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Excessive nitric oxide impairs wound collagen accumulation

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ABSTRACT

Background: Nitric oxide (NO) plays a major regulatory role in wound collagen synthesis. We hypothesized that this regulatory role is tightly controlled by the levels of NO in the wound environment and that supranormal wound NO generation impairs wound collagen accumulation.

Materials and methods: We used the model of turpentine-induced granuloma in male Sprague–Dawley rats as a sterile inflammatory stimulus generating large amounts of NO. In this environment, NO generation increased by 260%, whereas collagen deposition was significantly reduced by 38.5% (729.7 ± 81.5 *versus* 449.4 ± 76.3 μg hydroxyproline/100 mg sponge, $P < 0.05$). Inhibition of NO synthase activity using 300 mM L-N6-(1-iminoethyl)-lysine, a highly potent and selective inhibitor of inducible NO synthase, significantly reduced NO elevation by 43.3% and increased wound collagen deposition by 37.3% ($P < 0.05$). These effects occurred without any anti-inflammatory effects of L-N6-(1-iminoethyl)-lysine as assessed by the white blood cell counts and levels of interleukins 1 and 6.

Conclusions: The data show that high levels of NO within the wound environment significantly reduce wound collagen deposition. Inhibition of NO generation restores collagen levels to normal levels. The regulatory effects of NO on wound collagen appear to be highly correlated with the amount of NO generated.

1. Introduction

The process of wound healing, a complex and well-regulated cellular and biochemical cascade, is incompletely understood. The full extent of the signals that control the activation or downregulation of the proliferative and synthetic processes that characterize wound healing remains to be fully elucidated.

Shortly after the discovery of the nitric oxide (NO) pathway, it became clear that this remarkable molecule plays an important role in wound healing [1,2]. Most, if not all, cell

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types that participate in wound healing are capable of expressing the inducible NO synthase (iNOS), at least *in vitro* [3]. The wound-activated phenotype of several critical wound cells such as fibroblasts includes the production of NO, a characteristic that is essential for optimal collagen synthesis [4]. *In vivo* inhibition of NO synthesis results in impaired wound collagen accumulation [1], whereas transfection of wounds with the *iNOS* gene enhances wound collagen accumulation [5]. Clinical conditions characterized by impaired wound collagen accumulation and weakened scars such as protein–calorie malnutrition and diabetes are also characterized by a deficiency in NO synthesis in the wounds of both experimental animals [6,7] and humans [8].

We have postulated that NO generation within the wound is a very tightly regulated and critical process for adequate wound collagen synthesis. The "healthy" healing response requires a "normal" level of NO generation, whereas impaired wounds are characterized by decreased wound NO synthesis. In the present experiments, we hypothesized that excess wound NO production, as observed during heightened inflammatory responses, would be detrimental to healing responses, in particular collagen synthesis.

2. Materials and methods

All animal studies were approved by the Sinai Hospital of Baltimore Institutional Animal Care and Utilization Committee and were carried out according to National Institutes of Health Guidelines.

2.1. Animals

Male, 12- to 14-week-old, Sprague–Dawley rats weighing between 350 and 450 g were obtained commercially. The animals were certified as free of common pathogens and were housed in isolation for a week within the animal care facility of Sinai Hospital of Baltimore. All animals were kept at constant temperature (25°C) and humidity (50%) on a 12-h light–dark cycle. Rats were offered a complete laboratory chow (Teklad Inc, Monmouth, IL) and tap water *ad libitum*. Body weight was monitored every day during the length of the study (10 d) as a sign of overall well-being.

