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Propranolol impairs the closure of pressure ulcers in mice

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ABSTRACT

Aims: β-Adrenoceptors modulate acute wound healing; however, few studies have shown the effects of β-adrenoceptor blockade on chronic wounds. Therefore, this study investigated the effect of β_1-/β_2-adrenoceptor blockade in wound healing of pressure ulcers.
Main methods: Male mice were daily treated with propranolol (β_1-/β_2-adrenoceptor antagonist) until euthanasia. One day after the beginning of treatment, two cycles of ischemia–reperfusion by external application of two magnetic plates were performed in skin to induce pressure ulcer formation.
Key findings: Propranolol administration reduced keratinocyte migration, transforming growth factor-β protein expression, re-epithelialization, and necrotic tissue loss. Neutrophil number and neutrophil elastase protein expression were increased in propranolol-treated group when compared with control group. Propranolol administration delayed macrophage mobilization and metalloproteinase-12 protein expression and reduced monocyte chemoattractant protein-1 protein expression. Myofibroblastic differentiation, angiogenesis, and wound closure were delayed in the propranolol-treated animals. Propranolol administration increased neo-epidermis thickness, reduced collagen deposition, and enhanced tenascin-C expression resulting in the formation of an immature and disorganized collagenous scar.
Significance: β_1-/β_2-Adrenoceptor blockade delays wound healing of ischemia–reperfusion skin injury through the impairment of the re-epithelialization and necrotic tissue loss which compromise wound inflammation, dermal reconstruction, and scar formation.

Introduction

A pressure ulcer is defined as an area of necrosis in the integument developed as a result of compression of soft tissues between an osseous prominence and a hard surface during a sufficiently long period of time to induce local ischemia (Cullum et al., 2006). These ulcers have become a great clinical problem due to an increase in the disorder rates that impair sensation or mobility, especially in senior citizens (Saito et al., 2008). The prevalence of pressure ulcer is estimated at 3% to 10% of all hospitalized patients and from 20% to 32% of all elderly hospitalized patients (Kosiak, 1959). In Brazil, the incidence of pressure ulcer is 40% in surgical units, but higher in intensive care units (Rogenski and Santos, 2005). According to the National Pressure Ulcer Advisor Panel, annual costs directly related to pressure ulcer treatment have been estimated from 3.5 to 7.0 billion of dollars and the index of mobility and mortality is greater in patients who develop these types of lesions (Kosiak, 1959). Although the etiology of pressure ulcers is multifactorial and remains unclear, ischemia–reperfusion (IR)-induced injury can be a factor in the formation of these chronic lesions (Salcido et al., 1995; Peirce et al., 2000; Stadler et al., 2004). In animal models, repeated cycles of ischemia and reperfusion in skin develop tissue necrosis due to leukocyte migration, pro-inflammatory cytokine production, protease secretion, and high reactive oxygen species (ROS) levels (Salcido et al., 1995; Peirce et al., 2000; Stadler et al., 2004; Mustoe, 2004; Tsuji et al., 2005). However, the precise clinical or histopathological course and the mechanism of pressure ulcer formation in an animal model remain unknown.

β-Adrenoceptors, which are subdivided into β_1-, β_2-, and β_3-, are G-protein-coupled receptors highly expressed on cell types that participate in wound healing as fibroblasts, keratinocytes, leukocytes, and endothelial cells (Wallukat, 2002; Duell, 1980; McSwigan et al., 1981; Leicht et al., 2000). Various studies have demonstrated that activation

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or blockade of these receptors modulates keratinocyte and fibroblast function during tissue repair (Pullar et al., 2003; Pullar et al., 2006a; Pullar et al., 2006b; Pullar and Isseroff, 2006; Pullar et al., 2012). β_1-Adrenoceptor blockade with metipranolol may increase corneal epithelial wound healing in rabbit (Reidy et al., 1994). However, in cutaneous excisional lesions, β_1-adrenoceptor blockade with atenolol increases inflammatory cell number, impairs wound contraction, myofibroblast differentiation and angiogenesis, and reduces collagen deposition in rats (Romana-Souza et al., 2009a). β_2-Adrenoceptor activation decreases keratinocyte migration and re-epithelialization, whereas its antagonism has an opposite effect (Pullar et al., 2003; Pullar et al., 2006a; Pullar et al., 2006b). In addition, β_2-adrenoceptor antagonism or activation enhances fibroblast proliferation, endothelial cell migration and α-smooth muscle actin (SMA) expression (Pullar and Isseroff, 2006; Pullar et al., 2012). In rodent models, β_1-/β_2-adrenoceptor blockade prolongs inflammatory response and compromises wound closure, granulation tissue formation, and re-epithelialization in rat acute lesions (Romana-Souza et al., 2009a; Souza et al., 2006; Romana-Souza et al., 2009b). However, β_1-/β_2-adrenoceptor antagonism improves cutaneous wound healing of diabetic rats, chronically stressed mice and burn-injured rats (Romana-Souza et al., 2008; Romana-Souza et al., 2009c; Romana-Souza et al., 2010a). β_1-/β_2-/β_3-Adrenoceptor activation with isoproterenol enhances embryonic dermal fibroblast proliferation, but decreases dermal fibroblast-mediated contraction of collagen gel and keratinocyte migration (Pullar et al., 2003; Anesini and Borda, 2002; Pullar and Isseroff, 2005). However, neither study evaluated the effect of β_1-/β_2-/β_3-adrenoceptor blockade on cutaneous wound healing. Nevertheless, neither study demonstrated how β-adrenoceptors participate in cutaneous wound healing of chronic lesions, as pressure ulcers, in the animal model.

