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Mobilization of endogenous stem cell populations enhances fracture healing in a murine femoral fracture model

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Abstract

Background aims. Delivery of bone marrow–derived stem and progenitor cells to the site of injury is an effective strategy to enhance bone healing. An alternate approach is to mobilize endogenous, heterogeneous stem cells that will home to the site of injury. AMD3100 is an antagonist of the chemokine receptor 4 (CXCR4) that rapidly mobilizes stem cell populations into peripheral blood. Our hypothesis was that increasing circulating numbers of stem and progenitor cells using AMD3100 will improve bone fracture healing. *Methods.* A transverse femoral fracture was induced in C57BL/6 mice, after which they were subcutaneously injected for 3 d with AMD3100 or saline control. Mesenchymal stromal cells, hematopoietic stem and progenitor cells and endothelial progenitor cells in the peripheral blood and bone marrow were evaluated by means of flow cytometry, automated hematology analysis and cell culture 24 h after injection and/or fracture. Healing was assessed up to 84 d after fracture by histomorphometry and micro–computed tomography. *Results.* AMD3100 injection resulted in higher numbers of circulating mesenchymal stromal cells, hematopoietic stem cells and endothelial progenitor cells. Micro-computed tomography data demonstrated that the fracture callus was significantly larger compared with the saline controls at day 21 and significantly smaller (remodeled) at day 84. AMD3100-treated mice have a significantly higher bone mineral density than do saline-treated counterparts at day 84. *Conclusions.* Our data demonstrate that early cell mobilization had significant positive effects on healing throughout the regenerative process. Rapid mobilization of endogenous stem cells could provide an effective alternative strategy to cell transplantation for enhancing tissue regeneration.

Key Words: *AMD3100, bone regeneration, fracture repair, mobilization, stem cells*

Introduction

Bone marrow contains a variety of stem and progenitor cells that participate in skeletal repair, including mesenchymal stromal cells (MSCs) (1), endothelial progenitor cells (EPCs) (2) and hematopoietic stem and progenitor cells (HSPCs) (3). Each of these cell types has been independently proposed to enhance bone healing (4–6). EPCs revascularize the injury site and provide access for other types of stem cells to populate the callus (7); MSCs give rise to chondroblasts and osteoblasts for tissue repair and may have anti-inflammatory properties (4,8,9); and HSPCs, in addition to re-establishing the local bone marrow, provide precursors to osteoclasts, which are essential for converting cartilage to bone and ultimately remodeling the callus (6).

In an effort to improve fracture healing, much energy has been directed toward cell-based therapeutics that require the isolation of bone marrow and expansion or concentration of specific stem and progenitor cells *ex vivo* for subsequent delivery *in vivo*. Indeed, studies show that vascular infusion of such cells has significant positive effects on bone healing (4,10). Both transplanted MSCs (4) and endothelial/hematopoietic precursor cells (10) have been shown to enhance bone healing. An alternative to cell transplantation is to rapidly mobilize large numbers of endogenous progenitor cells directly into the peripheral blood that will home to the site of injury and take part in tissue regeneration.

Under normal physiological conditions, there are few circulating hematopoietic stem cells (HSCs)

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Table I. Animal numbers per experiment and end point.

Experiment	Figure	End points	Treatment						Total mice per end point
			No injection No fracture	Saline injection No fracture	AMD3100 injection No fracture	No injection Fracture	Saline injection Fracture	AMD3100 injection Fracture	
Advia, flow cytometry	1, 2, 3	24 h after fracture/1 h after second injection	4	7	9	4	7	8	39
Cell culture cytometry	4	1 h after 1st injection	4	3	4	–	–	–	11
Histomorphometry, micro-CT	5, 6	7 d after fracture	–	–	–	–	3	3	6
		14 d after fracture	–	–	–	–	3	3	6
		21 d after fracture	–	–	–	–	3	3	6
		42 d after fracture	–	–	–	–	6	5	11
		84 d after fracture	–	–	–	–	7	7	14

and EPCs in peripheral blood (11–15). Whereas the existence of circulating MSCs in peripheral blood is controversial, adherent fibroblast-like cells with adipogenic and osteogenic capacity have been detected in very low numbers (16,17). EPC numbers in peripheral blood are significantly increased in association with vascular injury, burns and fracture (7,15,18–21). Data suggest that similar to EPCs, in response to tissue trauma, bone marrow–derived MSCs and osteogenic progenitors also enter the peripheral blood (22–26). An emerging strategy is to utilize molecules that interfere with molecular mechanisms that retain stem and progenitor cells in the bone marrow niche to significantly increase circulating numbers of stem and progenitor cells in peripheral blood and enhance healing. One such molecule is AMD3100, a selective antagonist of chemokine (CXC motif) receptor 4 (CXCR4) (27). AMD3100 rapidly mobilizes CXCR4+ cells such as HSCs, EPCs, and possibly MSCs into the peripheral blood (28). Mobilization of these progenitor cells into the peripheral blood occurs because of disruption to stromal cell-derived factor-1 (SDF-1)/CXCR4 interaction, which anchors progenitor cells to their niches (29). AMD3100 has very few side effects in humans (30) and is Food and Drug Administration–approved and used in hospitals to mobilize HSCs for bone marrow transplantation (31,32).

Previous studies demonstrate positive effects of mobilizing endogenous stem cells with AMD3100 on bone. Wang *et al.* (33) demonstrated that 15 daily injections of AMD3100 enhanced healing of critical-sized calvarial defects in mice in as soon as 4 wk. Another study by McNulty *et al.* (34) asserts that a single dose of AMD3100, 3 h after murine bone marrow ablation surgery, significantly enhances intramedullary trabecular bone regeneration 21 d later. Another study by Kumar *et al.* (35) used a combination of insulin-like growth factor-1 and AMD3100 to treat a murine tibial defect and found improvement in bone healing after 8 wk.

In the present study, we examined the effect of a brief, 3-d AMD3100 treatment on mobilization of endogenous stem and progenitor cells into the peripheral blood and its effects on bone regeneration in a murine femoral fracture model. We expand on previous studies by simultaneously comparing HSC, MSC and EPC populations in both the blood and bone marrow from all combinations of non-fractured/fractured and saline/AMD3100-treated mice by means of flow cytometry. In addition, we evaluated bone healing through callus formation up to 12 wk after injury by examining both the soft and hard callus during fracture healing with histomorphometry and micro–computed tomography (CT) analysis.

We hypothesized that administration of AMD3100 would increase circulating numbers of HSPCs, EPCs and MSCs into the peripheral blood and enhance fracture healing.

Methods

Animals

The total number of animals used is detailed in Table I. A total of 39 13- to 14-wk-old male C57BL/6 mice (Jackson Laboratories, Bar Harbor, ME, USA) were used for hematological and flow cytometric analyses. Transverse fractures were created in the right femur of 19 animals, eight of which were administered two subcutaneous doses of 5 mg/kg AMD3100 (Sigma, St Louis, MO, USA), the first immediately after surgery and the second 23 h later (1 h before euthanasia). Seven fractured animals were administered an equal volume of saline carrier at these same time points and the remaining four fractured animals were not injected. An additional 16 animals that were not fractured received two equivalent doses of AMD3100 ($n = 9$) or saline ($n = 7$).