2.2. Wound models

2.2.1. Model 1

Twenty animals were anesthetized with 50 mg/kg of intraperitoneal sodium pentobarbital (Nembutal; Abbott Laboratories, North Chicago, IL). The backs of the animals were shaved and wiped with povidone–iodine (Medline, Inc, Mundelein, IL) solution. Using a scalpel, a 2-cm horizontal incision was then made in the dorsal midline followed by the creation of a caudal subcutaneous pocket (Fig. 1A). Polyvinyl alcohol sponges (Unipoint Industries, High Point, NC) of approximately 40 ± 1 mg weight were sterilized in boiling water for 20 min and soaked in sterile saline for 5 min before implantation into the subcutaneous pockets (one sponge per pocket per animal). Before insertion, the sponges were mopped dry and treated with 50 μL of either normal saline (NS; $n = 10$) or turpentine (TURP; $n = 10$) (obtained from Parks Corporation, Fall River, MA). The midline dorsal wound was closed with surgical staples, and animals were sacrificed on postoperative day 10.

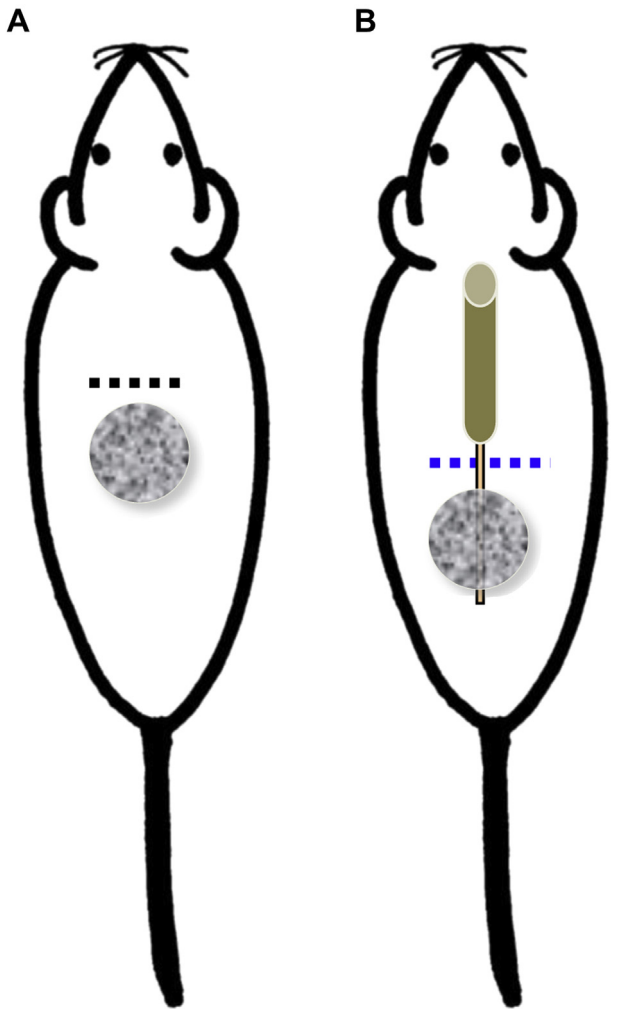

Fig. 1 – Schematic representation of the two wound models used: (A) implantation of polyvinyl sponge subcutaneously and (B) implantation of polyvinyl alcohol sponge connected to an osmotic pump for continuous treatment with either saline or NIL. (Color version of figure is available online.)

2.2.2. Model 2

In this set of experiments, we used a model that uses a continuously perfused and treated sponge, as previously described [9]. Briefly, a miniosmotic pump (Alzet; Alza Pharmaceuticals, Palo Alto, CA) was connected to each polyvinyl alcohol sponge by a silastic catheter perforated at the distal 1 cm and sealed at the end (Fig. 1B). Each pump was primed and loaded to perfuse locally into the sponges at a constant rate of 0.5 μL/h for 10 consecutive days either NS or 300 mM L-N6-(1-iminoethyl)-lysine (L-NIL), a well-known inhibitor of iNOS activity [10]. Each sponge was treated with 50 μL of sterile TURP before implantation into the subcutaneous pocket.

In one experiment examining collagen accumulation, 20 male Sprague–Dawley rats underwent the sponge-pump