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Adipose mesenchymal stromal cells minimize and repair radiation-induced oral mucositis

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Abstract

Background aims. Mesenchymal stromal cells (MSCs) have been used to minimize and repair radiation-induced normal tissue injury in the intestine, salivary gland, liver, skin, lungs and cardiac muscle. This study investigated the ability of adipose tissue–derived MSCs (aMSCs) to minimize and/or repair single dose radiation-induced oral mucositis (RIOM). *Methods.* Syngenic phenotypically and functionally characterized BALB/c mouse aMSCs were implanted intraperitoneally in a RIOM mouse model with different dosing protocols. Response was quantified macroscopically, microscopically and by using different histological and clinically relevant parameters. *Results.* Irradiation at 18 Gy generated a self-resolved single-dose RIOM BALB/c mouse model with 5.6 ± 0.3 days mean duration (95% confidence interval (CI) 4.233–7.1 days) and 100% survival rate. Intraperitoneal implantation of 5 doses of 2.5 million freshly cultured syngenic aMSCs significantly and reproducibly reduced RIOM ulcer duration to 1.6 ± 0.3 days (95% CI 0.0233–3.1 days, a 72% reduction in RIOM ulcer duration), ulcer size and ulcer floor epithelial height. The therapeutic benefits were significantly dependent on dose size and frequency, number of doses, and therapy onset time. aMSCs therapy significantly minimized the RIOM-related weight loss, accelerated the weight gain and improved irradiated animals' hydration and nutritional status. aMSCs therapy did not potentiate head and neck cancer *in vitro*. *Conclusions.* Syngenic freshly cultured aMSCs significantly minimized and repaired radiation-induced oral mucositis with a 72% reduction in ulcer duration. aMSCs dose size and frequency, number of doses and therapy onset time are the main keys for optimized therapeutic outcome. aMSCs therapy did not stimulate Head and Neck cancer cell growth *in-vitro*.

Key Words: *adipose tissue, cellular therapy, head and neck cancer, ionizing radiation, mesenchymal stromal cells, normal tissue injury, oral mucositis, radiotherapy*

Introduction

Radiation-induced oral mucositis (RIOM) is a normal tissue injury side effect of radiation (RT) therapy in head and neck cancer patients with a 100% incidence in altered fractionation radiotherapy [1,2]. RIOM is a form of four-phase mucosal barrier injury that is considered one of the major dose-limiting toxicities [3,4]. It is a challenge that leads to alteration in RT dose fractionation, treatment interruptions and poor local tumor control with such narrow therapeutic ratio. RIOM starts as localized asymptomatic inflammatory hyperemia and edema, then results in confluent desquamation, necrosis and deep ulceration with exposed oral connective tissue that may lead to secondary infection. Although considered a self-limited inflammation if the patient survives, RIOM can lead to lethal deterioration of patient's quality of life in elderly sick patients with altered fractionation RT [1,5,6].

Reducing the severity and the duration of RIOM are the two main goals for a satisfactory proposed

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treatment. Many therapies have been tried to minimize and/or repair RIOM using topical and systemic routes. Although pharmacological and nonpharmacological therapies and procedures have been applied for RIOM management, no single therapy has been identified to significantly minimize or repair RIOM mainly by reducing the injury severity and duration [2,4,5,7–40].

The recent clinical data on mesenchymal stromal cell (MSC) therapy in ionizing radiation-induced normal injuries other than RIOM (e.g., bone, lung, intestine and skin injury) has shown promising therapeutic benefits. In these therapies, MSC therapy helped improve hematopoiesis and osteoradionecrosis, breathing parameters, lung immune function, intestinal mucosal inflammation, hemorrhages, fistulization, pain, diarrhea and skin ulceration induced by RT [41–43].

