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Accelerated functional recovery after skeletal muscle ischemia–reperfusion injury using freshly isolated bone marrow cells

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ARTICLE INFO

Article history:
Received 8 October 2013
Received in revised form
18 December 2013
Accepted 30 December 2013
Available online 3 January 2014

Keywords:
Ischemia
Reperfusion
Stem cell
Skeletal muscle
Bone marrow
Injury

ABSTRACT

Background: Relatively little information exists regarding the usefulness of bone marrow–derived cells for skeletal muscle ischemia–reperfusion injury (I/R), especially when compared with I/R that occurs in other tissues. The objectives of this study were to evaluate the ability of freshly isolated bone marrow cells to home to injured skeletal muscle and to determine their effects on muscle regeneration.

Materials and methods: Freshly isolated lineage-depleted bone marrow cells (Lin^- BMCs) were injected intravenously 2 d after I/R. Bioluminescent imaging was used to evaluate cell localization for up to 28 d after injury. Muscle function, the percentage of fibers with centrally located nuclei, and the capillary-to-fiber ratio were evaluated 14 d after delivery of either saline (Saline) or saline containing Lin^- BMCs (Lin^- BMCs).

Results: Bioluminescence was higher in the injured leg than the contralateral control leg for up to 7 d after injection ($P < 0.05$) suggestive of cell homing to the injured skeletal muscle. Fourteen days after injury, there was a significant improvement in maximal tetanic torque (40% *versus* 22% deficit; $P < 0.05$), a faster rate of force production (+dP/dt) (123.6 *versus* 94.5 Nmm/S; $P < 0.05$), and a reduction in the percentage of fibers containing centrally located nuclei (40 *versus* 17%; $P < 0.05$), but no change in the capillary-to-fiber ratio in the Lin^- BMC as compared with the Saline group.

Conclusions: The homing of freshly isolated BMCs to injured skeletal muscle after I/R is associated with an increase in functional outcomes.

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1. Introduction

Skeletal muscle ischemia–reperfusion injury (I/R) occurs as a result of muscle ischemia followed by the reestablishment of blood flow. Tourniquet application, vascular injury, bone fracture, and skeletal muscle crush injury are among the list of causative factors (reviewed in [1]). There are a large number of options for reducing skeletal muscle I/R if treatments can be applied before, during, or soon after the injury [2–4]. However, in the case of traumatic injuries where I/R is not predictable (e.g., explosions), and an early intervention is desired, such strategies are not as relevant. This is especially the case for severe extremity trauma, wherein lifesaving surgical procedures are performed with the objective of stopping hemorrhage for the preservation of vital organ function and the extremities are not the primary focus [5].

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0022-4804/$ – see front matter Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.jss.2013.12.026

The application of therapies that can be applied and improve regeneration once the patient has been stabilized may be another means to improve outcomes after skeletal muscle I/R. In this regard, there have been a large number of reports describing the successful use of stem cell–based therapies after I/R injury for hepatic [6–8], renal [9–11], and cardiac [12–14] tissues. Consistent with this idea, we have previously demonstrated that muscle progenitor cells (MPCs) enhance tissue repair after skeletal muscle I/R [15]; MPCs were a logical choice given their well-documented role in skeletal muscle regeneration; however, the use of MPCs is not currently clinically applicable.

Bone marrow is a rich source of stem and progenitor cells, so it is not surprising that a number of treatments that have progressed to the clinic have used bone marrow cells [13,14,16–18]. Skeletal muscle I/R is a complex injury resulting in vascular, neural, and muscular damage [19], structures that have been shown to benefit from transplanted bone marrow–derived cells in traumatized and diseased conditions [17,20,21]. Recently, it was demonstrated that bone marrow cells (lineage-depleted; Lin^- BMCs) delivered *via* intramuscular injection can survive in a mouse I/R model for up to 1 mo after injury indicating that they may be suitable for the environment imposed with the injury, however, a functional improvement was not realized [22]. Given the ability of systemically delivered stem cells to localize to areas of injury [10,23–25], a logical progression is to evaluate the ability of systemically derived bone marrow cells to home to injured tissue. As the success of regenerative processes are ultimately judged by their capacity to restore function, neural-evoked muscle force or torque (which depends on the integrity of neural, vascular, and muscular elements) is an appropriate outcome for which therapies of skeletal muscle I/R injury can be evaluated. To this end, the primary objective of the present study was to determine if bone marrow–derived cells delivered intravenously (IV) would improve functional regeneration after skeletal muscle I/R.

