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Progressive tissue injury in burns is reduced by rNAPc2☆

Ajay L. Mahajan [a,1,2], Xavier Tenorio [a,2], Michael Sean Pepper [b,c], Danielle Baetens [b], Denys Montandon [a], Kai-Uwe Schlaudraff [a], Brigitte Pittet [a,*]

[a] *Department of Plastic and Reconstructive Surgery, University Hospitals of Geneva, Geneva, Switzerland*
[b] *Department of Morphology, University of Geneva Medical Centre, Geneva, Switzerland*
[c] *NetCare Molecular Medicine Institute, Unitas Hospital, Lyttelton and Department of Immunology, Faculty of Health Sciences, University of Pretoria, South Africa*

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Abstract

Introduction: Burn wounds are characterised by central necrosis surrounded by an area of stasis with compromised perfusion. Secondary aggravation of the burn wound due to ischaemia in the zone of stasis can also result in necrosis. This study aims to improve circulation in the zone of stasis by reducing microthrombus formation and thereby to reduce secondary aggravation.
Material and methods: Recombinant nematode anticoagulant protein (rNAPc2) was administered to Wistar rats at 3 or 30 μg/kg as a single or daily dose. A comb pattern burn was induced on the dorsum of these rats and its evolution monitored by serial photography, planimetry, laser doppler flowmetry and immunohistochemistry.
Results: In the 30 μg/kg daily group, extension of the burn wound was curbed, limiting the burn area to 1.99 ± 0.67 cm^2 on day 28, compared to 3.51 ± 0.37 cm^2 in the control group ($p = 0.015$). Laser doppler evaluation showed a significant ($p < 0.001$) increase in circulation in the first day post-burn. Significantly less ($p < 0.001$) microvascular fibrin formation was observed by immunohistochemistry.
Conclusion: Anticoagulation with rNAPc2 improved perfusion of the burn wound. The resultant reduction in the area of the burn led to earlier healing and less scar contracture.

Keywords: Burn wound; Anticoagulation; rNAPc2; Scar contracture

1. Introduction

Severe burns injuries can lead to debilitating chronic sequelae, of which scarring and contracture are a major concern. The severity of the scar is proportional to the severity of the burn. The deeper the tissue injury caused by the burn, the worse the contracture [1]. The size and depth of the burn wound generally increases after the injury. This phenomenon is known as secondary aggravation or conversion. Hence, the treatment of burn wounds would benefit from preventing extension of the wound, thereby decreasing sequelae.

Jackson has described the burn wound as composed of a central zone of necrosis, surrounded by a zone of stasis, that in turn is surrounded by a zone of hyperaemia [2]. The tissue in the central zone is damaged beyond recovery and the tissue in the outer zone will recover fully. The area of interest is the zone of stasis where blood flow in the capillaries slows down and can eventually stop. Although Jackson initially stated that this zone of stasis progresses to necrosis, he subsequently realised that some circulation is present in this zone [3] and that preventing dehydration by early covering of the burns with grafts [4] leads to healing of the tissue in this zone. Various co-existing factors contribute to the

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* Corresponding author. Tel.: +41 022 3727997.
E-mail addresses: almahajan@yahoo.com (A.L. Mahajan), Brigitte.Pittet-Cuenod@hcuge.ch (B. Pittet).
[1] Tel.: +41 772 5355903.
[2] These authors have contributed equally to the paper.

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progression of the burn wound [5], but tissue perfusion appears to be the deciding factor [6]. Ehrlich et al. [7] have shown that perfusion is decreased from 2 h after-burn. This decrease in circulation is dynamic [8] and gets progressively worse over the ensuing 24 h [9].

Tissue factor is released following a burn, directly by damage of the endothelium and indirectly by the associated inflammatory reaction [10,11]. This triggers the coagulation pathway leading to the formation of fibrin. It has been shown by others that on the one hand, fibrinogenesis increases after-burn [12], while on the other, fibrinolysis is suppressed [13,14]. It has also been shown that tissue factor is increased in ischaemic conditions [15]. Increased levels of fibrin result in the formation of thrombi that leads to vascular occlusion [16,17]. The ensuing ischaemia in the zone of stasis results in necrosis, and the zone coalesces with the central zone of coagulation.

