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# Mesenchymal stem cells reverse trauma and hemorrhagic shock-induced bone marrow dysfunction

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ARTICLE INFO

*Article history:*
Received 10 April 2015
Received in revised form
22 May 2015
Accepted 10 June 2015
Available online 16 June 2015

*Keywords:*
Mesenchymal stem cells
Trauma
Hemorrhagic shock
Hematopoietic progenitor cells
G-CSF

ABSTRACT

*Background:* Lung contusion (LC) followed by hemorrhagic shock (HS) causes persistent bone marrow (BM) dysfunction lasting up to 7 d after injury. Mesenchymal stem cells (MSCs) are multipotent cells that can hasten healing and exert protective immunomodulatory effects. We hypothesize that MSCs can attenuate BM dysfunction after combined LCHS.

*Materials and methods:* Male Sprague–Dawley rats ($n = 5–6$ per group) underwent LC plus 45 min of HS (mean arterial pressure of 30–35). Allogeneic MSCs ($5 \times 10^6$ cells) were injected intravenously after resuscitation. At 7 d, BM was analyzed for cellularity and growth of hematopoietic progenitor cell (HPC) colonies (colony-forming unit–erythroid; burst-forming unit–erythroid; and colony-forming unit–granulocyte, erythrocyte, monocyte, megakaryocyte). Flow cytometry measured %HPCs in peripheral blood; plasma granulocyte colony-stimulating factor (G-CSF) levels were measured *via* enzyme-linked immunosorbent assay. Data were analyzed by one-way analysis of variance followed by the Tukey multiple comparison test.

*Results:* As previously shown, at 7 d, LCHS resulted in 22%, 30%, and 24% decreases in colony-forming unit–granulocyte, erythrocyte, monocyte, megakaryocyte, burst-forming unit–erythroid, and colony-forming unit–erythroid colony growth, respectively, *versus* naive. Treatment with MSCs returned all BM parameters to naive levels. There was no difference in %HPCs in peripheral blood between groups; however, G-CSF remained increased up to 7 d after LCHS. MSCs returned G-CSF to naive levels. Plasma from animals receiving MSCs was not suppressive to the BM.

*Conclusions:* One week after injury, the persistent BM dysfunction observed in animals undergoing LCHS is reversed by treatment with MSCs with an associated return of plasma G-CSF levels to normal. Plasma from animals undergoing LCHS plus MSCs was not suppressive to BM cells *in vitro*. Treatment with MSCs after injury and shock reverses BM suppression and returns plasma G-CSF levels to normal.



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0022-4804/$ – see front matter 
http://dx.doi.org/10.1016/j.jss.2015.06.023

## 1. Introduction

Persistent injury-associated anemia and increased susceptibility to infection [1,2] are manifestations of the bone marrow (BM) dysfunction observed after severe injury with significant clinical ramifications. In a rodent model of lung contusion (LC) alone, this dysfunction is manifested as a reduction in BM cellularity and growth of hematopoietic progenitor cells (HPCs) within the BM itself and mobilization of HPCs to the periphery with an associated rise in plasma granulocyte colony-stimulating factor (G-CSF) early after injury; however, rats recovered within 7 d [3,4]. When animals undergo lung contusion following hemorrhagic shock (LCHS), BM dysfunction is both more acutely marked and persists for >1 wk [3,4].

Mesenchymal stem cells (MSCs) are a type of multipotent cell with multiple immunomodulatory and paracrine functions that are essential to maintain the BM microenvironment and support hematopoiesis [5–7]. They have been investigated in several disease states, such as graft-*versus*-host disease, aplastic anemia, and myocardial infarction, as a potential cellular therapy to expedite healing and restore homeostasis [8–10].

We have previously investigated the role of these cells in lung healing [11]; however, we have not examined their role in treating the BM dysfunction that ensues after injury. We hypothesize that treatment of rats undergoing combined LCHS with MSCs given early after resuscitation can reverse injury-associated BM dysfunction. Furthermore, we hypothesize that this protection occurs *via* a systemic effect by altering the plasma in shocked animals such that it is no longer suppressive to BM cells *in vitro*.

