Angiopoietin-1 improves endothelial progenitor cell–dependent neovascularization in diabetic wounds

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Background. *The diabetic phenotype of wound healing is in part characterized by impaired neovascularization and deficient endothelial progenitor cell (EPC) recruitment. Angiopoietin-1 (Ang-1) is a potent mobilizer of EPCs from the bone marrow (BM). A suggested mechanism for EPC mobilization from the BM is mediated by matrix metalloproteinase 9 (MMP-9) and stem cell factor (SCF). Taken together, we hypothesized that overexpression of Ang-1 in diabetic wounds will recruit EPCs and improve neovascularization and wound healing.*
Methods. *An endothelial lineage BM-labeled murine model of diabetes was developed to track BM-derived EPCs. FVBN mice were lethally irradiated and then reconstituted with BM from syngeneic Tie2/LacZ donor mice. Diabetes was induced with streptozotocin. Dorsal wounds in BM-transplanted mice were treated with Ad-Ang-1, Ad-GFP, or phosphate-buffered saline. At day 7 after injury, wounds were harvested and analyzed. A similar experiment was conducted in EPC mobilization deficient MMP-9 –/– mice to determine whether the effects of Ang-1 were EPC-dependent.*
Results. *Overexpression of Ang-1 resulted in greatly improved re-epithelialization, neovascularization, and EPC recruitment in diabetic BM-transplanted wounds at day 7. Ang-1 treatment resulted in increased serum levels of proMMP-9 and SCF but had no effect on vascular endothelial growth factor levels. According to our FACS results, peripheral blood EPC ($CD34^+/Cd133^+/Flk1^+$) counts at day 3 after wounding showed impaired EPC mobilization in MMP-9 –/– mice compared with those of wild-type controls. EPC mobilization was rescued by SCF administration, validating this model for EPC-mobilization–deficient mechanistic studies. In MMP-9 –/– mice, Ad-Ang-1 accelerated re-epithelialization in a similar manner, but had no effect on neovascularization.*
Conclusion. *Our results show that Ang-1 administration results in improved neovascularization which is dependent on EPC recruitment and has direct effects on wound re-epithelialization. These data may represent a novel strategy to correct the phenotype of impaired diabetic neovascularization and may improve diabetic wound healing. (Surgery 2015;158:846-56.)*

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CHRONIC DIABETIC ULCERS ARE RESPONSIBLE FOR MORE THAN 42,500, NONTRAUMATIC LOWER-LIMB AMPUTATIONS and 27% of diabetic health care costs in the United States annually.[1,2] The impairments in the phenotype of cutaneous diabetic wound healing are associated with several intrinsic and extrinsic factors.[3]

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This study is supported by NIH-NIDDK awards R01-DK072446 and R01-DK074055 (T.M.C.) and NIH-NIGMS awards K08 GM098831 and 1R01GM111808 (S.G.K).

The work described here was conducted in Cincinnati, OH.

Abstract Control Number: ASC20151363.

Presented at the 10th Annual Academic Surgical Congress in Las Vegas, NV, February 3–5, 2015.

Accepted for publication June 27, 2015.

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0039-6060/$ - see front matter

http://dx.doi.org/10.1016/j.surg.2015.06.034

Wounds in diabetic patients, as well as in murine models of Type I and Type II diabetes, show a defect in angiogenesis, re-epithelialization, and wound closure.[4] The initial re-epithelialization does not depend on angiogenesis, but the complete healing and maturation are regulated closely by vascular responses of several cells and cell–matrix interactions.[5] The deficiencies in angiogenesis have been attributed to poorly managed blood glucose levels and to the related vascular defects in both endothelial cells[6,7] and endothelial progenitor cells (EPCs).[8-14]