To inhibit microthrombus formation, we selected the recombinant nematode anti-coagulant protein (rNAPc2). This anticoagulant protein was identified in the hookworm *Ancylostoma caninum* [18,19]. It targets a specific site in the primary stages of the coagulation pathway: it binds to factor X (FX) or activated factor X (FXa) prior to the formation of an inhibitory complex with activated factor VII/tissue factor (FVIIa/TF) [20–22]. This inhibits coagulation and decreases the formation of fibrin. Anticoagulation in the treatment of burns has also been carried out by administration of heparin [23]. However, bleeding and thrombocytopenia are well-recognized complications [24]. Unlike heparin and vitamin K antagonists like warfarin, rNAPc2 acts upstream in the coagulation cascade. It also has minimal drug interactions compared to warfarin [24]. As the mechanism of action is different, close laboratory monitoring is not required. As rNAPc2 has a prolonged half-life of over 50 h [25] it can be conveniently used every other day instead of as a daily dose. This could be a disadvantage in the event of a bleeding episode. However, anticoagulation with rNAPc2 can be reversed with recombinant factor VIIA (rVIIA) [26] and rVIIA has been used in burns to promote a procoagulant state [27]. rNAPc2 has been used clinically to decrease the incidence of venous thromboembolism in patients undergoing total knee replacement surgery [28] and to prevent arterial thrombus generation during coronary angioplasty [29]. By administering rNAPc2, this study aims to assess: (1) whether microthrombus formation is reduced; (2) circulation in the zone of stasis is thereby improved; (3) whether secondary extension of the burn wound can be limited by this.

2. Materials and methods

All experiments were carried out in accordance with Swiss guidelines for animal experimentation and approved by the Geneva cantonal veterinary authority.

2.1. Experimental animals

Male Wistar rats weighing between 250 and 300 g were used for this study. They were observed to be disease free for 1 week prior to experiments, fed on rat chow and had access to water ad libitum. They were housed in special storage rooms.

2.2. Study drug and dose

We assigned the experimental animals randomly to five groups ($n = 5–8$ animals in each group). Group I comprised control animals to whom phosphate buffer saline (PBS) was administered. Animals in groups II and IV were administered rNAPc2 (Corvas International Inc., San Diego, CA, USA) at a dose of 3 μg/kg and those in groups III and V received the drug at a dose of 30 μg/kg. The drug was injected into the femoral vein 30 min prior to creation of the burn. rNAPc2 was diluted in PBS. Following the initial injection, animals in groups II and III continued to receive a daily subcutaneous dose of 3 and 30 μg/kg, respectively, for 1 week.

2.3. Creation of the burn

Animals were anaesthetised with inhalation anaesthesia using 2% isoflurane. Analgesia in the form of subcutaneous morphine was given at a dose of 30 μg/100 g, 8 h. The animals were prepared 12 h prior to the experiment by clipping the hair on the dorsum, followed by depilation using a depilatory cream. The comb burn model [30] was used to create the burn wound. This was achieved by immersing a brass template in boiling water for 15 min. The template was then placed 1 cm lateral to the midline on the dorsum of the animal for 30 s, first on the one side, then on the other. Each burn wound (Fig. 1A) comprised four areas of 1 cm × 2 cm separated by three intervening burn free spaces of 0.5 cm × 2 cm (total area of burn 8.00 cm^2). The intervening spaces represent the zone of stasis and were observed for their progression to necrosis.

2.4. Observations

Daily observations for the first week were followed by weekly observation for 4 weeks. We observed progression of the burn area and recorded it by digital photography. The individual burn wounds were traced onto transparent acetate sheets, and the areas measured with a polar planimeter. The four areas of burns on each side were summed up and considered as one area of burn (Fig. 1A). This was done as the four adjacent areas of burns in the control group coalesced into a single area with time. In a second part of the study, in one of the study groups (30 μg/kg daily dose group) fixed points were marked at a distance of 0.5 cm around the burn and the area inside these points was monitored by planimetry. Contracture of this area was