## 2. Materials and methods

### 2.1. Experimental groups

Male rats ($n = 5–6$ per group) were assigned to experimental groups as follows: LC alone, LC plus MSCs (LC + MSCs), LCHS, and LC followed by HS plus MSCs (LCHS + MSCs). A control naive group of uninjured animals undergoing daily handling was also used. On day 7, rats were sacrificed and BM and blood collected.

### 2.2. Animals

Male Sprague–Dawley rats weighing between 250 and 350 g (Charles River, Wilmington, MA) were maintained according to the recommendations of the Guide for the Care and Use of Laboratory Animals, and experiments were approved by the Rutgers New Jersey Medical School Animal Care and Use Committee. Rats were housed in a barrier-sustained animal facility at 25°C with 12-h light–dark cycle. Animals had free access to water and chow (Teklad 22/5 Rodent Diet W-8640; Harlan, Madison, WI).

### 2.3. LC model

After intraperitoneal administration of sodium pentobarbital (50 mg/kg), a 12-mm metal plate was secured to the rat's right axilla and a percussive nail gun (Craftsman 968514 Stapler; Sears Brands, Chicago, IL) was used to induce a unilateral LC.

### 2.4. HS model

As previously described [12], immediately after LC, rats in the LCHS and LCHS + MSC groups underwent cannulation of the right internal jugular vein and right femoral artery with polyethylene (PE-50; Becton Dickinson and Co, Sparks, MD) and Silastic (Dow Corning Corp, Midland, MI) tubing, respectively. To prevent clotting, all tubing was flushed with 10 U/mL of heparinized saline. The femoral artery catheter was then connected to a continuous blood pressure monitoring device for measurement of mean arterial pressure (MAP) and heart rate. Animals were bled to an MAP of 30–35 mm Hg for 45 min; additional blood was withdrawn or reinfused as needed to maintain MAP within range. Temperature was maintained at approximately 37°C with the use of an electric heating pad placed under the surgical platform. After the completion of the shock period, shed blood was reinfused at a rate of 1 mL/min.

### 2.5. MSC culture

As previously described [13], Sprague–Dawley rat MSC cultures (Cyagen Biosciences, Santa Clara, CA) were established and expanded. Briefly, cells were thawed and transferred into 15 mL OriCell MSC Growth Medium (Cyagen Biosciences, Santa Clara, CA). Cells were washed and resuspended in 2–3 mL of fresh growth medium. Cells were incubated in a 37°C humidified 5% $CO_2$ incubator after seeding into T25 flasks containing additional growth medium. Medium was changed at 24 h and then every 72 h. Cells were dissociated with trypsin–EDTA when 80%–90% confluent and reseeded at $3 \times 10^3/cm^2$. This continued until adequate cell counts were available for harvest. On the day of injection, $5 \times 10^6$ MSCs were quantified and aliquoted into individual vials. Cells were washed with Iscove's Modified Dulbecco's Medium (IMDM) and resuspended in 1-mL IMDM before injection.

### 2.6. MSC injection

A previously prepared suspension of $5 \times 10^6$ MSCs in 1-mL of IMDM was infused *via* the previously cannulated right internal jugular vein for >5 min, beginning within 10 min of injury in the LC and LC + MSC groups or within 10 min of return of shed blood in the groups undergoing HS.

### 2.7. BM cellularity

BM cells were harvested from the right femur to determine cellularity and establish cell culture. An 18-gauge needle was inserted into the femur and BM was aspirated into 1-mL IMDM. Cells were suspended, stained with 0.4% trypan blue, and hemocytometer was used to determine total viable cell count.

### 2.8. BM HPC cultures

Growth of HPCs from the BM was assessed by culturing colony-forming unit–granulocyte, erythrocyte, monocyte,