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Sex hormones and mucosal wound healing

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ABSTRACT

Wound healing studies, which have chiefly examined dermal tissues, have reported a female advantage in healing rates. In contrast, our laboratory recently demonstrated women heal mucosal wounds more slowly than men. We hypothesized sex hormones influence wound healing rates, possibly through their modulating effects on inflammation. This study involved 329 younger subjects aged 18–43 (165 women, 164 men) and 93 older subjects aged 50–88 (60 women, 33 men). A 3.5 mm diameter wound was created on the hard oral palate and videographed daily to assess wound closure. Blood collected at the time of wounding was used to assess circulating testosterone, progesterone and estradiol levels, and *in vitro* cytokine production in response to LPS. No strong associations were observed between healing times and estradiol or progesterone levels. However, in younger subjects, lower testosterone levels related to faster wound closure. Conversely, in older women higher testosterone levels related to (1) lower inflammatory responses; and (2) faster healing times. No such relationships were seen in older men, or in women taking oral contraceptives or hormone replacement therapy [HRT]. Older women (50–54 years) not yet experiencing menopause healed similarly to younger women and dissimilarly from age-matched post-menopausal women. This suggests that the deleterious effects of aging on wound healing occur secondary to the effects of menopause. Supporting this, there was evidence in post-menopausal women that HRT augmented wound closure. Overall, this study suggests that human mucosal healing rates are modulated by testosterone levels. Based upon when between-group differences were observed, testosterone may impact upon the proliferative phase of healing which involves immune processes such as re-epithelialization and angiogenesis.

1. Introduction

To date, wound healing studies have chiefly examined dermal wounds and reported a female advantage in healing rates (Ashcroft et al., 1997; Ashcroft and Mills, 2002; Jorgensen et al., 2002; Shimizu et al., 2004; Gilliver and Ashcroft, 2007). Conversely, when observing oral mucosal wounds our laboratory has found a male advantage in healing rates (Engeland et al., 2006). In addition, mucosal wound healing after oral surgical procedures has been associated with greater complications and longer recovery times in women (Conrad et al., 1999; Phillips et al., 2003; Benediktsdottir et al., 2004; Adeyemo et al., 2006). Thus, gender advantages in wound healing appear to be tissue specific.

Sex hormones, specifically estrogens and progesterone, play a role in mucosal inflammation as demonstrated in both gingivitis (Ashcroft et al., 1999) and periodontal disease (Mascarenhas et al., 2003), suggesting they are mechanistically related to mucosal wound healing. However, this association has not been verified. Importantly, the sexual dimorphism observed in dermal healing rates has been linked to the modulating effects of sex hormones on healing processes, specifically on inflammation (Ashcroft et al., 1997; Ashcroft and Mills, 2002; Gilliver and Ashcroft, 2007). Overall, androgens generally lengthen, whereas estrogens shorten, healing times in skin (for recent reviews see Gilliver et al., 2007; Marucha and Engeland, 2007).

Compared to dermal tissue, mucosal tissue heals much faster with less inflammation and scarring (Lee and Eun, 1999; Szpaderska et al., 2003; Heikkinen, 2006). This suggests that the level of inflammation needed for optimal healing is lower in mucosal tissue. Females mount higher cellular, humoral and inflammatory responses (Schuurs and Verheul, 1990; Miller and Hunt, 1996; Zuk and McKean, 1996; Giglio et al., 1994), have higher levels of circulating antibodies (Giglio et al., 1994; Miller and Hunt, 1996) and a greater ability to clear bacteria than males (Krzych et al., 1981; Miller and Hunt, 1996; Engeland et al., 2003) (for review see Bouman et al., 2005). These enhanced immune responses in females have been primarily attributed to differences in levels of circulating sex hormones (Gaillard and Spinedi, 1998; Lahita, 2000), and in particular the lack of circulating androgens (Bilbo and Nelson, 2001). Sex hormones can influence healing by modulating inflammation, which may explain the observed reversal in the gender

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advantage for healing between dermal and mucosal tissues. Testosterone generally has immunosuppressive and anti-inflammatory properties (McCruden and Stimson, 1991; Giglio et al., 1994; Wichmann et al., 1997; Savita and Rai, 1998), although there is evidence that testosterone promotes inflammation in dermal wound healing (Ashcroft and Mills, 2002; Ashcroft et al., 2003a).

Estrogens have been shown to generally have anti-inflammatory effects (Ashcroft et al., 1999; Ashcroft and Ashworth, 2003; Mascarenhas et al., 2003) whereas progesterone may promote inflammation (Leslie and Dubey, 1994; Cannon and St. Pierre, 1997; Cannon, 1998; Bouman et al., 2001a, 2001b, 2005; Mascarenhas et al., 2003). In line with these findings, it has been shown that women exhibit higher inflammatory responses during the luteal phase (characterized by high progesterone levels) compared to the follicular phase (characterized by high estrogen levels) of the menstrual cycle (Bouman et al., 2001a, 2001b; Leslie and Dubey, 1994; Cannon and St. Pierre, 1997; O'Brien et al., 2007). Although beyond the scope of this paper, it is important to note that that both estrogens and progesterone have complex interactions with immunity and may either inhibit or activate the immune system depending which immune responses are being observed (for reviews see Beagley and Gockel, 2003; Cutolo et al., 2002, 2006; Bird et al., 2008).

