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The effects of topical nitric oxide on healing of partial thickness porcine burns

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ABSTRACT

Background: Nitric oxide is a wound mediator that promotes wound healing. We hypothesized that topical application of nitric oxide would speed reepithelialization, enhance angiogenesis, and reduce scar thickness in a partial thickness porcine burn model.

Methods: While under general anesthesia, 20 partial thickness burns were created on the backs of four female Yorkshire swine using a 2.5 cm × 2.5 cm × 7.5 cm, 150-g aluminum bar, preheated to 80 °C and applied for 20 s. The necrotic epidermis was removed and the burns were randomized to low, medium, and high concentrations of a novel nitric-oxide (NO) releasing drug or its ointment vehicle applied 3 times weekly for 28 days. Full thickness punch biopsies were performed at 8, 11, 14 and 28 days after injury to determine percentage wound reepithelialization and scar thickness using H&E staining and blood vessel density using CD31 staining.

Results: At day 11, the percentages (SD) wound reepithelialization were: control, 26.3 (34.6); low NO, 23.9 (36.9); medium NO, 43.3 (42.9); and high NO, 59.9 (43.6); ANOVA, $P=0.02$. The number of CD31 stained blood vessels at days 8 and 11 were greater in wounds treated with high dose NO vs. controls (48.1 vs. 22.9 [$P<0.001$] and 44.0 vs. 33.5 [$P=0.05$] per 1 mm^2 respectively). Scar thicknesses (SD) in mm at day 28 by treatment allocation were: control, 4.8 (1.2); low NO, 4.7 (1.2); medium NO, 4.3 (1.2); and high NO, 4.1 (1.0); $P=0.22$.

Conclusions: Treatment of partial thickness porcine burns with high concentrations of topical NO resulted in earlier reepithelization and increased angiogenesis but not reduced scar thickness compared with its control vehicle in a partial thickness porcine burn model.

1. Introduction

Burns are common injuries that lead to significant disfigurement and dysfunction. Prior studies have suggested that burns that heal within 2–3 weeks are less likely to result in hypertrophic scarring compared with burns that take longer to heal [1,2]. Thus early healing of burns is an important component of ultimate burn outcomes.

Nitric oxide (NO) is a free radical gas produced from L-arginine by nitric oxide synthase (NOS) [3]. It is synthesized by multiple cells in the wound bed and is involved in many physiological and pathological responses during wound healing [4–8]. It is an important messenger molecule that acts

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partly through the expression of multiple growth factors and cytokines such as vascular endothelial growth factor (VEGF), transforming growth factor-β1(TGF-β1) and interleukin-8 (IL-8) [9–11]. A number of studies in small animals have demonstrated beneficial effects of topically applied NO on burn injuries. For example, topical nitric oxide promoted reepithelialization, accumulation of inflammatory cells, expression of myeloperoxidase (MPO), angiogenesis, and collagen synthesis in a full thickness burn model in rats [12]. A subsequent study demonstrated that NO enhanced reepithelialization, follicle stem cell recruitment, angiogenesis, and the number of procollagen-expressing fibroblasts in mice [13]. However, we are unaware of any studies that have evaluated the effects of topical NO on the healing of burns in larger animals such as pigs.

The aim of the current study was to evaluate the effects of topically applied NO on healing of partial thickness burns in a porcine model. We hypothesized that NO would accelerate reepithelization, enhance angiogenesis, and reduce scar thickness.

2. Methods and materials

2.1. Study design

In order to test the study hypothesis, we conducted a double-blind, randomized, controlled study. Our study was approved by the Institutional Animal Care and Use Committee (IACUC).

2.2. Animal use

The study was conducted on four female domestic Yorkshire pigs (20–25 kg). We chose the pig as the study animal because the structure and function of its skin is most similar to that in humans [14]. During an acclimation period of seven days the animals were kept in separate pens and fed with a standard diet in our Division of Laboratory Animal Research. The animals were exposed to 12 h of light and 12 h of darkness every 24 h. Feedings were withheld the night prior to conducting any experiment. During the entire study, we adhered to the National Research Council Guidelines [15].