Adipose tissue–derived mesenchymal stromal/stem cells (aMSCs) are multi-potent progenitor cells located in the stromal vascular fraction (SVF) of adipose tissue [44]. They are characterized by expressing expected MSC surface antigens (e.g., Sca1, CD106, CD105, CD73, CD29 and CD44) and lacking the expression of hematopoietic stem cell surface antigens (e.g., CD11b and CD45) [44–46]. In addition to their multi-lineage differentiation potential, aMSCs have anti-inflammatory/immunomodulatory and paracrine effects [47–52]. They also have the ability to home to the site of tissue injury after irradiation and inflammation [44,53,54]. aMSCs are promising for cellular therapies because of their prominent anti-inflammatory effects, enhancing interleukin (IL)-10 secretion, ease of isolation, high cell count after expansion and their source abundance [55]. In radiation-induced normal tissue injury, aMSCs have shown significant repair of RT-induced cutaneous syndrome [56–60], photo aging [61], acute salivary gland [62], intestinal injuries [63–67] and chronic injuries [57,67,68]. In addition, we have shown in a previous study that aMSCs are relatively resistant to ionizing radiation, a property that qualifies them to be a reliable cellular therapy candidate before and during RT [69].

An initial MSCs therapy for RIOM conducted in 2014 by Schmidt concluded that transplantation of bone marrow (BM) or BM-derived MSCs (bmMSCs) could modulate RIOM in fractionated RT mouse model depending on the time of transplantation relative to RT exposure time [70].

Nevertheless, in another study, they concluded that BM transplantation had no therapeutic effect on RIOM in single dose RT mouse model compared with the therapeutic benefit of mobilizing endogenous BM stem cells [69]. In our present study, we investigated the ability of aMSCs therapy to minimize and/or repair the single dose RIOM mouse model.

Methods

Isolation of mouse aMSCs

aMSCs were isolated according to our previous methodology [69]. Animals were purchased from Charles River Laboratories. After a 1-week adjustment period, animals were put to sleep (according to McGill University's Standard of Procedure, SOP110 for mouse anesthesia) with Isoflurane inhalation (oxygen flow of 0.4–0.8 L/min, and 2.5% isoflurane vapor maintenance). Sleeping animals were transported to a CO_2 chamber for euthanasia followed by cervical dislocation after confirmation of arrested breathing for 2 min (according to SOP501 for animal transport and SOP410 for humane intervention points) and McGill University Animal Care Committee's (UACC) ethics, after having the proper training and certificates for animal handling. Bilateral inguinal white adipose tissue of male BALB/c mice was sterilely collected, washed, minced and digested in 1 × sterile phosphate-buffered saline (PBS; Invitrogen), 2% heat-inactivated fetal bovine serum (iFBS, Wisent) and 2 mg/mL collagenase type II (Invitrogen) at 37°C for 15 min. After filtration, the cell suspension was spun down and the cell pellet (SVF) was re-suspended in 0.83% ammonium chloride (NH_4Cl) for erythrocytes lysis. SVF cells were plated in a 25-mL flask containing 1 × Dulbecco's Modified Eagle's Media (DMEM, Invitrogen), 10% iFBS (Wisent), 1% penicillin/streptomycin from Gibco (distributed by Invitrogen Canada) at 37°C and 5% CO_2 after counting and checking cell viability using trypan blue. Medium was freshly supplemented with 2–20 ng/mL mouse fibroblast growth factor-2 (Sigma-Aldrich) and 5 U/mL sodium-purified heparin (Sigma-Aldrich).

aMSCs functional differentiation assay

Mouse Mesenchymal Stem Cell Functional Differentiation Kit (R&D Systems, cat. # SC010) was used for differentiation of aMSCs to adipocytes, osteocytes and chondrocytes according to the manufacturer's protocol. For adipogenesis, cells were seeded in 24-well plate and cultured to 80% confluence. Medium was then replaced by 0.5 mL of adipogenic differentiation medium and kept in culture for 10–14 days. For osteogenesis, cells were seeded in 24-well plate and cultured to 70% confluence. Medium was then replaced by 0.5 mL of osteogenesis differentiation medium and kept in culture for 14–21 days. Both newly formed adipocytes and osteocytes were fixed with paraformaldehyde for immunohistochemistry (IHC) staining. For chondrogenesis, a freely mobile cell pellet of 15×10^3 cells was kept inside a vertically positioned falcon tube within chondrogenic differentiation medium for 17–21 days. The cell pellet was then fixed