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Systemic administration of hemoglobin improves ischemic wound healing

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ABSTRACT

Background: Oxygen plays multifaceted roles in wound healing, including effects on cell proliferation, collagen synthesis, angiogenesis, and bacterial killing. Oxygen deficit is a major factor in the pathogenesis of chronic wounds.

Materials and methods: We present a novel mechanism for oxygen delivery to ischemic wounds by systemic administration of an oxygen carrier substitute derived from bovine hemoglobin (IKOR 2084) in our ischemic rabbit ear wound model. The wound healing indexes, including epithelial gap and neo-granulation tissue area, were histologically analyzed. *In situ* expression of endothelial cells (CD31+) and proliferative cells (Ki-67+) were examined by immunohistochemistry analysis. The messenger RNA expression of collagen I, III, and vascular endothelial growth factor was measured by quantitative RT-PCR. Sirius Red staining was implemented for detection of collagen deposition, and terminal deoxynucleotidyl transferase dUTP nick end labeling analysis was performed to examine dermal cellular apoptosis.

Results: Systemic administration of IKOR 2084 significantly improved oxygen tension of ischemic tissue. When compared with saline controls, IKOR 2084 treatment enhanced wound repair as demonstrated by a reduced epithelial gap and increased granulation tissue area. The expression of Ki-67+, CD31+, vascular endothelial growth factor and collagen was also enhanced by IKOR 2084 administration. Moreover, apoptosis analysis in the wounds showed that cell survival in the dermis was increased by systemic IKOR 2084 administration.

Conclusions: Our study suggests that systemic delivery of IKOR 2084 ameliorates hypoxic state, subsequently promotes angiogenesis, cellular proliferation, and collagen synthesis, attenuates hypoxia-induced apoptosis, and improved ischemic wound healing.

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1. Introduction

Physiological wound repair is a complex and stepwise process consisting of overlapping biochemical events, including inflammation, proliferation, angiogenesis, tissue formation, and remodeling [1]. Oxygen plays a crucial role in nearly every step of the wound healing process by supplying biological energy (e.g., adenosine triphosphate, ATP), catalyzing the formation of reactive oxygen species, functioning as a signaling molecule, as well as regulating collagen composition

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and deposition by fibroblasts [2–5]. Therefore, constant and adequate oxygen supply to wound tissue, which is dependent on both blood flow volume and oxygen carrying capacity of the blood, is the decisive parameter for wound repair rate.

The microenvironment of the wound is virtually devoid of oxygen shortly after acute tissue injuries as a result of vascular damage caused by inciting trauma and the high oxygen consumption caused by increased cell activity and cell density in healing tissue [6,7]. In normal tissue repair, the shortage of oxygen supply is temporary. However, in chronic wound states, like pressure sores, diabetic foot ulcers, and venous stasis ulcers, long-term hypoxia occur due to pathologic alterations of the vascular network and peri-wound fibrosis. Ultimately, this leads to increased anaerobic metabolism and poor energy production. This ischemic milieu significantly impairs cutaneous wound healing and defines the chronic wound healing state [8]. Consistent with these observations, Ahn and Mustoe [9] demonstrated that decreased oxygenation in wound tissue markedly delayed wound healing in an ischemic rabbit ear model.

In light of the critical role of oxygen in wound healing, improvement of oxygenation in ischemic tissues has been pursued as an important therapeutic strategy in clinical applications and in various experimental animal models of chronic wound healing [10–12]. Currently, two major methods of oxygen therapy have been used to improve the tissue oxygen tension in ischemic wounds [13]. Systemic hyperbaric oxygen therapy (HBOT) has been used clinically in the treatment of diabetic ulcers and chronic lower limb ischemia for over five decades and involves the inhalation of pure oxygen under oxygen pressure of 2 atmosphere or higher in a sealed chamber [14,15]. Additionally, topical application of gaseous oxygen at normal or elevated partial pressures [16] and topical application of an oxygen carrier to the ischemic lesion have been attempted [17]. However, variable efficacy, inconvenience, expense, and cellular toxicity have prevented these therapies from gaining wide acceptance [15].