2.3. Animal sedation and anesthesia

The sedation protocol that we followed included intramuscular injection of a combination of acepromazine 0.1 mg/kg, atropine 0.02 mg/kg, ketamine 20 mg/kg, and xylazine 2 mg/kg. Following their sedation, the animals underwent endotracheal intubation. The pigs were then anesthetized with isoflurane 1–5.0% in O_2 USP titrated to a surgical plane of anesthesia. The pigs' hair over their backs and flanks was clipped with an electric clipper and the skin was scrubbed with soap and water.

2.4. Burn creation and management

In this study we utilized a burn model that produces partial thickness burns, which undergo vertical progression over the first 24–48 h [16]. After achieving an adequate level of anesthesia, the investigator placed five rows of four burns each for a total of 20 burns per animal over the para-spinal region of the pigs. The partial thickness burns were created by applying an aluminum bar (Small Part Inc., Miami Lakes, FL) weighing 150 g directly over the skin after heating it to a temperature of 80 °C in a temperature controlled water bath. The surface area of the burn was 2.5 cm × 2.5 cm. Using gravity alone, the heated aluminum block was placed on top of the skin for a period of 20 s. In order to simulate blister formation and sloughing of the necrotic epidermis, we removed the necrotic epidermis immediately after creating the burns, by scraping it off with the blunt end of surgical forceps. The burns were then randomly assigned to one four treatment groups applied topically as a thin 1–2 mm layer: a petrolatum based ointment, a low concentration of a nitric-oxide releasing ointment, a medium concentration of a nitric-oxide releasing ointment, or a high concentration of a nitric-oxide releasing ointment. Randomized assignment of treatment was based on a computerized table of random numbers.

The wounds were then covered with non-adherent gauze (Telfa, Kendall Healthcare Products Company, Mansfield, MA). A secondary dressing (Sof-Form, Medline Industries, Inc., Mandekein, IL) was then applied and kept in place with an outer adhesive elastic bandage (Tensoplast, BSN Medical S.A. S., Vibraye, France). Following the initial injury, we removed the dressings and reapplied the topical treatments on Mondays, Wednesdays and Fridays for a period of four weeks. At the end of the 4-week study period, the animals were euthanized by the veterinary staff with a lethal dose of intravenous pentobarbital. We controlled post-operative pain with a combination of buprenorphine given by intramuscular injection in combination with transdermal fentanyl patches.

2.5. Histopathological assessments

In order to perform histomorphological analyses of the tissue we obtained 3 mm full thickness punch biopsies at day 8 from the upper left corner of the burn, at day 11 from the bottom right corner of the burn, and at day 14 after injury from the upper right corner of the burn. At 28 days we took 8 mm punch biopsies from the center of the burn. Preparation of tissue sections was performed by sequential alcohol-dehydration, xylene-clearance, and paraffin embedding. After bisecting the biopsies, they were sectioned at 5 μm intervals, and stained with Hematoxylin & Eosin (H&E).

The primary outcome was the percentage wound reepithelialization. The percentage reepithelialization was determined under low power microscopy by dividing the length of the wound that was covered by a neo-epidermis by the entire length of the wound section on the slide. This measure has excellent inter-observer agreement [17]. Secondary outcomes included wound infection, angiogenesis and scar thickness. While there is considerable debate regarding the exact definition of wound infection, wounds were considered infected in the presence of erythema of the surrounding non-injured skin, warmth and/or purulent discharge. Scar thickness was measured as the vertical distance from the epidermal basement membrane to the lowest level of the scar. The scar was recognized by the abnormal orientation of the collagen bundles. Normally distributed in all three planes, scar collagen fibers are thinner than normal and mostly oriented in

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