2. Materials and methods

This study has been conducted in compliance with the Animal Welfare Act and the Implementing Animal Welfare Regulations and in accordance with the principles of the Guide for the Care and Use of Laboratory Animals and was conducted in the animal facility at the US Army Institute of Surgical Research. FVB-L2G85 transgenic and FVB mice (The Jackson Laboratory, Bar Harbor, ME) were housed individually in a temperature-controlled environment with a 12-hour light–dark cycle.

2.1. Experimental design

To evaluate cell survival and distribution after I/R, Lin^- BMCs derived from male FVB-L2G85 transgenic mice were injected IV into female FVB mice 2 d after I/R. Bioluminescent imaging (BLI) was performed at 0, 1, 3, 7, 14, 21, and 28 d after cell injection (add 2 d for postinjury time) to evaluate cell survival and distribution. To test the hypothesis that BMCs improve regeneration after I/R, 2 d after injury, 15 female FVB mice were allotted to one of two experimental groups: (1) injured with IV injection of saline (Saline, $n = 8$) or injured with IV injection of Lin^- BMCs derived from male FVB-L2G85 transgenic mice (Lin^- BMCs, $n = 7$). *In vivo* functional assessments were made up to 1 wk before and 16 d after I/R on mice from both experimental groups, after which tissues were collected for histology.

2.2. I/R injury

I/R injury was induced in FVB mice similar to that described previously [26]. Mice were maintained under anesthesia with isoflurane gas (1.5%–2.5%) for the induction of I/R. A pneumatic digit tourniquet (D.E. Hokanson, Inc, Bellevue, WA) was placed as proximal as possible around the elevated upper hind limb of FVB syngeneic mice and inflated to the pressure of 250 mm Hg for a duration of 2 h using a Rapid Cuff Inflator (D.E. Hokanson, Inc). All mice received buprenorphine (0.1 mg/kg, subcutaneously) 30 min before tourniquet release and every 12 h thereafter for the first 24 h.

2.3. Isolation of bone marrow–derived cells

On the day of injection, Lin^- BMCs were isolated from FVB-L2G85 transgenic donor mice (The Jackson Laboratory) for IV delivery to FVB syngeneic recipient mice injured 2 d earlier (The Jackson Laboratory). Only male mice were used as a source of Lin^- BMCs to minimize potential variability because of the effect of cell gender on regenerative capacity [27]. Magnetic-activated cell sorting (MACS) with lineage depletion was used to enrich for stem and progenitor cells, similar to that previously described by us [22] and others [28–33]. Briefly, bone marrow cell suspensions derived from tibia and femurs were labeled with a cocktail of biotin-conjugated antibodies against lineage-specific antigens (CD5, CD45 R [B220], CD11 b, Gr-1 [Ly-6 G/C], 7–4, and Ter-119) and subsequently magnetically labeled with Anti-Biotin MicroBeads (Miltenyi Biotec Inc, Auburn, CA). The lineage-positive cells (T cells, B cells, monocytes, macrophages, granulocytes, erythrocytes, and their committed precursors) were depleted by retaining them on a MACS column in the magnetic field of the autoMACS Pro Separator (Miltenyi Biotec Inc, Auburn, CA) while unlabeled lineage-negative cells (Lin^- BMCs) pass through. Lin^- BMCs from the FVB-L2G85 transgenic donors were suspended in saline and injected IV into FVB syngeneic animals injured 2 d earlier to determine (1) their survivability and distribution and (2) the ability to improve muscle function after I/R.

2.4. IV injection

Two days after the induction of I/R mice received either 50 μL of saline only or saline containing $0.2–0.25 \times 10^6$ Lin^- BMCs. Cells were injected through the tail vein using a tuberculin syringe (Tyco Healthcare, Mansfield, MA) and immediately flushed with an additional 50 μL of saline.

2.5. BLI

Cells derived from FVB-L2G85 transgenic mice express firefly luciferase allowing for *in vivo* measurements of survival and distribution over time when transplanted into syngeneic FVB recipients [25,34]. Two days after the induction of I/R, FVB