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The presence of tissue renin-angiotensin system components in human burn wounds and scars

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ABSTRACT

Objective: Healing of severe and large surface burn wounds is faced with hurdles such as aberrant wound healing and excessive scar formation. The tissue renin-angiotensin system (tRAS) is involved in dermal wound healing, and fibrosis of other organs. However, little is known about the presence of tRAS during burn wound healing in human skin. This study investigated the presence of tRAS components in human burn wounds and scars.

Methods: Dermal tissue biopsies were collected from 39 patients and divided into six categories: burn wounds post burn day (PBD)0–9, PBD11–21 and PBD22–37; young scars (1.5–3.5 months), mature scars (>12 months) and control skin from 9 patients. The tRAS components angiotensin converting enzyme (ACE), chymase, angiotensin receptor 1 (AT1) and Mas receptor were detected via immunohistochemistry. Digital images were acquired and analyzed using image analysis software.

Results: Burn wounds from PBD22–37 showed a decreased expression of ACE and chymase compared to earlier time points or control, respectively. In contrast, ACE expression was increased in young scars compared to control skin but was normalized in mature scars. In comparison to control, mature scars showed increased AT1 expression.

Conclusions: These results show the presence of components of tRAS in human burn wounds and scars. In addition, they suggest that tRAS has a time-dependent response during burn wound healing. Reduced tRAS might play a role in delayed healing, while an increase during remodeling phase might contribute to scar formation. This research provides a basis for future studies exploring tRAS involvement in burn wounds and scars.

1. Introduction

Generally, uncompromised human dermal wounds heal with minimal scarring. However, disturbance of the tightly orchestrated wound healing processes can result in severe scarring. This type of scarring occurs more often in deep partial-thickness and full-thickness burn wounds compared to superficial cuts or abrasions [1,2]. These scars not only lack the functionality of the original tissue including limited joint mobility but also cause disfiguration. The quality of life of patients is negatively affected by these problems. To alleviate these problems, they often require multiple reconstructive surgeries [3,4].

Scarring is a form of fibrosis; however, the onset of fibrosis and the underlying exact mechanisms that lead to excessive or hypertrophic scarring in burn wounds are still unknown. The local tissue renin angiotensin system (tRAS) is one of the proposed mechanisms to play a role during wound healing and fibrosis. It is generally accepted that tRAS is involved in fibrosis of other organs (e.g. heart, kidneys, liver, lungs).

Tissue RAS is an autocrine/paracrine system which is localized in most organs and tissues including heart, lungs, kidneys, liver and skin [5]. A review by Paul et al. [6] describes the different functions of tRAS in several tissues. Generally, tRAS regulates tissue homeostasis which involves proliferation and tissue metabolism [6], whereas the main function of the systemic RAS is regulation of the cardiovascular system and body fluid homeostasis. In the skin, tRAS activation is initiated upon tissue damage and it is proposed that activation of tRAS occurs during the inflammatory and granulation phases of wound healing [7].

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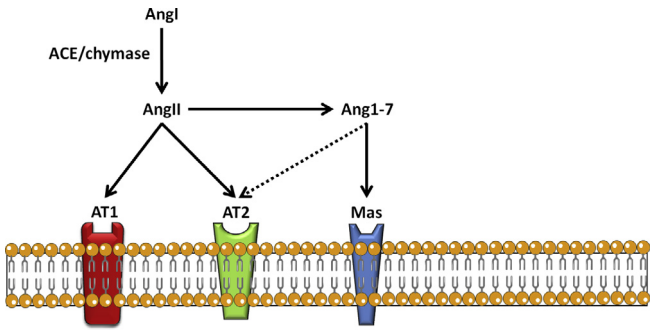

Fig. 1. tRAS system overview. Simplified scheme of the main tRAS components. Angiotensin I (AngI) is converted to Angiotensin II (AngII) by the enzymes angiotensin converting enzyme (ACE) and chymase. Angiotensin1–7 (Ang1–7) is a smaller derivative of AngII). The ligands AngII and Ang1–7 bind to the different angiotensin receptors: angiotensin receptor 1 (AT1), angiotensin receptor 2 (AT2) and Mas receptor.

Similar to the systemic RAS, tRAS is composed of several components (Fig. 1). The main effector molecule of tRAS is the peptide angiotensin II (AngII) which can bind to either Angiotensin receptor 1 (AT1) or Angiotensin receptor 2 (AT2). Most known effects of RAS activation, including fibrosis, are thought to be mediated by the AT1 receptor.

