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Early intervention by Captopril does not improve wound healing of partial thickness burn wounds in a rat model

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ABSTRACT

The Renin Angiotensin System is involved in fibrotic pathologies in various organs such as heart, kidney and liver. Inhibition of this system by angiotensin converting enzyme antagonists, such as Captopril, has been shown beneficial effects on these pathologies. Captopril reduced the inflammatory reaction but also directly influenced the fibrotic process. Prolonged and excessive inflammatory response is a major cause of hypertrophic scar formation in burns. We therefore evaluated the effect of Captopril on the healing of partial thickness burn wounds in a rat model.

Partial thickness contact burns were inflicted on the dorsum of the rats. The rats received either systemic or local treatment with Captopril. The inflammatory reaction and wound healing (scar) parameters were investigated and compared to control animals.

In this study we could not detect positive effects of either administration route with Captopril on the inflammatory reaction, nor on wound healing parameters. The local treatment showed reduced wound closure in comparison to the systemic treatment and the control group.

Early Captopril treatment of burn wounds did not show the beneficial effects that were reported for fibrotic disorders in other tissues. To influence the fibrotic response Captopril treatment at a later time point, e.g. during the remodeling phase, might still have beneficial effects.

1. Introduction

Excessive scar formation after partial and full-thickness burn wounds is still an unresolved problem and prevention remains a major challenge. Tightly regulated processes which are necessary for successful regeneration of the skin have become uncontrolled in burn wounds, resulting in fibrosis. The onset of fibrosis in various tissue types is diverse, although common features are also present. One of the common factors, and the

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main effector of fibrosis, is transforming growth factor beta one (TGF-ß1) [1]. This growth factor is involved in the transition of fibroblasts into myofibroblasts. These myofibroblasts are major contributors to the production of excessive extracellular matrix proteins such as collagens, as well as to the process of wound contraction. Ultimately this process results in red, stiff, contracted and disfiguring scars. To date, the underlying mechanisms leading to scar formation are still a puzzle that needs to be solved and an effective therapy to prevent excessive scarring has not been discovered yet.

A possible other player in the fibrotic process is the Renin Angiotensin System (RAS). The main function of this system is the regulation of blood pressure and electrolyte balance. Several studies have shown the activation of a local RAS (local or tissue RAS: tRAS) upon tissue damage in various tissues [2]. It was shown that tRAS is involved in fibrotic processes of many organs including heart, kidney and liver [2,3]. In addition tRAS has been linked to scar formation in the skin [4–6]. The main bioactive component of RAS is angiotensin II (AngII) which is derived from cleavage of angiotensin I by angiotensin converting enzyme (ACE). Subcutaneous administration of AngII in C57BL/6 mice induced skin fibrosis characterised by an increased deposition of collagen, α smooth muscle actin (αSMA) expression and number of inflammatory cells [7]. In addition increased ACE activity was shown in human scars compared to control and wounded skin [4].

AngII exerts its effects by binding to one of the two main RAS receptors; angiotensin receptor I (AT1) and angiotensin receptor II (AT2). AT1 activation is linked to fibrosis through the induction of the production of various cytokines (e.g. TGF-ß1) and extracellular matrix proteins, and by inducing cell proliferation and inflammation [2,3]. In mice, scars showed an increased expression of AT1 compared to unwounded skin. More contraction of the granulation tissue and enhanced fibroblast migration was observed through AT1 activation [5]. In humans, two days after wounding, both AT1 and AT2 expression are upregulated, while at later time points AT2 expression in skin biopsies was more pronounced compared to AT1 [8]. In contrast to the AT1, the exact biological functions of AT2 are not completely clear yet. It has been suggested that AT2 can counteract the effects of AT1 and thereby has an anti-fibrotic function. However, literature is equivocal about the exact role of AT2 in fibrosis.

Studies on fibrotic processes in other tissues than skin have demonstrated beneficial effects of RAS inhibition. These inhibitors were shown to elicit an anti-fibrotic as well as an anti-inflammatory response to ameliorate fibrosis. One of the methods to inhibit the RAS is inhibiting the formation of AngII by ACE-inhibitors such as Captopril or Enalapril. Systemic treatment with Enalapril was shown to reduce pro-fibrotic collagen III expression and reduce hypertrophic scar formation in the rabbit ear wound model [6]. The effect of ACE-inhibition on burn wound healing and scar formation is largely unknown. One case-study describes the topical treatment of a patient with a 5-month old hypertrophic scald burn scar. Captopril improved the scar by reducing hypertrophy and redness. However, an appropriate placebo-treated scar was missing in this study [9]. Inhibition of AngII production using ACE-inhibitors early during wound healing might be effective to reduce scar formation in burn wounds. In this paper we describe the effects of systemic and topical Captopril administration early during the wound healing process on partial thickness contact burns in a rat model.

2. Materials and methods

2.1. Animals

The experimental protocol of this study was approved by the institutional Animal Experiments Committee of the VU University Medical Center Amsterdam, The Netherlands in accordance with the governmental and international guidelines on animal experimentation. Animals had access to water and food ad libitum. Before the start of the experiment, seventy-eight male Wistar rats of 6–7 weeks old, weighing 280 g ±11 g, received an acclimatization period of 12 days of which the last three days included handling of the animals. During the acclimatization period animals were housed in groups of 4–5.

After the infliction of the burn wound the animals were individually housed in order to prevent detachment of the bandage by other animals and disturbance of the wound healing process. Two animals died several hours after the bandage procedure at PBD 2 (topical Captopril treatment group) and PBD 8 (systemic Captopril treatment group) due to unknown reasons.

2.2. Application of the burn wound

Approximately 30 min before the infliction of the burn wound, animals received the analgesic Temgesic 0.05 mg/kg via subcutaneous injection. Anesthesia was induced by inhalation of 4% isoflurane combined with O_2 with a flow rate of 0.3 L/min, the maintenance dose was 2.5% isoflurane. During surgery the animals were placed on a warming pad to prevent hypothermia. The dorsum and abdomen of the animals were shaved from neck till the hind legs with electric clippers. Additionally the dorsum (the area of the burn wound) was shaved wet with a razor blade to remove possible remaining stubbles.

A partial thickness contact burn wound of 2 × 2 cm was inflicted on the dorsum, just behind the forelegs of the animal, with a brass stamp (100 g) of 100 °C for 10 s without applying pressure.

To prevent contamination of the wound and further damage from scratching, the burn wounds were covered with sterile gauze and fixed with adhesive Curafix® (Lohmann & Rauscher GmbH & Co., Neuwied, Germany). Subsequently the Curafix® bandage was covered with an elastic self-adhesive PetFlex® (Andover Healthcare, Inc.; Almelo, The Netherlands) bandage forming a jacket which was fixed with silk tape.

Bandage changes were performed at PBD 1, 4, 7, 11 and 15 under inhalational anesthesia which was induced with 4% isoflurane and maintained with 2.5% isoflurane. On these days the burn wound was covered with Multisorb® (3 × 3 cm) (BSN medical Limited, Willerby, United Kingdom) which was subsequently covered and fixed with adhesive Curafix®. The rest of the bandage procedure was similar to the procedure after burn wound infliction (PBD 0).

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