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Effect of tadalafil on reduction of necrosis in the ischemic zone in a rat comb burn model

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ABSTRACT

Objectives: A major goal of burn management is to reduce the progression of necrosis in the zone of ischemia surrounding the central zone of necrosis. A rat comb burn model is often used to assess the progression of necrosis in the zone of ischemia. We compared various combinations of naproxen [NPX], *N*-acetyl cysteine [NAC], and tadalafil [TD] (a phosphodiesterase-5 inhibitor used as a vasodilator to treat erectile dysfunction) in a rat comb burn model to determine their effects on injury progression.

Methods: We created two comb burns on the backs of 40 anesthetized Sprague-Dawley ratsusing a brass comb with four rectangular prongs preheated in boiling water and applied for 30s, resulting in four rectangular 10 × 20mm full-thickness burns separated by three 5 × 20mm unburned interspaces, representing the ischemic zones. We randomized five animals each to daily oral gavage with TD (1mg/kg), NPX (10mg/kg), NAC (500mg/kg), NAC+NPX, TD+NPX, TD+NAC, TD+NPX+NAC, or normal saline [NS]. Wounds were observed daily for gross evidence of necrosis in the unburned interspaces and full-thickness biopsies from the interspaces were evaluated with Hematoxylin & Eosin seven days after injury for histological evidence of necrosis.

Results: The percentages of interspaces with histological evidence of necrosis at day seven were TD-40%, NPX-93%, NAC-97%, NS-87%, TD+NPX-50%, TD+NAC-40%, TD+NPX+NAC-33%, and NPX+NAC-60% ($P < 0.001$). Repeated measures ANOVA demonstrated reduced gross percentage of interspace area undergoing necrosis in all groups that included TD, compared with all groups not including TD ($P < 0.001$). There were no differences among the various treatments within the groups that did or did not include TD.

Conclusions: Daily oral therapy with tadalafil reduces necrosis in the unburned interspaces compared with naproxen, NAC, or their combination in a rat comb burn model. Addition of naproxen or NAC to tadalafil does not further reduce injury progression.

1. Introduction

Burns are dynamic wounds in which the extent of injury tends to progress over time due to a combination of ischemia-reperfusion due to decreased perfusion, inflammation and oxidative stress [1–5]. In his monumental paper in 1953 Jackson described how burns classically affected the skin [6]. The zone directly in contact with the thermal insult underwent immediate and complete necrosis and was termed the zone of necrosis. Immediately surrounding this inner core was a zone of ischemia or stasis in which the microcirculation was

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diminished, but if improved, was potentially reversible. Since then, efforts to prevent the conversion of the zone of stasis to a zone of necrosis have been made.

While numerous classes of medications (e.g., vasodilators, anti-inflammatory agents, and anti-oxidants) have been studied in an attempt to mitigate burn injury progression [7–17], there is no FDA-approved therapy. Thus, efforts to find a safe and effective therapy aimed at preventing or reducing burn injury progression (also named burn conversion) have continued. While numerous agents have been previously investigated [7–16], it is unclear whether a cocktail of medications with different mechanisms of action may be more effective than single agents alone.

Tadalafil is an FDA approved phosphodiesterase 5 inhibitor (PDE5I) used to treat erectile dysfunction. It augments the vasodilatory effects of nitric oxide by inhibiting PDE thus increasing the intracellular levels of cyclic guanosine monophosphate (cGMP) [17]. This secondary messenger then leads to relaxation of smooth muscle cells within blood vessels and vasodilation. We are unaware of any previous studies that have specifically evaluated the effects of a PDE5I on burn wound progression. In this study, we tested the hypothesis that tadalafil, either alone or in combination with an anti-inflammatory agent and/or an anti-oxidant would reduce injury progression in the zone of stasis in a rat comb burn model.

2. Methods

2.1. Study design

We conducted a prospective, double-blinded, randomized controlled experiment to test the study hypothesis. The study was approved by the Institutional Animal Care and Use and Committee conducted following guidelines issues by the National Research Council [18].

2.2. Animals

We conducted our study on 40 Sprague-Dawley rats weighing 250–300 g each. All animals were housed in separate cages and given a standard rodent diet (Purina RMH 3000 Rodent Laboratory Chow [Purina Animal Nutrition, Gray Summit, MO]) 15 g/100 g body weight daily for seven says prior to experimentation to allow for acclimation. The animals were exposed to 12 h cycles of light and darkness.

2.3. Experimental protocol

The animals were fasted overnight and anesthetized with inhalational isoflurane 0.5–5% with a mask. Each animal received an intramuscular injection of buprenorphine for post-operative analgesia. The hair over the experimental region was clipped with an electric clippers and a depilating cream was applied for five minutes and then wiped off.

On all animals, we created two comb burns, one on each side of the animal placed lateral to the spinal column between the forepaws and hind paws. The burns were created using a previously validate animal model with a 20 by 20 by 55 mm brass comb which had four 10 by 20 mm prongs separated by three 5 by 20 mm notches (Fig. 1) [13]. The brass comb was preheated in boiling (100° C) water for a period of 3 min and applied directly to the skin over the dorsum of the animals for 30 s with gravity alone. This resulted in four 10 by 20 mm full-thickness burns separated by three 5 by 20 mm unburned interspaces, which represented the ischemic zones surrounding the other burns.

Using a random numbers table, the animals were randomized, in groups of five animals each, to one of the following eight treatments: tadalafil (TD) 1 mg/kg, naproxen (NPX) 10 mg/kg, N-acetyl cysteine (NAC) 500 mg/kg, NAC+NPX, TD+NPX, TD+NAC, TD+NPX+NAC, or normal saline (NS) that served as a control. Half of the animals were burned one week and the other half in a subsequent week. The experimental agents were given approximately 60 min after the injury and then once daily by oral gavage till the end of the study. The burns were left uncovered. Wounds were observed and imaged daily for evidence of necrosis in the unburned ischemic areas. At day seven, the wounds were imaged and the animals were euthanized with lethal CO_2 inhalation. Immediately after euthanasia, skin pelts including the entire burns were removed from the animals and fixed in formalin for 24 h. The specimens were than alcohol-dehydrated, xylene-cleared, paraffin-embedded, and sliced with a microtome into 5 micrometer sections. The sections were stained with Hematoxylin and Eosin and viewed under a standard microscope by a board-certified dermatopathologist masked to treatment assignment.

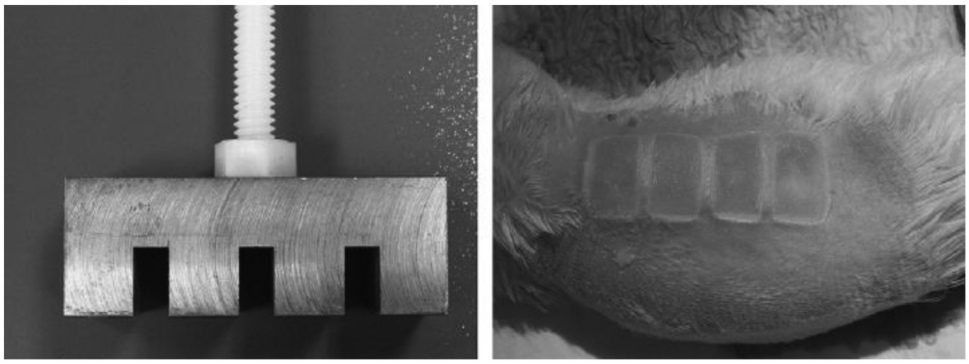

Fig. 1 – Brass comb (left) used to create comb burns (right).

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