Selectin Blockade Decreases Postischemic Recruitment of Bone Marrow Stromal Cells

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Objectives/Hypothesis: Investigate the localization mechanisms of bone marrow stromal cells following transient ischemia-reperfusion injury in a murine flap model.

Study Design: Controlled laboratory study.

Methods: A cutaneous flap based on the inferior epigastric artery was elevated, and transient ischemia of 3.5 hours using a microvascular clamp was achieved. Fucoidan was injected intravenously 24 hours before the ischemic period. Following the period of ischemia, radiolabeled bone marrow stromal cells were injected intravenously, and radioactivity was determined postoperatively.

Results: Attenuation of the uptake of bone marrow stromal cells into postischemic tissue was observed in those mice treated with fucoidan as indicated by gamma counts measured in the flaps when compared with controls (P < .001).

Conclusions: Decreased uptake of radiolabeled bone marrow stromal cells into postischemic tissues pretreated with fucoidan indicates selectin-mediated bone-marrow stromal cell recruitment in a murine cutaneous flap model.

Key Words: Stem cells, ischemia-reperfusion, selectin, fucoidan.

Level of Evidence: N/A.

INTRODUCTION

Surgical reconstruction of head and neck defects often requires transferring tissue, which must undergo a period of ischemia before reperfusion. Although the interval of ischemia will result in the formation of free radicals and subsequent tissue damage, the reperfusion of the tissue also results in tissue injury, likely related to inflammatory processes. Attempts at modulating the cellular and molecular factors involved in these types of injuries have typically been aimed either at protecting and promoting cell survival with growth factors and protective molecules or at attenuating the inflammatory phase thought to be involved in cellular injury.

To promote cell survival after ischemia/reperfusion (I/R) injury, various types of exogenous stem cells have been applied experimentally with promising results. It is thought that these benefits are related to the release of growth factors and stabilizing substances directly into the reperfused tissue. However, to provide this benefit, the stem cells need to home in and migrate to the specific tissue that is injured.

Previous studies at our institution in a model of cerebral I/R injury have demonstrated increased gene expression of relevant molecular factors involved in cellular protection, regeneration, and angiogenesis with the administration of bone-marrow stromal cells (BMSCs). Other studies have suggested a similar benefit from stem cell administration in I/R of soft-tissue flaps and grafts. Many of these studies suggest a causal relationship of tissue survival with the administration of stem cells without investigating the mechanisms by which these benefits occur. To begin to understand the therapeutic benefits of stem cells in I/R, it is important to understand the mechanisms by which the stem cells localize to the specific tissue site of injury. The purpose of this study is to explore the molecular factors involved in stem cell migration in a flap model of I/R injury. Knowledge of stem cell migration mechanisms may allow for modulation of their migration with therapeutic potential.

The homing of BMSCs to areas of I/R injury following cerebral vascular infarct has been found to involve selectin adhesion molecules. Various selectin subtypes (E, P, and L) have long been known to mediate the rolling and adhesion of individual cells undergoing migration. Primarily selectin-mediated migration is associated with the transit of leukocytes and platelets with inflammation. In limited studies, selectin blockade has been shown to decrease the inflammatory injuries associated with I/R. However, previous studies in a stroke model have demonstrated that selectin blockade also decreased stem cell migration and therefore therapeutic benefit from stem cell application. Thus,
selectin-mediated cell rolling seems to play a role in both the inflammatory injury associated with I/R and also in the migration of stem cells, which provide a cell survival benefit. This contrasting role suggests that a better understanding of stem cell migration is essential to defining the possible therapeutic benefits (or disadvantages) involved in the application of stem cells in the clinical model of flap I/R injury.

In the present study, pedicled epigastric island flaps in mice were implemented to address the following objectives: 1) to determine if BMSCs would localize to the tissue in which I/R injury was induced and 2) to determine if recruitment and migration of these BMSCs were dependent on selectins.

MATERIALS AND METHODS

Before the study, approval of the protocol was obtained through the Louisiana State University Health Sciences Center at Shreveport Animal Care and Use Committee. The bone marrow–derived stem cells were isolated from H-2Kb-tsA58 mice expressing the temperature-sensitive SV40 large-T antigen (large T; CBA/ca X C57Bl/10 hybrid; Charles River Laboratories, Wilmington, MA), CD44 knockout mice (B6.Cg-Cd44tm1Hbg/J; Jackson Laboratories, Bar Harbor, ME), or WT mice by Jonathan Steven Alexander, PhD, Department of Molecular and Cellular Physiology, LSUHSC-Shreveport. They were cultured in high-glucose Dulbecco's modified Eagle's medium with 10% fetal bovine serum and 1% antibiotic/antimycotic. Cells were split when confluence was noted. Cells were mobilized using trypsin–ethylene diamine tetraacetic acid before injection. During surgical procedures, the mice were anesthetized using inhalational isoflorane and intramuscular buprenorphine.

