et al., 2003). Although, metformin has pleiotropic role in controlling many diseases; metformin is the most widely used anti diabetic for controlling hyperglycemia (Arunachalam et al., 2014). There is an increase in the incidence of heart related disease which is one among many complications of diabetes (Kravchuk et al., 2011). As revealed by the accumulated evidence metformin is the only antidiabetic drug available for clinical use in the biguanide class. In addition to its major glucose-lowering effect, it also exerts several pleiotropic effects including beneficial changes in blood rheology, serum lipid profile, and putative anti-ischemic effects (Lotfinegad et al., 2014). Currently available therapies for diabetes fail to give the consistent glycemic control so there is a dire need to look for an alternative treatment option. Mesenchymal stem cells (MSCs) have been proven to be immunomodulatory, anti-inflammatory, anti aging and Angiogenic (Dormady et al., 2001). MSCs are widely known to be multipotent. They synthesize various cytokines and growth factors naturally which are primarily influenced by local microenvironments around them (Aggarwal and Pittenger, 2005). These trophic mediators not only promote the survival of surrounding cells but also play important roles in MSCs regenerative/regulatory properties both in vitro and in vivo (Berman et al., 2010). It has been reported earlier that systemic syngenic injection of
adipose derived stem cells reduces blood glucose and improves sensitivity to insulin in DIO mice (Cao et al., 2015). Preconditioning of MSCs with a suitable compound or media has been shown to be extremely effective in treating a diabetic heart (Khan et al., 2013). Metformin possesses neuroprotective activity in MPTP induced Parkinson’s disease in mice and provides preclinical support for therapeutic prospective of MPTP (Patil et al., 2014). There are no reports so far stating the effect of metformin preconditioned ADSCs in insulin resistance. Here, we demonstrated for the first time the beneficial effects of intramuscular injection of metformin preconditioned ADSCs (Met ADSCs) in ameliorating insulin resistance.

Figure 36: Experimental design for Chapter 6

6.2. RESULTS

DIO model development: As described in chapter 5.

Reduction in body weight: There was a significant reduction in the body weight of the mice treated with MetADSCs as shown in Figure 37A which was comparable to that of metformin despite of continued high fat diet (p<0.01). There was no significant change in the body
weight of the mice treated with ADSCs. Figure 37B depicts the body weight at the end of the study (Week 4 after treatment).

**Figure 37: Body Weight measurements.** A. Weekly body weight from week 0 to week 4 showed MetADSCs significantly reduces body weight as compared to untreated control \( p < 0.01 \). B. Body weight at the end of the study.

**Improvement in glycemic status:** Intramuscular delivery of ADSCs preconditioned with metformin resulted in decreased glucose levels one week after the treatment dramatically which was near to normal on third week of treatment and was maintained till the end of the study (Figure 38).

**Figure 38: Fasting Glucose levels.** A. Fasting glucose levels of the mice from week 0 to week 4 of the treatment. All the treatment groups showed significant reduction in glucose levels with a \( p \) value of <0.001. B. Bar graph showing fasting glucose at the end of the study.

**Effect on lipid profile:** At the end of 4th week of treatment, serum triglycerides and oxidized LDL were measured after overnight fasting and showed significant increase in the DIO
control mice as compared to the lean control (p< 0.001). The MetADSCs treated group showed significant decrease in triglyceride levels (Figure 39A) and serum oxidised LDL (Figure 39B) which was comparable to that of metformin.

**Figure 39: Status of lipids in serum.** A. Serum triglyceride levels at the end of the study displays significant reduction of triglyceridemia when treated with MetADSCs (p<0.001). B. Serum levels of oxidised LDL showed drastic reduction in MetADSCs group when compared to the untreated control (p<0.001).

**Improvement in hyperinsulinemic conditions:** Untreated DIO control attained hyperinsulinemia as compared to the lean control (p<0.001). On the other hand, all the treatment groups showed significant reduction in the serum insulin levels as shown in Figure 40. MetADSCs treated group being the best of all the treatment groups.

**Figure 40: Insulin levels in the serum.** Fasting serum insulin levels denoted as ng/ml showed a remarkable reduction in hyperinsulinemic condition when treated with MetADSCs (p<0.001).
Measurement of Interleukin-6 in serum: We measured serum IL6 and found a drastic increase in IL6 levels of the DIO mice. In contrast, only MetADSCs treated group showed significant decrease in IL6 levels (Figure 41).

**Figure 41: Interleukin-6 levels in the serum.** Serum analysis at the end of the study demonstrated reduction in pro-inflammatory cytokine Interleukin-6 when treated with MetADSCs (p<0.01)

HOMA IR and TyG: In accordance with the earlier reports which states that HOMA IR and TyG are the useful and reliable indicators of insulin resistance our data shows a remarkable decrease in HOMA IR and TyG in all the treatment groups as compared to DIO control (Figure 42A and 42B).

**Figure 42: Assessment of Insulin resistance:** A. Homeostatic model assessment of Insulin resistance (HOMA-IR) confirms the significant reduction in insulin resistance when treated with MetADSCs (p<0.001). B. Triglyceride glucose index measurement results showed
remarkable reduction in MetADSCs treated group (p<0.01) as compared to untreated control.

**Gene Expression Analysis:** Gene expression analysis was carried out for liver and muscle tissue samples. Figure 43A shows significant increase in the skeletal muscle GLUT4 gene expression levels in MetADSCs treated group indicating enhanced sensitivity to insulin. Gene expression analysis for the liver tissues showed remarkable decrease in Il-6 and Pai-1 depicted in Figure 43B and 43C. Downregulation of these genes in MetADSCs treated group indicates the reduction in the amount of inflammation in the liver.

