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Prednisolone but not selenium and rtPA reduces edema and improves angiogenesis after burn in mice

O. Goertz [a,b,*], H. Over [a], L. von der Lohe [a,b], H. Lauer [a], A. Ring [a], A. Daigeler [a], M. Lehnhardt [a], J. Kolbenschlag [a]

[a] Department of Plastic and Hand Surgery, Burn Center, BG-University Hospital Bergmannsheil, Ruhr University Bochum, Germany

[b] Department of Plastic, Reconstructive and Hand Surgery, Martin-Luther-Hospital, Berlin, Germany

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ABSTRACT

Objective: Despite dramatic improvements in burn care, the major part of the therapy of thermal injuries remains symptomatical in nature. A targeted approach to accelerate angiogenesis and woundhealing and reduce edema formation remains to be found. We therefore aimed to investigate the impact of anti-inflammatory, anti-coagulative and thrombolytic agents on microcirculation after thermal injuries on the mentioned parameters.

Methods: Full thickness burns were inflicted on the ears of hairless mice ($n = 48$). The effects of five intraperitoneal injections of either recombinant tissue plasminogen activator (rtPA), selenium, prednisolone or sodium chloride on microcirculation, edema formation, leukocytes and angiogenesis were investigated over a 13 day period using intravital fluorescent microscopy.

Results: Prednisolone slightly improved angiogenesis (100.0% day 0 vs. 91.4% non-perfused area on day 1 post burn, $p < 0.05$) and reduced edema formation (93.3% vs. 123.1% control on day 3, $p < 0.05$). The rtPA-group showed the highest number of sticking leukocytes up to day 7 post burn (233%, 265%, 254% on days 1, 3, and 7, $p < 0.05$ compared to baseline). A post-traumatic expansion of the non perfused area could only be observed in the selenium group (100.0% day 0, 103.1% day 1 post burn). In addition, selenium caused an increase of rolling leukocytes over the complete observation time.

Conclusion: The often described positive influences of selenium for the treatment of burn patients could not be confirmed, on the contrary we found a post-traumatic expansion of the non perfused area and an increase of leukocytes in this group. The expectations to rtPA did not fulfill. Prednisolone improved angiogenesis and reduced the edema formation, both Parameters are essential for wound healing and survival of burned patients.

* *Corresponding author at*: Department of Plastic and Hand Surgery, Burn Center, BG University Hospital Bergmannsheil, Ruhr-University Bochum, Buerkle de-la-Camp Platz 1, 44789 Bochum, Germany. Tel.: +49 234 302 3814; fax: +49 234 302 6379.
E-mail address: ole.goertz@rub.de (O. Goertz).

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1. Introduction

The care for severly burned patients has dramatically improved over the last decades, leading to higher survival rates and quality of life. However, these advancements are mainly based on the general progress in intensive care and symptomatic care. Specific treatments to decrease the systemic impact of thermal injuries or to improve re-epitheliaziation of the burned skin have become the focus of ongoing research, but are rarely clinically implemented [1]. Based on our previously published findings in the same model, both prostaglandine (PGE1) and acetylsalicyclic acid (ASA) showed a positive effect on woundhealing [2]. This can most likely be attributed to the anti-inflamatory and platelet-inhibiting properties of ASA and the vasoactive aspects of PGE1. Based on these findings, a further insight into the impact of anti-inflammatory and anti-coagulatory drugs seems desireable. We therefore chose to apply rtPA as stimulus of fibrinolysis as well selenium and prednisolone for their anti-inflammatory modes of action in the same model to research their respective effects.

rtPA, selenium and prednisolone were shown to improve angiogenesis and overall wound healing and could therefore potentially aid in burn recovery. Tissue plasminogen activator (tPA), a serine protease, is physiologically located on the endothelial cells and catalyzes the conversion of plasminogen to plasmin [3]. The recombinant tissue plasminogen activator (rtPA) used in this trial is manufactured using biotechnology. Since it is known, that blood clotting especially in the zone of stasis can cause burn depth conversion and therefore extension of the irreversible damaged burn area, a protection from further clotting and a dissolution of already existing blood clots is desirable [4].

