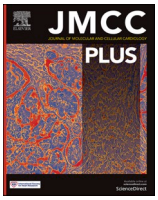
Journal of Molecular and Cellular Cardiology Plus 10 (2024) 100086

Contents lists available at ScienceDirect

Journal of Molecular and Cellular Cardiology Plus

journal homepage: www.journals.elsevier.com/journal-of-molecular-andcellular-cardiology-plus



Applying adipose tissue-derived stem cell therapies as a novel treatment for atherosclerotic plaque development: Importance of appropriate dosing

1. Introduction

The manifestation of atherosclerosis underlies many cardiovascular diseases (CVDs), which are the leading causes of death worldwide [1]. The onset of atherosclerosis stems from accumulating risk factors such as sedentariness, obesity, aging, hypertension, high-cholesterol, dyslipidemia, and smoking, all of which are known to create pro-inflammatory and pro-oxidative environments in the vasculature [1]. These environments have been found to trigger the manifestation of atherosclerosis, mediated by endothelial cell damage which increases the susceptibility for the development of atherosclerotic lesions and impaired blood flow in the peripheral tissue that contributes to the vasculopathies in individuals with CVDs [1]. Unfortunately, these effects can be intensified for those living with chronic metabolic diseases such as diabetes mellitus (DM). DM is a chronic metabolic disease characterized by systemic hyperglycemia and hyperinsulinemia which leads to increases in systemic inflammation, making those with DM 2-4× more likely to develop atherosclerosis and CVD-mediated mortality compared to individuals without DM [2]. Additionally, patients with DM have been found to have greater atherosclerotic plaque instability that are more prone to rupture, which is the most common cause of heart failure and death [3]. This creates a problematic crisis for the nearly 537 million individuals currently living with DM, a number that is expected to rise to nearly 783 million by 2045 [2]. Therefore, therapeutics which target atherosclerotic plaques may be an effective strategy to treat the vasculopathies that underly the manifestation of CVDs and early mortality in patients with DM.

Stem cell therapies (SCTs) have received growing interest as a novel therapeutic option due to their ability to undergo cell differentiation which may potentially treat a variety of cardiovascular, neurological, respiratory and musculoskeletal diseases [4]. Interestingly, SCTs using mesenchymal stem cells (MSCs), a type of multipotent, non-hematopoietic, stromal precursor cell, capable of multi-pathway differentiations, has emerged as the dominant cell type in clinical research [4]. This may be due to the discovery that MSCs can be harvested from a variety of tissues (e.g., bone marrow, adipose tissue, placenta) and may have distinct tissue specific characteristics [5]. In regenerative medicine, harvesting MSCs from bone marrow has emerged as the traditional approach due to high concentration of neutrophils which are crucial for immune function, although this process is highly invasive [6]. On the contrary, isolating stromal cells from adipose tissue is far less invasive, yielding adipose derived MSCs (ASCs) that display potential vascular protective properties through anti-inflammatory and angiogenic mechanisms [6]. Collectively, this suggests ASCs may prove to be a potentially potent therapeutic for treating the multi-faceted pathology of DM and atherosclerosis [5,7]. However, much of this is based on positive outcomes observed in murine models, as outcomes in clinical trials have been insufficient to support the efficacy of SCTs in human trials. In the current issue of *Journal of Molecular and Cellular Cardiology Plus*, Korn et al. strategically identified that this may be because SCTs doses in animal trials are vastly disproportional to those investigated in clinical trials, and instead investigated how a clinically relevant dose of ASCs impacted atherosclerotic plaque characteristics in a hyperglycemic induced mouse model.

