Marrow-Derived Stromal Cell Delivery on Fibrin Microbeads Can Correct Radiation-Induced Wound-Healing Deficits

Michael W. Xie[1], Raphael Gorodetsky[2], Ewa D. Micevicz[1], Natalia C. Mackenzie[1], Elena Gaberman[2], Lilia Levdansky[2] and William H. McBride[1]

Skin that is exposed to radiation has an impaired ability to heal wounds. This is especially true for whole-body irradiation, where even moderate nonlethal doses can result in wound-healing deficits. Our previous attempts to administer dermal cells locally to wounds to correct radiation-induced deficits were hampered by poor cell retention. Here we improve the outcome by using biodegradable fibrin microbeads (FMBs) to isolate a population of mesenchymal marrow-derived stromal cells (MSCs) from murine bone marrow by their specific binding to the fibrin matrix, culture them to high density *in vitro*, and deliver them as MSCs on FMBs at the wound site. MSCs are retained locally, proliferate in site, and assist wounds in gaining tensile strength in whole-body irradiated mice with or without additional skin-only exposure. MSC-FMBs were effective in two different mouse strains but were ineffective across a major histocompatability barrier. Remarkably, irradiated mice whose wounds were treated with MSC-FMBs showed enhanced hair regrowth, suggesting indirect effect on the correction of radiation-induced follicular damage. Further studies showed that additional wound-healing benefit could be gained by administration of granulocyte colony-stimulating factor and AMD3100. Collagen strips coated with haptides and MSCs were also highly effective in correcting radiation-induced wound-healing deficits.

Journal of Investigative Dermatology (2013) **133,** 553–561; doi:10.1038/jid.2012.326; published online 6 September 2012

INTRODUCTION

There is a growing concern about the dearth of medical countermeasures for the treatment of ionizing radiation injuries in the event of either a radiological or nuclear incident (Williams and McBride, 2011). This has led to many studies directed at correcting the acute radiation syndrome. Much is known about how different tissues individually respond to radiation, but less about how damage to one tissue affects healing of another and how effects are compounded by nonradiation injuries. The skin is particularly susceptible to compounded injuries.

In an acute radiation syndrome situation, rapid wound closure, re-epithelialization, and reestablishment of the skin tissue integrity are top clinical treatment priorities. These processes are compromised by irradiation, which seriously impedes the healing of physical or thermal skin wounds. Indeed, radiation-impaired wound healing is a specific form of the general clinical challenge posed by nonhealing wounds for which few effective treatment options are available (Olascoaga *et al.*, 2008).

Healing of skin wounds involves complex, well-orchestrated interactions between different cell types and extracellular matrix materials (Wu *et al.*, 2007b). After exposure to ionizing radiation, the time to expression of skin damage is determined by the slow turnover of dermal cells (Withers, 1967). However, trauma speeds the proliferation rate of the affected cells and thereby greatly accelerates the expression of any radiation-induced latent damage, and wounded irradiated skin displays healing defects acutely (Gorodetsky *et al.*, 1988). The dose and the body volume are critical variables. The dermis is relatively radiation resistant, but local stem/progenitor skin cells can be damaged and thus they fail to replace those lost through normal turnover or through physical or thermal injury. On the other hand, damage to the more sensitive hematopoietic system through whole- or partial-body radiation exposure can compromise the pool of bone marrow (BM)–derived cells that contribute to the healing process. This includes stem cells, immune cells,

[1]*Department Radiation Oncology, David Geffen School Medicine, University of California, Los Angeles, Los Angeles, California, USA and* [2]*Sharett Institute of Oncology, Hadassah-Hebrew University Medical Center, Jerusalem, Israel*

Correspondence: William H. McBride, Department Radiation Oncology, David Geffen School Medicine, University of California, Los Angeles, 10833 Le Conte Avenue Box 951714, B3-109 CHS, Los Angeles, California 90095-1714, USA.
E-mail: wmcbride@mednet.ucla.edu

Abbreviations: AMD3100, plerixafor; BM, bone marrow; FMB, fibrin microbead; G-CSF, granulocyte colony-stimulating factor; GFP, green fluorescent protein; MSC, mesenchymal marrow-derived stromal cell; PBS, phosphate-buffered saline; SI, skin-only irradiation; WTS, wound tensile strength; WBI, whole-body irradiation

Received 7 March 2012; revised 19 July 2012; accepted 25 July 2012; published online 6 September 2012

© 2013 The Society for Investigative Dermatology

endothelial progenitor cells, and fibrocytes, all of which can contribute to healing either structurally or as regulatory influences (Wu *et al.*, 2007b). Sublethal whole-body irradiation (WBI) therefore requires a far less dose compared with skin-only irradiation (SI) to delay healing of full-thickness incisional wounds in mouse skin (Vegesna *et al.*, 1993). In a radiological incident, the whole-body dose received is therefore critical to the assessment of potential deficits in wound healing. The dose received by the hematopoietic system may, however, be very different from that received by the skin because of proximity to the radiation source.

In recent years, multiple animal injury models, as well human studies (Garcia-Gomez *et al.*, 2010), have shown that mesenchymal marrow-derived stromal cells (MSCs) are excellent candidates for enhancing tissue repair, including damage caused by radiation (Leclerc *et al.*, 2011). This is enhanced by reports that they may be effective across histocompatibility barriers (Shi *et al.*, 2010), although their true potential in this regard is still controversial.