The gender differences in mucosal healing rates previously reported by this laboratory (Engeland et al., 2006) encouraged us to look into the role of sex hormones in mucosal wound healing. The current study determined circulating sex hormone levels from three past human wound healing studies using available blood samples. Testosterone levels were ascertained for all subjects since this hormone is the principal androgen in men, and is produced by the ovaries and adrenals in women. Estradiol and progesterone levels were only determined in naturally cycling young women, as the use of oral contraceptives (OCs) alters the production of these endogenous hormones (Chabbert et al., 1998). Also, after menopause the predominant estrogen becomes estrone, rendering estradiol a poor measure of biological function in post-menopausal women.

The objective of this study was to determine the relationships between sex hormones and mucosal wound healing rates in several comparison groups: younger and older men, naturally cycling women versus women taking OCs, naturally cycling women in the follicular phase versus the luteal phase of the menstrual cycle, and post-menopausal women with and without hormone replacement therapy (HRT). Due to previously observed gender differences, we hypothesized that sex hormone levels would be predictive of wound healing rates, possibly through the modulation of inflammatory responses.

2. Methods

2.1. Participants

This study involved 329 younger subjects aged 18–43 years (165 women, 164 men) and 93 older subjects aged 50–88 years (60 women, 33 men). All individuals gave written informed consent and received monetary compensation for their participation. Questionnaires were used to determine demographics, health history and behaviors, and current medication use. Participants were excluded only if they had an oral disease needing emergency intervention or medical problems that would make them a high surgical risk (e.g., unstable angina or an infectious disease such as hepatitis, tuberculosis, or AIDS). All of the wounding was performed by a licensed periodontist (P.T.M). All of the procedures were carried out in The Ohio State University and the analysis of the data was carried out at the University Of Illinois College Of Dentistry and met with institutional review board and ethics committee approval at both institutions.

2.2. Wound placement

Subjects arrived and were seated in the dental clinic between 9:30 and 10:30 AM. Wounds were created between the first and second molar approximately 3 mm from the marginal gingival. The site was anesthetized with 2% lidocaine. The wound area was outlined using a 3.5 mm tissue punch. A scalpel was used to remove the surface epithelium and superficial connective tissue to create a wound with a uniform depth of 1.5 mm. No dressing was used over the wound site. Participants were encouraged to resume their normal oral hygiene procedures but refrain from using alcohol-based mouth wash. A longitudinal wound ($1 \times 5 \times 1.5$ mm) was placed anterior to this first wound, and a $2 \times 5 \times 1.5$ mm biopsy of this second wound was obtained at either 6 h or 24 h post-wounding. Gene expression for inflammatory mediators was determined from all tissues obtained (0, 6, and 24 h) using real-time PCR.

2.3. Wound size assessment

Wounds were videographed at 24 h intervals for 7 days after wounding. A standard 6 mm label was placed around the wound to account for variations in magnification and angulation. These images were transferred to a Macintosh computer, blind coded and measured for area. The same person measured all the wounds. All values were expressed as a ratio of the wound to the standard label. These values were then expressed as a ratio to the original wound size. This is an objective measure of wound closure which has been used extensively in dermal wound studies in humans (Kiecolt-Glaser et al., 1995) and animals (Padgett et al., 1998), and in mucosal wound studies in humans (Bosch et al., 2007; Engeland et al., 2006)

2.4. Sex hormone assessment

Blood was drawn at the time of wounding, as well as 15 and 30 min post-wounding, via catheter. A total of 5 ml blood was drawn in injectable EDTA-coated tubes and blood plasma was used to measure sex hormones levels. Enzyme immunoassays (EIAs) were performed on available blood samples using commercial kits to determine plasma testosterone and estradiol levels (ALPCO Diagnostics, Salem, NH), as well as progesterone levels (Immuno-Biological Laboratories, Inc. IBL America, Minneapolis, MN). Standard protocols provided with the kits were followed. Testosterone levels were measured for all subjects. Estradiol and progesterone levels were measured only in naturally cycling young women.

2.5. Determining the stage of the menstrual cycle

Due to the sharp rise in progesterone levels shortly after ovulation, progesterone concentration can be used to predict the stage of the menstrual cycle (Hampson and Young, 2008). The United States National Institutes of Health reported in 2004 that normal cycling women produce 0.2–1.4 ng/ml of progesterone in the follicular phase and 4.0–25 ng/ml in the luteal phase. Based on this report, values of 4.0 ng/ml or higher were designated to the luteal phase and values below 1.4 ng/ml were designated to the follicular phase. The phase of the menstrual cycle for all subjects was further validated by self-report obtained at the time of wounding. By using this method we were able to determine the phase of the menstrual cycle in the majority of subjects. Of 99 naturally cycling young women, three subjects had progesterone levels between 1.4 and 4.0 ng/ml and two subjects had self-reports which did not agree