Therefore, this study investigated the effect of β_1-/β_2-adrenoceptor blockade on wound healing of pressure ulcers using a murine model of IR-induced skin injury.

Material and methods

Animals

Male Swiss mice (8–12 weeks) had free access to food and water and were maintained in a room with controlled humidity (50%) and temperature (22 °C) on a 12-h light/dark cycle and air exhaustion cycle (15 min/h). All experimental animal work was carried out in accordance with the Brazilian Legislation (no. 11.794, from October 8th, 2008) and approved by the Ethical Committee for Animal Use of State University of Rio de Janeiro (CEUA/049/2012).

IR-induced injury model and propranolol treatment

Mice (n = 60) were daily treated by gavage with 25 mg/kg of propranolol hydrochloride (a non-selective β_1-/β_2-adrenoceptor antagonist) (Sigma-Aldrich, St. Louis, MO, USA) dissolved in water, until euthanasia (Romana-Souza et al., 2010a). Another group (n = 60) was treated by gavage with vehicle. The treatment did not induce deaths in animals during the period observed. One day after the beginning of propranolol administration, all animals were intraperitoneally anesthetized with ketamine (150 mg/kg) and xylazine (15 mg/kg) and their dorsa were shaved and cleaned. Dorsal skin was gently pulled up and placed between a pair of magnet disks that had an 8-mm diameter and 4-mm thickness, with an average weight of 1.47 g and 300 G magnetic force (Eudes Angelo de Almeida Produtos ME, São Paulo, Brazil). Epidermis, dermis, and hypodermis were pinched between the magnet plates. This process created a compressive pressure of above 50 mm Hg between the magnets (Peirce et al., 2000; Stadler et al., 2004). Two IR cycles were performed in each mouse to initiate chronic ulcer formation. A single IR cycle consisted of a 16-hour period of magnet placement, followed by a release period of 8 h. After magnet application, animals were left to emerge from anesthesia and individually housed. After the second IR cycle, all mice developed two circular ulcers separated by a bridge of normal skin and located 2 cm from the occipital bone of the cranium (Fig. S1A), and this point was considered day 0. After the second IR cycle, all mice developed two circular ulcers, and this point was considered day 0. Thus, IR injury model was used to create a chronic lesion similar to a pressure ulcer of stage II as previously described (Peirce et al., 2000; Stadler et al., 2004).

Laser Doppler flowmeter

Skin blood flow of the compressed area and the contralateral untreated area was evaluated using a scanning laser Doppler flowmeter (0.5–1 mm of measuring depth) (PeriFlux System 5000; Perimed Inc., Stockholm, Sweden), after second IR cycle (day 0) and 7, 24, and 72 h later. To measure skin blood flow of the compressed area, the dorsal skin was again pulled up in order to keep left and right ulcers aligned (Fig. S1B). Since in lesions the thickness was really thin, we measured lesion blood flow and not just adjacent muscle blood flow. Subsequently, the probe of the laser Doppler flowmeter was positioned on the right ulcer (Fig. S1B). To measure skin blood flow of the contralateral untreated area, the probe of the laser Doppler flowmeter was positioned on skin 1 cm below the right ulcer (Fig. S1C). Results were expressed as percentage of perfusion units of normal skin (untreated area).

Evaluation of wound closure and re-epithelialization

To evaluate wound closure, a transparent plastic sheet was placed over the ulcer and its margins were traced soon after application of second IR cycle (day 0), 3, 7, 14, and 21 days later (Nascimento and Costa, 2006). After digitalization, wound area was measured using ImageJ software (National Institute of Mental Health, Bethesda, MD, USA). Results were expressed as percentage of original wound area. To evaluate re-epithelialization, the margins of total wound area and non-re-epithelialized wound area were measured 7, 14, and 21 days after wounding as described (Nascimento and Costa, 2006). Results were expressed as percentage of re-epithelialized wound area. Furthermore, animals were daily observed to determine the day of necrotic skin loss.

The blood pressure and heart rate were not measured in control and propranolol-treated mice, since previous studies have already shown that propranolol administration does not influence blood pressure and heart rate in normotensive rats (Souza et al., 2006; Lewis, 1976; Brum et al., 2002; Altman et al., 1999; Calvillo et al., 2014).

Sampling and histological examination

Mice (n = 15 per group) were killed by CO_2 inhalation 3, 7, 14, or 21 days after wounding. Ten lesions (two in each animal) and adjacent normal skin per group were formalin-fixed (pH 7.2) and paraffin-embedded. Twenty lesions per group (two in each animal) were collected, frozen at −70 °C, and destined to perform hydroxyproline levels (n = 10 each group) and immunoblotting (n = 10 each group). Sections (5 μm) were stained with hematoxylin–eosin to analyze, microscopically, the wound area, to quantify migratory tongue length and neo-epidermis thickness, and to estimate the volume density of blood vessels, using point counting. Sections were also stained with Sirius red and observed under polarization, to evaluate collagen fiber organization.

Measurement of migratory tongue length and neo-epidermis thickness

The length of migratory tongue provides an indication for the extent of keratinocyte migration in the wound tissue. Migratory tongue length was measured 3 and 7 days after wounding. Images of wound edge were digitalized using ×10 objective lens and videomicroscopic system