Our approach to correcting radiation-induced wound-healing problems using MSCs was tempered by our previous experience. On the basis of an earlier study in pigs (Kruegler *et al.*, 1978), we reported that implanted neonate skin fibroblasts could partly correct radiation-induced wound-healing deficits in mice (Gorodetsky *et al.*, 1991), findings that Dantzer *et al* (2003) later extended in a rat model using BM-derived stromal cells. The primary factor responsible for our limited success at the time was that cells implanted directly into a wound site rapidly disappeared, with $<1\%$ remaining for more than a few days. To circumvent this problem, we developed a novel fibrin microbead (FMB) cell carrier (Gorodetsky *et al.*, 1999, 2004; Gorodetsky, 2008). Matrix-dependent cells, including MSCs, attach to the FMBs in three dimensional suspension culture, allowing easy removal of the nonattaching hematopoietic and epidermal cells. Attachment is mediated by newly described cell-binding homologous C-terminal short peptides on β- and γ-chains of fibrin, termed haptides (Gorodetsky *et al.*, 1998, 2003; Levy-Beladev *et al.*, 2010). MSCs isolated on FMBs proliferate to high density (up to $10^8\,ml^{-1}$ packed beads) *in vitro*, yielding up to a log more cells than conventional plastic adhesion-based culture methods.

When MSC-FMBs are implanted in skin wounds *in vivo*, the FMBs degrade slowly and the MSCs are retained in high numbers to proliferate and differentiate normally within the target tissue. In addition, FMBs can support the viability of MSCs for up to 10 days at room temperature, making cell transportation easy in emergencies (Gorodetsky *et al.*, 2011).

Here we use the incisional wound-healing model to examine the ability of MSC-FMBs to correct radiation-induced damage. The ability of MSC-FMBs to act across an allogeneic barrier was examined, as was the effects of addition of granulocyte colony-stimulating factor (G-CSF) and plerixafor (AMD3100). Finally, the ability of MSCs on haptized collagen strips to reduce radiation wound-healing deficits was assessed.

RESULTS

Dose/time effects of WBI and SI on gain in skin wound tensile strength (WTS)

A dermal wound-healing model was established to examine the effect of irradiating only the skin with or without total body exposure of the hematopoietic system, with the aim of examining how different doses to these different organs, as might easily happen in a radiological situation, would interact.

Sublethal WBI of C3H mice using gamma rays compromised the ability of wounds in their skin to gain WTS by 2 weeks (Figure 1a). After 4 Gy, WTS was approximately 10% lower, after 6 Gy 20% lower, and after 8 Gy 30% lower. These data agree closely with what we found previously (Vegesna *et al.*, 1993). In contrast, doses of around 15 Gy 150 kVp X-rays to the SI were required to reduce the gain in WTS by 50% at 2 weeks (Figure 1b), rather more than the 13 Gy that we first reported 20 years ago; however, considering that the irradiator and tensiometer were different, the reproducibility of radiation effects on WTS measurements is remarkable.

The gain in WTS with time after radiation was also reexamined. We confirmed our earlier finding that WTS increased in unirradiated C3H skin in two phases with nearly half normal strength being recovered within 2 weeks (Figure 1c) and 90% by 4–5 weeks (Figure 1c) (Gorodetsky *et al.*, 1988). C57Bl/6 skin responded in a very similar manner, although with slightly higher WTS values (not shown), which could have been due to sex or strain differences. The combination of 4 Gy WBI and 21 Gy SI, a scenario that mimics the expected scenario of a radiological incident where the skin might be compromised by a high dose, whereas the whole body might receive a more moderate dose, resulted in a fairly consistent delay of around 10 days to gain the unirradiated level of WTS in C3H mice at 3 or 4 weeks (Figure 1c). The first ''phase'' of wound healing is most affected by radiation exposure (Gorodetsky *et al.*, 1988), so much so that the 2-week values are too low to be reliable after these doses. We therefore chose to measure the effects of 6 Gy WBI and 15 Gy SI on WTS measured at 4 weeks, the combined WBI and SI deficit in WTS being more than additive (Figure 1d).

MSC-FMB compared with plastic-adherent MSC populations

FMBs were used as a substrate for the culture of MSCs to allow their rapid purification from hematopoietic cells and high-density expansion (Gorodetsky, 2008). Because culture on FMBs may change the MSC phenotype, we have previously compared the flow-cytometric profiles of MSCs downloaded from FMBs with those cultured on plastic (Rivkin *et al.*, 2007) using a wide range of putative MSC markers that were more characteristic to a pure population of MSCs than the plastic-isolated cells. For these experiments, the major differences were that MSCs cultured on FMBs (Figure 2a) showed bimodal distributions for CD44, CD49e, and CD105, with increases in expression of CD49e and CD105. The bimodal distribution was largely contributed to by Sca1+, $CD44^{lo}$, $CD49e^{hi}$, and $CD105^{hi}$ cells of smaller size (not shown). CD45+ and CD19+ cells were absent, indicating purification from hematopoietic cells, which was not the case for cells grown

Download English Version:

https://daneshyari.com/en/article/6078023

Download Persian Version:

https://daneshyari.com/article/6078023

Daneshyari.com