Recently, improvement of the oxygenation in ischemic tissue by systemic application of artificial oxygen carriers has emerged. Hemoglobin-based oxygen carriers and perfluorocarbon emulsions are two major classes of artificial oxygen carriers. The artificial oxygen carriers were initially developed as blood substitutes for systemic administration in patients with high-volume blood loss. More recently, artificial oxygen carriers have been extended as potential treatments for the ischemic condition. Similar to hemoglobin, blood substitutes administered systemically acquire oxygen from the respiratory system, carry and release oxygen to the tissues through the cardiovascular system, and subsequently ameliorate local hypoxia in ischemic organs and tissues with limited blood supply. Plock *et al.* [18] demonstrated that systemic administration of hemoglobin within phospholipid bilayer membrane vesicles reduced hypoxia-related inflammation in a hamster ischemic flap model and improved survival of critically ischemic skin through nitric oxide synthase-induced neovascularization [19]. However, the efficacy of hemoglobin-based oxygen carriers to improve the healing of chronic wounds has not been demonstrated. We proposed that administration of hemoglobin-based oxygen carriers increase the oxygen tension in ischemic tissue with limited blood supply, and subsequently improves wound repair.

In the present study, using our rabbit ear ischemic wound model [9], the biological efficacy of systemic delivery of modified bovine hemoglobin (IKOR 2084) on chronic wound healing was evaluated with respect to epithelialization and granulation tissue formation. In addition, its effect on cellular proliferation, angiogenesis, collagen synthesis, and cellular survival in cutaneous wound tissue was also investigated.

2. Materials and methods

2.1. *Rabbit ear ischemic wound model*

Young (3–6-mo-old) female New Zealand White rabbits were purchased from Covance (Denver, PA) and housed in the animal facilities of Northwestern University Center for Comparative Medicine at 22°C and 50% humidity with a 12-h light–dark cycle. All animal procedures used in this study were approved by the Animal Care and Use Committee of Northwestern University. After 1 wk of acclimatization, an ischemic rabbit ear model was created as previously described [20]. Briefly, rabbits were sedated with intramuscular injection of ketamine (45 mg/kg) and xylazine (7 mg/kg). Ears were shaved, depilated, and sterilized. After instillation of a local anesthetic of 1% lidocaine, the central, caudal, and minute arteries were divided by an incision down to the cartilage around the base of the ear with preservation of the central, caudal veins. The inflow source to the rabbit ear was supplied by the intact rostral artery, which was preserved by keeping a 2-cm cuff of muscle tissue undisturbed around the rostral pedicle. The incision was closed with a single running 5-0 nylon suture. Immediately after ischemia surgery, three full-thickness 7-mm punch wounds to the perichondrium were created on the ventral surface of the rabbit ear and covered with semi-occlusive dressings (Tegaderm; 3M Health Care, St. Paul, MN).

2.2. *Treatment with IKOR 2084*

Chemically modified bovine hemoglobin (IKOR 2084, 6.0 g/dL) was supplied by the IKOR Company (IKOR life sciences Inc, Aberdeen, SD). Two doses of IKOR 2084, 200 and 400 mg per kg body weight, or saline control, were intravenously administered through the cephalic vein on postoperative days (POD) as described in results. The efficacy of IKOR 2084 on wound healing was evaluated by measuring the epithelial growth and granulation tissue area (G-A) in all wounds.

2.3. *Measurement of tissue oxygenation*

Tissue oxygen tension was examined by the OxyLite system (Oxford Optronix, Milton Park, Abingdon, UK), which consists of the OxyLite 2000E monitor and pO2/temperature fiber optic probe with automatic temperature compensation according to manufacturer instructions. Briefly, under mild anesthesia, the fiber optic probe was introduced through a 20-G needle inserted subcutaneously on the dorsum of the ear near the punch wounds. The monitor was allowed to stabilize for 5 min. Measurements were obtained before systemic delivery and 3 h after delivery of IKOR 2084. Oxygen tension in the normal tissue of the ear base was measured as a normoxia control.

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