A deficit in neovascularization in diabetes is known to be associated with a compromised response to ischemia in wound healing as a consequence of metabolic derangement,[3,15] but beyond this vague understanding, the compromised ability to revascularize ischemic tissues in diabetes is poorly understood.[10] A potential mechanism to explain this impairment is a decrease in the expression of angiogenic growth factors, such as vascular endothelial growth factor (VEGF), angiopoietin-1 (Ang-1), and their receptors.[16,17] Skin samples taken from the peri-wound area from lower extremities (skin within 1 cm from wound margin) of type 2 diabetic subjects has significantly less expression of both VEGF (46%) and Ang-1 (36%) than the skin tissue of nondiabetic subjects.[18] Many groups have shown evidence linking impaired neovascularization with delayed closure of diabetic wounds, and further, that supplementation of angiogenic growth factors, such as VEGF, Ang-1, epidermal growth factor, basic fibroblast growth factor, and hypoxia inducible factor–1α (HIF-1), or a combination of these factors via recombinant growth factor therapy or gene transfer, has positive effects on improving neovascularization and outcomes of diabetic wound closure.[8,19-25]

The angiopoietin family (Ang-1–4) has been shown to play a critical role in the modulation of physiologic angiogenesis and pathologic neovascularization. VEGF and the angiopoietins function together, playing independent roles during vascular development and embryogenesis; VEGF acts early during vessel formation[26] and Ang-1 acts later during vessel remodeling, maturation, and stabilization.[27,28] Angiopoietins 1 and 2 have been studied in in vivo and in vitro models, particularly in relation to diabetic retinopathy.[29,30] Ang-1, a well-established secreted 70-KDa ligand that shares many of the proangiogenic properties of VEGF, protects blood vessels from increased plasma leakage by counteracting transendothelial permeability stimulated by VEGF.[31] Ang-1 signals primarily through the transmembrane receptor tyrosine kinase (Tie2), which is expressed ubiquitously in vascular endothelium and is phosphorylated in quiescent vessels. Ang-1 interacts with several cells, such as neutrophils, endothelial cells, and fibroblasts, through integrins to mediate survival, cell adhesion, and migration.[32-34] Ang-1 is an essential and critical regulator of blood vessel development, as evidenced by the Ang-1–null mouse, which is embryonically lethal.[35] In contrast, Ang-2, an antagonist of Ang-1 and Tie2 signaling, generally is not expressed in tissues of healthy adults but is expressed in secretory tissues undergoing inflammation and vascular remodeling, such as healing wounds and tumors.[36,37] The systemic levels of Ang-2 increase early in sepsis.[38] Compared with wounds in nondiabetic wild-type (WT) control mice,[39] wounds in diabetic db/db mice show decreased expression of Tie-1 and -2 proteins, which is paralleled by increased expression of the ligand Ang-2. Conversely, Ang-1 treatment was associated with suppressed development of diabetic retinopathy and decreased both vascular endothelial injury and breakdown of the blood–retinal barrier in a rat diabetic model.[29] Ang-1 gene transfer also improved the delayed wound repair in diabetes by inducing angiogenesis, albeit in a VEGF-independent manner.[25] A recent study suggested that Ang-1 and Ang-2 have a specific regulatory role in endothelial development from circulating $CD34^+$ progenitors; Ang-1 regulates the initial commitment of EPCs, whereas Ang-2 enhances expansion of the endothelial cell progeny.[39] The effects of Ang-1 on EPC mobilization and vasculogenesis are not defined completely.

The paradigm to improve therapeutic angiogenesis has focused on enhancing the formation of neovessels from preexisting, terminally differentiated endothelial cells to accelerate neovascularization. Several reports have shown that EPCs incorporate into areas of neovascularization via vasculogenesis. A novel approach to accelerate neovascularization is site-specific recruitment of bone marrow (BM)-derived EPCs to drive both the angiogenic and vasculogenic components of neovascularization. The mechanisms of EPC mobilization have been studied vigorously during the last decade.[40] EPCs are primarily derived from BM and are characterized by antigenic markers defining the stemness and hematopoietic lineage (CD34, CD133), in combination with markers showing endothelial commitment (Flk-1).[41,42] In response to tissue injury, EPCs mobilize from their BM niche into the circulation and home to sites of tissue