BURNS XXX (2016) XXX–XXX

Available online at www.sciencedirect.com
ScienceDirect
journal homepage: www.elsevier.com/locate/burns

Administration of adipose-derived stem cells enhances vascularity, induces collagen deposition, and dermal adipogenesis in burn wounds

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ARTICLE INFO

Article history:
Accepted 7 December 2015

Keywords:
Adipose-derived stem cell
Burn wounds
Collagen
Adipocytes
Vascularization

ABSTRACT

Current treatment options for severe burn wounds are often insufficient in reconstructing skin and soft tissue defects. Adipose-derived stem cells (ASCs), a readily available source of multipotent stem cells, represent a promising therapy for the treatment of full-thickness burn wounds. Full-thickness burn wounds were created on the paraspinal region of athymic mice. A one-time, sub-eschar injection of 6.8×10^6 ASCs in PBS or PBS alone was administered at 24-h postoperatively. Time to healing was quantified using Image J analysis. At days 4, 7, 14, and 21, mice were sacrificed and tissues were excised for molecular and histological analysis. ASCs were able to survive in burn wounds as determined by the presence of PKH labeling and human PPARγ expression within the wounds. CD-31 staining demonstrated increased vascularity in ASC-treated wounds at POD 4 ($p < 0.05$). Molecular studies showed enhanced adipogenesis, as well as type III and type I collagen deposition in the ASC treated group ($p < 0.05$). An increase in the mRNA expression ratio of type III to type I collagen was also observed following ASC treatment ($p < 0.05$). By enhancing vascularity, collagen deposition, and adipogenesis, ASCs show promise as an adjunctive therapy for the current treatment of full thickness burn wounds.

1. Introduction

With over 11 million people seeking medical attention for burn injuries annually, burns are a significant global concern. For deep second degree and third degree burns, current treatments, such as skin grafting, fasciocutaneous flaps, and muscle flaps, result in large donor-site disfigurement [27] and detrimental psychological effects [26]. Burns can also lead to considerable loss of mobility due to secondary scar formation and contracture, especially if the injury site traverses a joint or affects hand function [1,2].

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http://dx.doi.org/10.1016/j.burns.2015.12.007
0305-4179/

As mortality improves and more severely burned patients survive, treatment strategies should aim to be less invasive, minimize donor-site morbidity, and reduce secondary scar formation.

ASCs are an expendable source of multipotent mesenchymal adult stem cells that have shown efficacy in enhancing the regeneration and repair of multiple tissue types, including nerve [17] and bone [3]. It is hypothesized that these cells contribute to healing by upregulating angiogenic factors, such as vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF) [20]. ASCs have also been shown to directly differentiate into certain cell types in regenerating tissues, including vascular endothelial cells [18], adipocytes [4], etc.

For these reasons, ASCs have been investigated as a potential therapy to modulate wound healing. Zografou et al. displayed that skin grafts treated with ASCs have increased viability and less necrosis compared to untreated grafts [32]. Nie et al. also enhanced epithelialization and granulation of wounds with ASC administration in a rat streptozocin-induced diabetes model [18].

Interestingly, ASCs have also shown a beneficial impact in reducing scar formation in a number of different models. Zhang et al. showed a reduction in scarring following injection of ASCs into the infarcted myocardium [30]. Glial scar formation following hemorrhagic stroke was also reduced with intravenous ASC delivery [13]. At the present time, it is unclear whether this decrease in scarring is due to the immunomodulatory or pro-regenerative impact of ASC administration. However, it is evident that ASC therapy may be valuable in enhancing burn healing and reducing secondary scar formation.

Currently there are very few studies focused on utilizing ASCs to enhance burn wound healing. Many of these studies evaluate ASC treatment in delayed thermal injury, mature scar tissue formation, or study whole fat or the stromal vascular fraction (rather than isolated ASCs) at the burn wound site [6,21,24,29]. Rigotti et al. demonstrated enhanced healing and vascularization in patients treated with fat grafting following irradiation-induced lesions [21]. Similarly, Klinger et al. utilized fat injections to treat hypertrophic and keloid scars resulting from burn injuries. Biopsies of the scars revealed improved vascularity, collagen formation, and dermal thickening, which investigators largely attributed to the regenerative and paracrine affects of ASCs within the injected lipoaspirate [14]. However, these studies focused on delivering lipoaspirate injections to the affected region, not pure ASCs.

