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Gonadal hormones differently modulate cutaneous wound healing of chronically stressed mice

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ABSTRACT

Gonadal hormones influence physiological responses to stress and cutaneous wound healing. The aim of this study was to investigate the role of gonadal hormones on cutaneous wound healing in chronically stressed mice. Male and female mice were gonadectomized, and after 25 days, they were spun daily at 115 rpm for 15 min every hour until euthanasia. Twenty-eight days after the gonadectomy, an excisional lesion was created. The animals were killed 7 or 14 days after wounding, and the lesions were collected. Myofibroblast density, macrophage number, catecholamine level, collagen deposition, and blood vessel number were evaluated. In the intact and gonadectomized groups, stress increased the plasma catecholamine levels in both genders. In intact groups, stress impaired wound contraction and re-epithelialization and increased the macrophage number in males but not in females. In addition, stress compromised myofibroblastic differentiation and blood vessel formation and decreased collagen deposition in males but not in females. In contrast to intact mice, wound healing in ovariectomized female mice was affected by stress, while wound healing in castrated male mice was not. In conclusion, gender differences contribute to the cutaneous wound healing of chronically stressed mice. In addition, androgens contribute to the stress-induced impairment of the healing of cutaneous wounds but estrogens inhibit it.

1. Introduction

Cutaneous wound healing is a complex process that includes overlapping events such as inflammation, granulation tissue formation, and scar remodeling. After injury, neutrophils and macrophages invade the wound area and destroy foreign particles. Endothelial cells develop new blood vessels that sustain tissue formation. Fibroblasts proliferate and deposit a collagen-rich matrix; some of them differentiate into myofibroblasts that contract the lesion. Keratinocytes also proliferate, migrate, and reconstruct the epidermis. After the formation of the neo-epidermis, cellularity is progressively reduced, and fibroblasts remodel the extracellular matrix, forming a scar. Several studies have demonstrated that excessive stress-induced secretion of glucocorticoids and catecholamines through the activation of the hypothalamic–pituitary–adrenal (HPA) and sympathetic–adrenal medullary axes affect cutaneous wound healing (Mercado et al., 2002; Padgett et al., 1998; Romana-Souza et al., 2010a; Sivamani et al., 2009; Vileikyte, 2007). Moreover, high levels of stress hormones blunt the immune response and increase susceptibility to infections (Cohen et al., 1999; Glaser and Kiecolt-Glaser, 2005; Kiecolt-Glaser et al., 1996; Vedhara et al., 1999). It was recently demonstrated that stress also reduces T-cell-dependent antibody production, T-cell proliferation, and the phagocytic abilities of phagocytic cells in mice, and it increases the neutrophil number in the wound area of mice submitted to restraint stress (Gajendrareddy et al., 2013; Tymen et al., 2013). Gender may also modulate the activation of the HPA axis by stress in humans and animals (Frankenhaeuser et al., 1978; Papadopoulos and Wardlaw, 2000; Seale et al., 2004a,b; Viau and Meaney, 1996).

It has been suggested that premenopausal women could exhibit a reduced cortisol and catecholamine response to mental stress in comparison to age-matched men (Frankenhaeuser et al., 1978). In rats, high testosterone levels may inhibit the HPA axis response to stress by enhancing glucocorticoid feedback at the level of the medial preoptic area and reducing hypophysial arginine vasopressin (AVP) levels (Viau and Meaney, 1996). Similarly, castration of stressed rats increases the plasma secretion of the adrenocorticotropic hormone (ACTH) and corticosterone via an increase in the AVP as well as increases corticotrophin-releasing hormone mRNA expression in the paraventricular nucleus and pro-opiomelanocortin mRNA expression in the anterior pituitary (Seale et al., 2004a,b). In addition, testosterone replacement inhibits the release of ACTH and corticosterone induced by interleukin-6 (IL-6) in castrated rats (Papadopoulos and Wardlaw, 2000). In intact and

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castrated cockerels, estradiol supplementation depresses the plasma corticosterone response to heat stress (Wang and Edens, 1993). Similarly, estrogen administration attenuates the glucocorticoid and catecholamine responses to stress in ovariectomized sheep, most likely via a reduction in ACTH secretion by the pituitary (Komesaroff et al., 1998). Furthermore, postmenopausal women exhibit a greater increase in systolic blood pressure from the baseline and a corresponding increase in norepinephrine and epinephrine when compared to premenopausal women during the speech task (Komesaroff et al., 1999; Owens et al., 1993; Saab et al., 1989). However, estrogen treatment may blunt the mental stress-induced increase of systolic blood pressure in postmenopausal women (Lindheim et al., 1992). In ovariectomized rats, estrogen supplementation prevents emotional stress-induced cardiovascular responses, increasing the expression of heat shock protein 70 and atrial natriuretic peptide in the heart and decreasing c-fos mRNA expression in the paraventricular hypothalamic nucleus (Ueyama et al., 2007). Thus, the enhanced cardiovascular stress responses of postmenopausal women may play a critical role in determining their risk for subsequent cardiovascular morbidity and mortality (Owens et al., 1993). Sexual hormones also present different effects on wound healing. In male mice, castration promotes the wound healing of cutaneous lesions and collagen deposition, reducing macrophage migration and the production of IL-6 and tumor necrosis factor-α by macrophages (Ashcroft and Mills, 2002; Gilliver et al., 2006). However, estrogen treatment limits macrophage and neutrophil migration to the wound granulation tissue and promotes a rapid resolution of inflammation in ovariectomized mice through a reduction in the production of macrophage migration inhibitory factor by macrophages (Ashcroft et al., 2003; Brufani et al., 2009; Hardman et al., 2008). In addition, estrogens, but not progesterone, downregulate wound inflammation, increasing wound healing and matrix deposition (Routley and Ashcroft, 2009). In elderly men and women, estrogen administration increases the rate of cutaneous wound healing and collagen deposition through an increase in transforming growth factor-β1 and a reduction in the neutrophil infiltration and elastase activity in the wound area (Ashcroft et al., 1997, 1999). In men, decreased estrogen levels attenuate the expression of classic pro-healing growth factors, such as transforming growth factor-α, and the expression of arginase-1, which modulates inflammation and matrix deposition (Hardman and Ashcroft, 2008). In addition, the skin of male mice is more susceptible to damage from environmental insults, such as ultraviolet irradiation, than the skin of female mice (Oblong, 2012). In young men and naturally cycling young women, lower testosterone levels are related to faster wound closure in mucosal wound healing (Engeland et al., 2009). However, none of those studies evaluated whether gonadal hormones affect the adverse effects of stress on cutaneous wound healing.

