JOURNAL OF SURGICAL RESEARCH 190 (2014) 367–377

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.JournalofSurgicalResearch.com

Association for Academic Surgery

Adenoviral-mediated gene transfer of insulin-like growth factor 1 enhances wound healing and induces angiogenesis

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ARTICLE INFO

Article history:
Received 8 January 2014
Received in revised form
19 February 2014
Accepted 25 February 2014
Available online 2 March 2014

Keywords:
Wound healing
IGF-1
Gene therapy
Diabetes
Neovascularization
db/db
Organ culture

ABSTRACT

Background: Chronic wounds are characterized by a wound healing and neovascularization deficit. Strategies to increase neovascularization can significantly improve chronic wound healing. Insulin-like growth factor (IGF)-1 is reported to be a keratinocyte mitogen and is believed to induce angiogenesis *via* a vascular endothelial growth factor (VEGF)-dependent pathway. Using a novel *ex vivo* human dermal wound model and a diabetic-impaired wound healing murine model, we hypothesized that adenoviral overexpression of IGF-1 (Ad-IGF-1) will enhance wound healing and induce angiogenesis through a VEGF-dependent pathway.
Methods: Ex vivo: 6-mm full-thickness punch biopsies were obtained from normal human skin, and 3-mm full-thickness wounds were created at the center. Skin explants were maintained at air liquid interface. *Db/db murine model:* 8-mm full-thickness dorsal wounds in diabetic (db/db) mice were created. Treatment groups in both human *ex vivo* and *in vivo* db/db wound models include 1×10^8 particle forming units of Ad-IGF-1 or Ad-LacZ, and phosphate buffered saline ($n = 4–5$/group). Cytotoxicity (lactate dehydrogenase) was quantified at days 3, 5, and 7 for the human *ex vivo* wound model. Epithelial gap closure (hematoxylin and eosin; Trichrome), VEGF expression (enzyme-linked immunosorbent assay), and capillary density (CD 31 + CAPS/HPF) were analyzed at day 7.
Results: In the human *ex vivo* organ culture, the adenoviral vectors did not demonstrate any significant difference in cytotoxicity compared with phosphate buffered saline. Ad-IGF-1 overexpression significantly increases basal keratinocyte migration, with no significant

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0022-4804/$ – see front matter
http://dx.doi.org/10.1016/j.jss.2014.02.051

effect on epithelial gap closure. There was a significant increase in capillary density in the Ad-IGF-1 wounds. However, there was no effect on VEGF levels in Ad-IGF-1 samples compared with controls. In db/db wounds, Ad-IGF-1 overexpression significantly improves epithelial gap closure and granulation tissue with a dense cellular infiltrate compared with controls. Ad-IGF-1 also increases capillary density, again with no significant difference in VEGF levels in the wounds compared with control treatments.

Conclusions: In two different models, our data demonstrate that adenoviral-mediated gene transfer of IGF-1 results in enhanced wound healing and induces angiogenesis *via* a VEGF-independent pathway. Understanding the underlying mechanisms of IGF-1 effects on angiogenesis may help produce novel therapeutics for chronic wounds or diseases characterized by a deficit in neovascularization.

1. Introduction

Chronic wounds, including diabetic ulcers, are characterized by the disruption of normal healing process, which results in a pathologic state of nonhealing cutaneous inflammation and delayed wound closure [1,2]. These wounds account for an increasingly significant economic burden as their prevalence is rising commensurate with an aging population, and an increase in obesity and diabetes [3,4]. 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 [5,6]. Despite standard treatment protocols and active investigation on several fronts including novel cell, gene and molecular therapies [7,8], successful treatment of diabetic ulcers is limited, and currently there is no effective therapy.

Although the pathogenesis of impaired diabetic wound healing is multifactorial, dysfunctional angiogenesis is a primary contributing factor, which is characterized by hyperglycemia-related microvascular changes and deficiencies in both endothelial cells [9,10] and endothelial progenitor cells [2,11–14], which leads to impaired diabetic wound healing. The development of novel therapeutics that promotes angiogenesis has the potential to improve diabetic tissue repair and would be a boon for patients suffering with chronic nonhealing wounds. Diabetic wounds are also known to have reduced growth factor levels [12,15–17], some of which promote the angiogenic response to injury. Many groups have demonstrated that the supplementation of exogenous growth factors *via* recombinant growth factor therapy or gene transfer at the cellular and molecular level has vulnerary effects and improves wound healing outcomes [2,18–21]. Among the several growth factors studied, insulin-like growth factor (IGF-1) levels have been shown to be reduced in diabetic wounds and are associated with delayed wound healing [22–24]. Streptozotocin-induced diabetic rats demonstrated reduction in wound fluid IGF-I levels. Normal induction of IGF-1 messenger RNA is delayed and impaired in diabetic mice [25]. Importantly, endothelial dysfunction observed in diabetes has been shown to be associated with a blunted insulin-IGF signaling response in endothelial cells [26].

IGF-1 is a 7.6 kDa molecule that has 70 amino acids in a single chain with three intramolecular disulfide bridges. In the skin, IGF-1 is produced by cells of mesenchymal origin, such as fibroblasts of the dermis and dermal papilla [27,28]. IGF-1 has several roles in skin homeostasis. It is recognized as a proliferation and survival factor for the skin and hair morphogenesis [29,30]. Deficiency in IGF-1 is associated with decreased epidermal thickness and sparse hair growth [31]. The importance of IGF-1 signaling in the skin is evident from the original studies with IGF-1 receptor null (IGF-1R_/_) mice, which exhibited hypotrophic skin with reduced number and size of the hair follicles [32]. IGF-1 also has an important role in wound healing. It is a keratinocyte mitogen, motogen and morphogen [33,34]. IGF-1 was shown to stimulate keratinocyte proliferation and migration as well as collagen production by fibroblasts [34–36]. In addition, IGF-1 is a potent stimulator of hair follicle morphogenesis and cycling, and of re-epithelialization of skin wounds [37]. IGF-1 has also been shown to increase angiogenesis in several different models of tissue injury, potentially through an increase in hypoxia-inducible factor 1α and vascular endothelial growth factor (VEGF)-dependent mechanisms [38–42]. Furthermore, it has been shown that the addition of IGF-1 to diabetic wounds using nonviral gene transfer in combination with cell therapy improves diabetic wound closure significantly [43]. However, the underlying mechanism of how IGF-1 enhances angiogenesis and wound healing are not completely understood.

The limited data available support the concept that IGF-1 therapy has the potential to improve diabetic wound healing. One of the inherent challenges of developing novel therapeutics for wound healing clinical trials is the species-specific differences between preclinical animal models and humans. To address this, we have developed a novel model that uses wounded human skin that is maintained in culture *ex vivo* to screen candidate therapeutics for toxicity and wound healing effects in a rapid and efficient manner. These data can then be compared with *in vivo* data in animal models to develop a more complete understanding of the translational capability of the therapeutic agent. For diabetic animal studies, the best available model for type II diabetic wound healing is the leptin receptor deficient db/db murine model. Wounds created in db/db wounds demonstrate increases protease activity and have a significant impairment in neovascularization and wound healing [1,2], similar to the impaired wound healing phenotype observed in diabetic patients.

Taken together, we hypothesize that overexpression of IGF-1 *via* a VEGF-dependent pathway will enhance angiogenesis and improve wound healing. To test this hypothesis, we will first screen adenoviral overexpression of IGF-1 in a novel human *ex vivo* skin organ culture wound model to assess toxicity and vulnerary effects. We will then validate these data in a known diabetic impaired wound healing murine db/db mouse for similar end points.