AngII can be generated locally in tissues independently from the systemic RAS, and can exert autocrine and paracrine actions. AngII is spliced from its precursor Angiotensin I (AngI) by angiotensin converting enzyme (ACE), but also by chymase [8,9]. ACE was found to be expressed in human skin [10,11], and was found to be upregulated after wounding [10–12]. Morihara et al. [10] and Steckelings et al. [13] hypothesize that ACE plays a role in pathological wound healing; however, the presence of ACE in burn wounds and scars has not been studied yet.

The other AngI-converting enzyme chymase is released from granules within mast cells. During wound healing, these mast cells play a role in the activation of the early inflammatory response by releasing cytokines and growth factors which stimulate migration and proliferation of skin cells [14]. In mice a decreased mast cell number and chymase-like activity were observed in partial thickness burns at PBD 3 [15]. Not only do mast cells play a role in the inflammatory phase and re-epithelialization of wounds but increased mast cell activity and numbers have also been linked to hypertrophic scarring [16,17].

After generation of AngII by ACE or chymase, AngII exerts its effects through binding to the AT1 or AT2 receptors. In relation to wound healing, it has been reported that AT1 as well as AT2 are upregulated upon skin injury [13,18]. In dermal wound healing AT1 was shown to be involved in re-epithelialization [19]. More recently another biologically active peptide was identified in the RAS; Ang1–7. This peptide is a smaller derivative of AngII and acts via the Mas receptor. The Mas receptor is believed to counteract the actions of the AT1 receptor by binding of Ang1–7. It was shown that the Mas receptor was reduced shortly after the injury (<PBD 5) in partial thickness burn wounds in mice [20,21]. Although several studies have demonstrated that tRAS plays a role in dermal wound healing as well as in burns, information about the presence and course of tRAS during the several wound healing phases in burns of human tissue is still scarce. For that reason, we determined the presence of several tRAS components in tissue from burn wound patients and scars at different time points in comparison to healthy human skin tissue.

2. Materials & methods

2.1. Patient materials

Normal uninjured skin biopsies were derived from patients who underwent abdominoplastic surgery at the Red Cross Hospital, Beverwijk, the Netherlands. Eschar tissue was obtained from burn wound patients undergoing escharotomy in the burn centre of the Red Cross Hospital Beverwijk, Netherlands. Eschar tissue was removed between 0 and 37 days post burn injury (PBD). The samples were derived from patients with large burned body surface areas and were predominantly full thickness burns. Because in some cases the depth of the burn wound was heterogeneous and/or could not be determined accurately at early time points after burn, the surgical treatment was postponed to allow healing of the partial thickness wound areas under conservative treatment. In addition extensive burns are not excised in total within a few days but operated during multiple sessions over a longer period

Table 1
Groups and patient data.

Groups	N	Gender	Age (years) (mean ± SD)	Age (range in years)	TBSA of burns (%) (mean ± SD)	TBSA third-degree burn (%) (mean ± SD)	
Control	9	F:7 M:2	47 ± 9	34–61	N/A	N/A	N/A
Burn wound PBD 0–9	10	F:3 M:7	35 ± 22	5–71	Mean: 18.1 ± 13.7 Range: 1.2–37.5	Mean: 8.7 ± 7.4 Range: 0–18.5	Contact: n = 1 Flame: n = 7 Scald: n = 1 Unknown: n = 1
Burn wound PBD 11–21	10	F:4 M:6	47 ± 18	11–68	Mean: 30.2 ± 29.0 Range: 3.0–81.0	Mean: 19.0 ± 24.9 Range: 0–77.0	Flame: n = 7 Scald: n = 2 Unknown: n = 1
Burn wound PBD 22–37	7	F:3 M:4	52 ± 23	24–76	Mean: 22.1 ± 17. Range: 0.5–51.0	Mean: 14.3 ± 13.6 Range: 0.5–36.0	Contact: n = 1 Flame: n = 5 Scald: n = 1
Young scar: 1.5–3.5 months	5	F:0 M:5	51 ± 22	16–68	Mean: 28.0 ± 30.2 Range: 3.0–72.0	Mean: 10.8 ± 17.6 Range: 0–37.0	Flame: n = 3 Scald: n = 2
Mature scar: >12 months[a]	7	F:2 M:4	31 ± 26	4–59	N/A[b]	N/A[b]	Flame: n = 2 Scald: n = 1 Unknown: n = 4
Total[a]	48	F:19 M:28	43 ± 20	4–76	Mean: 23.1 ± 21.8 Range: 0.5–81.0	Mean: 12.8 ± 16.7 Range: 0–77.0	N/A

N = number of patients; N/A = not applicable; PBD = post burn day; F = female; M = male; SD = standard deviation; TBSA = total body surface area.
[a] Information gender and age is unknown for one patient.
[b] Information of total TBSA and/or third-degree TBSA is unknown for five patients.

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