Therefore, the aim of this study was to compare wound repair in male and female mice subjected to chronic stress. In addition, this study also evaluated the individual contribution of gonadal hormones on the healing of skin wounds in chronically stressed mice.

2. Methods

2.1. Animals

Male and female adult Swiss mice ($n = 120$ each gender) were used in this study. Mice were housed in plastic cages ($30 \times 20 \times 13$ cm) with a stainless steel grid cover (Insight, Ribeirão Preto, Brazil) containing groups of five animals per cage in a room with controlled humidity (50%) and temperature (22 °C) on a 12-h light/dark cycle and an air exhaustion cycle (15 min/h). Female and male mice were housed in the same room but in different cages. Moreover, cages were put on separated shelves with 90 cm between them. All procedures were carried out in accordance with the Brazilian legislation regarding animal experimentation (no. 11.794, from October 8th, 2008). This study was approved by the Commission on Ethics in Research at the Rural Federal University of Rio de Janeiro (no. 087/2010).

2.2. Gonadectomies (castration and ovariectomy)

Female and male mice were intraperitoneally anaesthetized with ketamine (150 mg/kg) and xylazine (15 mg/kg). In males ($n = 30$), a vertical incision was made at the middle of the scrotal sac, and the inner sacs, testes and epididymis were exposed. The epididymis was sutured with 4-0 Ethicon (Johnson & Johnson, Brazil), and the testes were removed (Fugger et al., 2000; Koshibu and Levitt, 2008). Incisions were closed using 3-0 Ethicon (Johnson & Johnson, Brazil). In freely cycling females ($n = 30$), an abdominal incision was made, and the ovaries and uterine tubes were exposed. Tubes were sutured with 4-0 Ethicon (Johnson & Johnson, Brazil), and the ovaries were removed. The incision was sutured with 3-0 Ethicon (Johnson & Johnson, Brazil) (Fugger et al., 2000; Koshibu and Levitt, 2008). Sham-operated mice ($n = 30$ of each gender) were subjected to the same surgery, but the gonads were not removed. Other animals ($n = 60$ of each gender) were not subjected to surgery and maintained their intact gonads.

To confirm the efficacy of the gonadectomy and normal synthesis of gonadal hormones, plasma levels of testosterone for male mice and of 17β-estradiol for female mice were measured using an ELISA assay (Cayman Chemical Company, Ann Arbor, MI) at the end of the experiment. The assay was performed according to the manufacturer's instructions. In addition, vaginal smear cytology was performed weekly in females until the end of the experiment (Mendonça et al., 2007). Furthermore, the uterus mass was measured at the end of the experiment.

2.3. Stress model and wounding

The groups studied are described in Fig. 1. Twenty-five days after the gonadectomies, intact ($n = 30$), sham ($n = 15$), and gonadectomized ($n = 15$) animals of each gender were subjected to

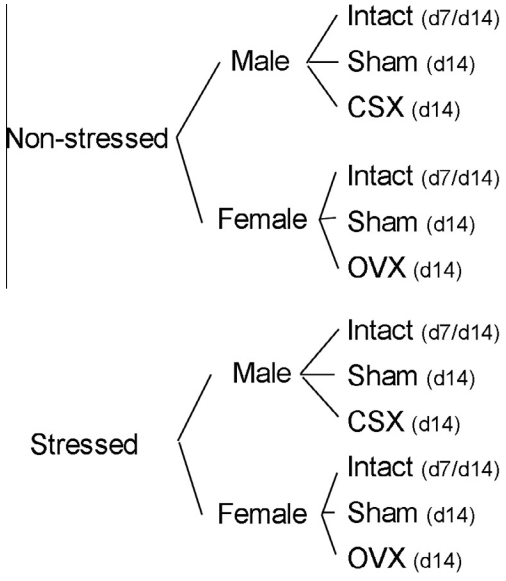

Fig. 1. Schematic representation of the studied groups. Each gender includes intact, sham, and castrated (CSX) or ovariectomized (OVX) groups. Animals were killed 7 (d7) or 14 (d14) days after wounding, and the lesions and plasma were collected.