The few studies that have focused on utilizing ASCs in burn wound healing [10,16] have not thoroughly investigated the molecular environment produced by ASC administration and how this could potentially augment healing. The aim of this study was to investigate the effect of concentrated ASC therapy in acute direct thermal injury with the hypothesis that ASCs would improve the quality of burn wound healing. We hypothesized that ASC injection post thermal injury would enhance vascularity, contribute to enhanced dermal coverage, as well as increase collagen deposition within full-thickness burn wounds.

2. Materials

Type II collagenase (Worthington Biochemical Corp., Lakewood, NJ), standard plating media: Dulbecco's Modified Eagle Medium/Nutrient Mixture F12 (Corning™, Manassas, VA), 10% fetal bovine serum (Atlas Biological, Fort Collins, CO), 1% penicillin/streptomycin, 1% Fungizone (all from Lonza BioWhittaker™, Portsmouth, NH), and 0.001% dexamethasone (Sigma–Aldrich, St Louis, MO), ketamine (Ketathesia; Butler Schein Animal Health Supply, North Dublin, OH), xylazine (Vedco, Inc., St. Joseph, MO), ketoprofen (Fort Dodge Animal Health, Overland Park, KS), PKH26 Red Fluorescent Cell Linker Kits for General Cell Membrane Labeling (Sigma–Aldrich PKH26GL, St Louis, MO), phosphate buffered saline (PBS; GIBCO®, Carlsbad, CA), 1-cm diameter brass stamps (Granger®, Chicago, Il), Anti-CD31 antibody produced in rabbit (Abcam, ab32457, Cambridge, MA), Biotinylated Anti-Rabbit IgG produced in goat (Vector Laboratories, Burlingame, CA, ba-1000)

3. Methods

This study was approved by the University of Pittsburgh Institutional Animal Care and Use Committee (protocol #13041711). Female athymic nude mice, age 7–9 weeks, (Harlan Laboratories, Indianapolis, IN) were used in this study. All animals acclimated for one week prior to experiments, under controlled conditions (20–23 °C, 40–60% humidity, constant laminar air flow, 12 h light–dark cycle). All experimental manipulations were performed under a sterile hood using aseptic technique.

3.1. *A non-lethal mouse burn model*

A non-lethal burn wound model was established by applying direct thermal injury with a heated, 1-cm diameter round brass stamp. On the day prior to burn, stamps were wrapped in aluminum foil and heated in a Fisher convection oven (Fisher Scientific, Hanover Park, Il) overnight at 70 °C; this allowed them to maintain core heat during transfer from oven to mouse. Ten minutes prior to burn application, mice were anesthetized with a mixture of ketamine (80 mg/kg) and xylazine (12 mg/kg) delivered via intraperitoneal (IP) injection. The entire flank and back region was prepped with an alchohol swab. Burns were created under a sterile hood by applying the stamps with direct pressure due to that of gravity alone to the lumbar paraspinal region of anesthetized mice for 10 s. Mice were placed in the same position each time and the weight of the brass stamps were held constant. This method has been described by many authors and ensures that the burns created are homogeneous [23]. Immediately after burn, mice received buprenorphine 0.03 mg/kg subcutaneously (SQ) every 8 h for two days. All animals also received DietGel® Recovery (ClearH_2O, Portland, ME) post-burn, in addition to standard food and water. No wound splints were used. This technique yields non-lethal, histologically verified full-thickness wounds that heal by contraction with a small 2–3-mm scar within 3 weeks. All mice tolerated burn injury alone without complications.

Please cite this article in press as: Bliley JM, et al. Administration of adipose-derived stem cells enhances vascularity, induces collagen deposition, and dermal adipogenesis in burn wounds. Burns (2016), http://dx.doi.org/10.1016/j.burns.2015.12.007