Three C57BL/6 male mice were used as controls with a 1 × 2-cm cutaneous flap based on the inferior epigastric artery that was elevated and underwent a 3.5-hour period of transient ischemia. The flaps were moistened using saline application every 15 minutes. The clamp was released following the ischemic period, thus allowing for reperfusion. The flaps were then inset using 6-0 Vicryl sutures (Ethicon, Inc., Somerville, NJ) in an interrupted fashion. Chromium-51 (150uCi per mouse) radiolabeled BMSCs (8 × 10⁶) were injected intravenously into the contralateral femoral vein, and the mice were monitored postoperatively according to protocol. On the third postoperative day, the mice were sacrificed and the ischemic flap and a contralateral control flap were harvested. Radioactivity was determined using a gamma counter to determine BMSC migration into the tissues. Epigastric flaps in which I/R injury was induced were compared to the normal epigastric flaps on the contralateral side of the mouse, and a ratio of cell counts was calculated (I/R cpm : normal cpm) to account for differences in physiology of each individual mouse.

RESULTS

To first examine whether BMSCs were selectively recruited to I/R injury in soft-tissue flaps, a control group was used to identify radiolabeled cells in the injured tissue compared to normal tissue. Despite compromising the blood flow to the raised (and temporarily ischemic) flap, radiolabeled BMSCs were detected at higher levels when compared to the normal (fully vascularized) epigastric tissues on the contralateral side.

When comparing the I/R injury flaps to the flaps on the other side of the mouse, the stem cells preferentially localized into the injured tissue with an average 6.8-fold (±2.28) increase in gamma counts. This suggests that stem cells are able to preferentially migrate into tissue that had undergone I/R injury and do so at levels much higher than the rest of the body.

To elucidate the transit mechanisms in stem cell migration, the pan-selectin blocker fucoidan was administered before stem cell injection in five additional mice. Upon measuring the gamma counts of the I/R injury flap...
and the normal contralateral flap, these mice exhibited much lower counts relating to radiolabeled BMSC migration. In fact, comparing the ratio of the radiolabeled counts between experimental flap and normal tissue flap resulted in an average ratio of 0.77 (±0.28). By contrasting this to the intense cellular uptake in the control mice, it can be inferred that less stem cell transit occurred due to blocking selectins (Fig. 2). A two-tailed t test was performed comparing the ratios of the I/R flaps compared to the normal tissue flaps in both the control and the fucoidan group, which indicated statistical significance was achieved ($P = 0.0077$).

**DISCUSSION**

Exogenously administered BMSCs hold great potential for the treatment of ischemic tissue injury. However, the mechanisms of I/R injury are complex and involve a cascade of variable reactive oxygen species, growth factors, and signaling molecules. Localization of stem cells to the injured tissue depends on this signal cascade. Modulating the mechanisms of cellular migration may prove beneficial both to increase stem cell therapeutic efficacy and perhaps to block inflammatory cell homing.

**CONCLUSION**

The current study demonstrates the ability of BMSC to localize directly to the site of I/R damage in soft-tissue flaps following simple intravenous injection. This study also demonstrates that this specific cellular homing is mediated by selectins, as blockage of selectins results in failure of BMSC migration. This finding is significant, as limited studies have shown blockage of selectins with fucoidan to actually have some benefit to tissue survival by decreasing neutrophil- and platelet-mediated injury. Therefore, an increased understanding of the various signaling factors involved in I/R injury is necessary before modulating the factors involved in cellular migration, as attempts at increasing selectin-mediated stem cell homing may also increase inflammatory cell homing and tissue injury.

**BIBLIOGRAPHY**


Fig. 2. Bone-marrow stromal cell localization expressed as mean ratio (counts per minute) of ischemia/reperfusion flaps : normal flaps. Control group mean, 6.8 (standard deviation, 2.28); fucoidan blockade group mean, 0.77 (standard deviation, 0.28). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]