**Figure 43: Gene expression Profile:** A. Glut4 gene expression in skeletal muscle tissue showed an upregulation of Glut4 gene expression in MetADSCs treated group (p<0.05). B. Il-6 gene expression in liver tissue demonstrated reduction in inflammation when treated with MetADSCs (p<0.05). C. Pai-1 gene expression in liver tissue showed significant reduction in the MetADSCs treated group as compared to the untreated control with a p value <0.05.
6.3. DISCUSSION

Insulin resistance in obesity and type 2 diabetes is manifested by decreased insulin-stimulated glucose transport and metabolism in adipocytes and skeletal muscle (Kahn and Flier, 2000; Reaven, 1995). Our study mainly focused on insulin resistance developed due to obesity and its reversal by i.m. injection of preconditioning ADSCs with metformin. For that, diet induced obese (DIO) model of mice was developed as reported earlier (Wang and Liao, 2012). The primary finding of the present study is that MetADSCs showed better reversal of insulin resistance as compared to metformin and ADSCs alone. It has been established that i.v. treatment with ASC show decrement in hyperglycemia in DIO mice (Cao et al., 2015; Ji et al., 2015). It has also been established that supplementation of metformin may be a potential choice for overcoming the damaging effects of aging on ADSCs and might be considered in the pretransplantation period, especially in older patients, to enhance the effects of ADSCs based regenerative therapies. Administration of metformin has an important influence on ADSCs morphology, proliferation rate, and osteogenic/adipogenic differentiation ability, all of which are essential features of tissue regeneration (Marycz et al., 2016). It has also been shown that metformin affects stromal stem cells proliferative activity in a dose-dependent manner (Śmieszek et al., 2015). The uniqueness of our study lies in intramuscular delivery of the MetADSCs. A cross talk between skeletal muscle and pancreatic beta cell has been reported earlier (Gopurappilly and Bhonde, 2012). We were interested in examining the effect of MetADSCs injection on the glycemic status and lipid profile which is invariably affected in DIO mice. A study by Nicpoń et al. showed an inhibitory effect of metformin on adipogenic differentiation potential (Nicpoń et al., 2013). The most exciting feature of our study is the remarkable reduction in the body weight of the MetADSCs treated mice group which was similar to that of mice treated with metformin alone. However, mice treated with ADSCs alone did not show significant reduction in the
body weight. We also observed a significant decrease in hyperglycemia as a result of single injection of MetADSCs right from the first week till third week when normoglycemia was restored and was maintained till the end of the study. It is demonstrated in several previous reports that high plasma triglycerides are associated with insulin resistance (Haffner et al., 1992). It is reported earlier that metabolic syndrome hypertriglyceridemia results from IR or both IR and hypertriglyceridemia are the result of a common cause (Hölzl et al., 1998). Reaven proposed the term “Syndrome X” for the cluster of insulin resistance and hyperinsulinemia, impaired glucose tolerance, abnormalities of plasma lipids, and hypertension, now commonly called the metabolic syndrome (Reaven, 1988). Our study shows the reduction in the major factors that add on to the development of metabolic syndrome viz. serum triglycerides, insulin. Two to three fold elevated levels of circulating IL6 has been reported in insulin resistant state (Pradhan et al., 2001). Our data shows a significant reduction in the serum IL6 levels in all the treatment groups. Circulating oxidized low-density lipoprotein (LDL), a marker of oxidative stress, is associated with obesity, insulin resistance, metabolic syndrome, and cardiovascular disease in adults (Kelly et al., 2010). We observed a remarkable reduction in serum oxidised LDL levels in Met ADSCs treated group. It is well known that IR is the root cause of metabolic syndrome (Roberts et al., 2013). Reversal of insulin resistance was the best seen in the mice treated with MetASC as evidenced by HOMA-IR and TyG measurements (Unger et al., 2014). It is stated earlier that muscle GLUT4 protein and mRNA expression in reduced in case of severe insulin resistance (Atkinson et al., 2013). Glut4 gene responsible for glucose uptake in muscle showed drastic increase in MetADSCs treated mice as compared to Metformin and ADSCs alone. Further, it has been reported that there is an altered gene expression in non alcoholic fatty liver disease (Auguet et al., 2014). Genes involved in inflammation in the liver such as Il6 and Pai-1 as a
result of severe IR leading to further complications showed significant reduction in MetADSCs treated group.

Although daily oral treatment with metformin was found to be effective in decreasing IR, it did not show its beneficial effect in decreasing IL6 at gene and protein levels. In all these conditions, MetADSCs has surpassed the role of metformin signifying its dominant role only with single injection which supports the earlier reports stating that metformin can be used orally before collection of adipose tissue for isolation of ADSCs for clinical application, or metformin might be used as an active agent that may be added to the culture environment to increase activity of ADSCs before patient transplantation (Marędziak et al., 2016). Here, we chose i.m. route in order to facilitate slow release of their paracrine secretion into the systemic circulation. Moreover, muscle insulin resistance is the hallmark of high fat diet induced type 2 diabetes. Metformin is known as insulin sensitizer. A combination of ADSCs with metformin is likely to exert synergistic action in counteracting insulin resistance.

6.4. CONCLUSION

Our data clearly demonstrates the impact of MetADSCs single injection in reversal of type 2 diabetic conditions in DIO mice as evidenced by decrease in body weight, hyperglycemia, hypeinsulenima, triglyceridemia and reduction in IL-6 and oxidised LDL.