Korn et al. induced hyperglycemia in male C57BL/6 ApoE −/− mice (6–8 weeks old, $n = 24$) by injecting streptozotocin (0.05 mg/g, STZ) intraperitoneally for 5 consecutive days. Blood glucose was measured 10 days after the first STZ injection to determine successful induction of hyperglycemia (blood glucose >10 mmol/L). ASCs were derived from stromal cells harvested from the abdominal subcutis and inguinal fat pads from healthy male C57BL/6 mice (5–7 weeks old, $n = 5$). At 16 weeks from the first STZ injection, mice were anesthetized and injected with a single dose of ASCs (100,000 cells/100 μL) ($n = 9$) or vehicle ($n = 14$). This dose was determined based on doses used in human clinical trials (1–4 million MSCs/kg), and Korn et al. established a clinically relevant dose between 25,000 and 100,000 ASCs based on an average mouse weight of 25 g. Blood samples were collected from the tail vein 3 days before treatment, 3 days after treatment, and immediately prior to termination 4 weeks after treatment via cardiac punction. After termination, the heart and connected thoracic aorta were excised for immunohistochemical analysis. STZ induced hyperglycemia led to the development of atherosclerotic plaques in both conditions. Although the authors found no significant difference in plaque characteristics between ASC and vehicle mice, the authors did note a positive trend in plaque stability following ASC treatment compared to vehicle ($p = 0.07$). Peripheral mononuclear blood cells (PBMCs) were obtained from blood samples to determine the concentration of classical (pro-inflammatory) and non-classical (anti-inflammatory) monocytes. 3 days after treatment, classical monocyte levels were elevated while non-classical monocytes were decreased compared to pre-treatment only in the ASC group. Although this was reversed at termination in both ASC and vehicle groups, which had increased non-classical monocyte levels and decreased classical monocyte levels compared to post treatment. However, these results are not enough to conclude that a single clinically relevant dose of ASCs administration is effective at improving atherosclerosis in hyperglycemic mice. Instead, these findings strategically point out the inadequate representation of stem cell therapies in translational application of treating atherosclerosis and DM between murine

https://doi.org/10.1016/j.jmccpl.2024.100086
Received 25 July 2024; Accepted 30 July 2024
Available online 3 August 2024

and human trials.

2. Experimental considerations and future directions

The pathologies of DM and atherosclerosis create a coupled relationship that uniquely fuels the progression of CVDs and early mortality. Individually, each condition presents with its own pathology, and is often treated in kind. However, the combination of the two leads to unique abnormalities that creates significant challenges for researchers in finding effective therapeutics. Growing evidence supports the potential use of MSCs in treating both atherosclerosis and diabetes [5,7]. While the effects of MSCs are becoming more established, there is still much that researchers and clinicians must determine before solid conclusions can be formed, many of which were highlighted by Korn et al. First, it is clear that the doses used in clinical trials are likely not potent enough to elicit significant improvements, as the present study acknowledges these doses typically range from 1 to 4million MSCs/kg, whereas the doses typically used in murine models is exponentially greater equating to 40–60 million MSCs/kg. This alone would be enough to explain the lack of translation to human trials. Second, the present study examined the effects of a single clinically relevant dose of ASCs on atherosclerotic plaque characteristics and inflammatory markers 4 weeks after the ASCs were administered. Whereas a previous MSC investigation found that positive outcomes on atherosclerotic and inflammatory markers occurred following 3 doses (500,000 MSCs) administered every other day for 8 weeks [8]. Therefore, it may be possible that the single clinically relevant dose was not potent enough to elicit significant effects, as MSCs often have lower survival rate after administration due to harsh inflammatory environments typically observed in atherosclerotic lesions [9]. To this end, routine administration of clinical concentrations of MSCs may be more potentially effective than a single high dose administration, or in combination of anti-inflammatory therapeutics which may help to increase the survival rate of MSCs in these advanced conditions, however this requires additional investigations.

3. Conclusion

The present work by Korn et al. provides valuable insight that shows the significant mismatch in MSC therapies between animal and human studies. This study also demonstrated that a single dose of clinically relevant MSCs administration was not potent enough to significantly change characteristics of atherosclerotic plaques or reduce inflammation thereby, highlighting the importance for establishing an appropriate clinical dose that is capable of improving atherosclerosis in DM murine models that also translates to patients with DM. Investigations building off this work should consider developing effective strategies that focus on identifying clinically effective doses of MSCs that are able to elicit significant improvements in atherosclerosis and DM. Once a clinically effective dose has been established, future studies should then consider developing an optimal dosing regimen by investigating how multiple doses administered over time may affect characteristics of atherosclerosis and DM. These investigations will help to document the use of MSCs as a potential therapeutic capable of improving the vasculopathies common in both atherosclerosis and DM, which may help to treat CVDs in these populations.

CRediT authorship contribution statement

Michael F. Allen: Writing – original draft, Writing – review & editing. **Song-Young Park:** Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare no conflict of interest.

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