CHAPTER 4: OBJECTIVE 2

EVALUATION OF THE ROLE OF hADSCs AND ITS CONDITIONED MEDIA ON NORMALISING ACUTE INFLAMMATION INDUCED BY CARAGENNAN IN db/db MICE

4.1. INTRODUCTION

Inflammation is a combination of biological host reaction to stimuli and is characterized by heat, pain, redness and swelling (Park et al., 2013). Any type of inflammation, either chronic or acute is strongly accompanied by diseases such as obesity, diabetes, vascular disease, obstructive pulmonary disease, cancer and many more. (Brevetti et al., 2010; Hummasti and Hotamisligil, 2010; Christaki et al., 2010; Lambrecht and Hammad, 2010; Mantovani et al., 2008). Mesenchymal stem cells (MSCs) treatment has flourished recently. There are several reports stating that MSCs have variety of role to be played in tissue repair and injury. They are strongly known to immunomodulatory and anti-inflammatory. Apparently, MSCs play a role as a guardian of inflammation (Prockop and Oh, 2012). Paw swelling, or footpad oedema, is a suitable method for measuring inflammatory responses to antigenic challenges and irritants. The protocol described by Whiteley et al. describes the development of paw oedema using carrageenan as an irritant. Generally, the test compounds are evaluated for acute anti-inflammatory activity by examining their ability to reduce or prevent the progress of carrageenan-induced paw swelling (Whiteley and Dalrymple, 2001). The role of MSCs in carragennan induced paw oedema has already been established. Nevertheless, the role of conditioned media from ADSCs has not been reported so far. There are some limitations in the usage of stem cells as a treatment of choice regarding source, age of donor, autologous versus allogeneic (Hentze et al., 2009)(Wang et al., 2012b)(Ryan et al., 2005). However,
using conditioned media would be more appropriate and less risky as it is a blend of all growth factors and cytokines secreted by MSCs. Conditioned media endorses a cell free treatment with all the advantages of stem cells without cells. Conditioned media can be stored for a longer period of time and can be frozen and dried as well; their storage and transportation appears to be easier than MSCs. Hence, usage of conditioned media is user friendly, easy and suitable than using MSCs (Kim et al., 2011; Peng et al., 2015). We investigated the role of ADSCs and its CM in improving carrageenan induced paw oedema in \( db/db \) mice.

**Figure 16: Experimental design for Chapter 4**

4.2. RESULT

**Interleukin-10, anti-inflammatory cytokine in the CM**

We performed ELISA to check for anti-inflammatory cytokines, we observed good amount of anti-inflammatory cytokine, Interleukin-10 is present in the CM used for the treatment of acute inflammation. Figure 17 shows the amount of IL-10 present in crude CM and 50% diluted CM.
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Figure 17: Graphs representing levels of IL10 in the conditioned media

Decreased paw volume in the treated group

Paw volume when measured using plethysmometer showed significant reduction in the ADSCs and CM treated group hence decreasing acute inflammatory response developed by carrageenan induced paw oedema as depicted in Figure 18.

Figure 18: Measurement of paw volume using plethesmometer.
Reduction in paw oedema in the treated group

After 24 hours of treatment with ADSCs and CM, the redness and swelling due to the inflammation induced by carrageenan injection remarkably reduced which can be witnessed through naked eye as shown in Figure 19.

![Figure 19: Images showing paw oedema after 24h of Carrageenan injection](image)

Reduced inflammatory exudate in the treated group

Histological examination of paw shows inflammatory exudates in carrageenan treated paw and was significantly inhibited in ADSCs and CM treated paw (Figure 20).

![Figure 20: Histological examination of the paw](image)

Reduction in pro inflammatory cytokines

To confirm the reduction in inflammation observed in tissue sections we analysed serum for the levels of interleukin-6 (IL6) in the treated and untreated group. As expected, drastic inhibition in IL6 levels by ADSCs and CM treatment was observed when compared to that of carrageenan treatment correlating to the histology of the paw (Figure 21).
4.3. DISCUSSION

Adipose tissue is widely known to be a rich, ubiquitous and easily accessible source for multipotent stem cells and has, therefore, several advantages over other sources of mesenchymal stem cells. The most complicated attempt is to identify the origin of the stem cell population within adipose tissue in situ because no marker has currently been described which explicitly identifies native adipose-derived stem cells (Baer, 2014). It has been reported that ADSCs treatment, beyond its protective properties, also displays a therapeutic potential and suggests that ADSCs may provide a promising approach for treating Rheumatoid Arthritis (Zhou et al., 2011). The earlier report demonstrated that 50% CM exerts the best results when compared to 70% and 100% CM (Hao et al., 2014). We used 50% CM for the present study. Our study demonstrates the desired effect of ADSCs and CM in reducing acute inflammatory response developed by carrageenan induced paw oedema. We found a significant amount of IL10 in the CM compared to the control supporting the fact that human MSCs irrespective of source of tissue exert anti-inflammatory action due to their paracrine secretion/secretome which contain anti-inflammatory cytokines. When there is a tissue injury the blood vessels in the damaged area constrict momentarily (vasoconstriction), the dilation
of blood vessels (vasodilation) follows, increasing blood flow into the area, which may last from 15 minutes to several hours. It is widely known that the walls of these blood vessels, which normally allow only water and salts to pass through, become permeable resulting in protein-rich fluid, called exudate, to exit into the tissues. This is followed by emigration of white blood cells into the extravascular space of the tissue leading to oedema (Gupta et al., 2015). Histological examination of paw shows inflammatory exudates in Carrageenan treated paw and was significantly inhibited in ADSCs and CM treated paw. The concept of the formation of proinflammatory cytokines (e.g., TNF-α, IL-1β, and IL-6) play important roles in the pathophysiology of inflammation has been widely accepted (Amdekar et al., 2012; Luo et al., 2010). To confirm the reduction in inflammation observed in tissue sections we performed IL6 ELISA and found a significant increase in the IL6 levels as a result of carrageenan treatment which was drastically inhibited by ADSCs and CM treatment indicating the probable mechanism of action of ADSCs and CM via reduction of IL6 levels. Overall, a single subcutaneous injection of ADSCs or CM have the potential to decrease the inflammation. Our study indicates that reduction in IL6 as a result of ADSCs or CM injection is the probable mechanism of action in the reversal of inflammation.

4.4. CONCLUSION

We demonstrate that ADSCs and its CM exert anti inflammatory effect in inhibiting the carrageenan induced paw oedema in db/db mice. This study might pave the way for treatment modality in acute inflammation.