The antioxidant and anti-inflammatory trace element selenium showed promising results in critically ill patients and especially critically ill burns patients. Although there are heterogeneous results in the available literature concerning the reduction of pneumonia and mortality [5–7]. Experimental studies for wound healing in rats after burns could underline the positive findings of selenium [8–10].

The use of synthetic glucocorticoids like prednisolone in burn patients is still controversial. However, its anti-inflammatory effects, especially on the endothelium, might help to reduce leukocyte activation but also to attenuate the loss of capillary integrity and therefore edema formation.

This integrity loss and the subsequent edema formation greatly contribute to the extension of thermal injuries over time and burn sickness, directly influencing the prognosis of burn patients [4,11–13]. The mentioned parameters are all crucial for wound healing, which in turn is paramount for the survival of burn patients.

To assess the impact of these drugs on microcirculation, angiogenesis and cell interaction, a well established model was used. Due to its highly standardized no-touch nature this model allows for reliable observations of the before mentioned parameters via intra-vital fluorescent microscopy [2,14,15].

2. Materials and methods

2.1. Animals

48 male hairless mice ($n = 12$ in each group; SKH-1/h, bodyweight 24 g (24.2 ± 2.2 g)) were used (Charles River, Sulzfeld, Germany). Standard laboratory food and tap water was available ad libitum. Each procedure was approved by the regional authorities according to German animal care regulations, which comply with the international guidelines of animal care and use in scientific experiments (AZ: 8.87-50.10.32.08.322).

2.2. Anesthetizing/preparation

Mice were anesthetized by spontaneous inhalation of isoflurane-N_2O (F_iO_2 0.35, 0.015 l/l isoflurane, Forene®, Abbott GmbH, Wiesbaden, Germany). Extension of the ear was achieved by two microsurgical loops (8/0 Surgipro®, Covidien, Neustadt (Donau), Germany) which were pulled through the ear in addition to adhesional flattening utilizing physiological saline solution.

2.3. Applications and drugs

Fluorochromes were administered via the tail-veins (tube 29G, Braun, Melsungen, Germany). 25 μl FITC labeled dextran (1.0%, MW 150 kDa) served as plasmamarker, and 25 μl rhodamine 6G (0.1%) for staining leukocytes (Sigma–Aldrich Chemicals Co., St. Louis, MO, USA). The drugs were injected intraperitoneally 30 min post burn, on day 1, 3, and 5 after intravital fluorescent microscopy and on days 2 and 4 in a short anesthesia. All drugs were dissolved in sodium chloride in a volume of 250 μl. The four groups include thrombolytic acting rtPA (volume: 250 μl, concentration: 1 mg/ml, dose: 250 μg; 10 mg/kg; $n = 12$; Actilyse®, Boehringer Ingelheim, Ingelheim am Rhein, Germany), selenium (volume: 250 μl; concentration: day 0: 6 μg/ml, days 1–5: 3 μl/ml; dose: 1.5 resp. 0.75 μg; 60 μg resp. 30 μg/kg; $n = 12$; Selenase®, Biosyn, Fellbach, Germany), prednisolone (volume: 250 μl; concentration: 1.5 mg/ml; dose: 375 μg; 15 mg/kg; $n = 12$; Solu-Decortin®, Merck, Darmstadt, Germany) and sodium chloride as control (250 μl, 0.9% sodium chloride, $n = 12$; isotone Natriumchloridlösung, B. Braun Melsungen AG, Melsungen, Germany).

2.4. Burn model

The exact model including histological evaluations was previously described [2,14]. A full thickness burn (3rd degree) was induced on the dorsal side of the air via a hot air stream (117 ± 2.1 °C) applied for 1 second in a no-touch manner Fig. 1.

2.5. Recordings

The microcirculatory parameters of the regions of interest (ROI, six per mouse) were assessed 1.5 monitor widths beside the non perfused area. For the intravital fluorescence microscopy (Axiotech vario, Carl Zeiss, Oberkochen, Germany) a fourfold objective and a 20-fold water immersion objective