Role of sex hormones in acute and chronic wound healing

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Abstract

The sex hormones oestrogen and testosterone are important mediators of wound repair. Oestrogen enhances wound healing by reducing inflammation and enhancing matrix production. However, in post-menopausal women, when oestrogen levels are decreased, wound healing is impaired and susceptibility to chronic non-healing wounds increases. Surprisingly, although the speed of healing is impaired, an improvement in the quality of scarring is observed. Testosterone, in contrast, inhibits wound repair and is associated with enhanced inflammation.

One of the most important consequences of hormonal changes is the age-related delay in cutaneous wound healing observed in both males and females, which leads to substantial morbidity and mortality. Manipulation of hormone levels may provide alternative treatments for promoting healing in non-healing wounds and may be of value in promoting reduced scarring.

Strudwick X, Powell BC & Cowin AJ. Role of sex hormones in acute and chronic wound healing. Primary Intention 2006; 14(1):35-38.

Steroid sex hormones and wound repair

Steroid sex hormones play a vital role in the development of sexual characteristics essential for reproduction, yet they also have important roles in many other tissues. In the skin, oestrogens and androgens are involved in the proliferation and differentiation of epithelial cells and the activity of fibroblasts and skin immune cells and they play important roles in wound healing.

In the skin, marked structural and functional changes, including a decrease in dermal collagen and reduced skin thickness, occur after menopause [1]. Oestrogen can reverse age-related impaired healing in females when applied topically or given systemically and is associated with reduced local inflammation and enhanced matrix deposition [2]. In this regard, recent reports have shown that hormone replacement therapy (HRT) prevents the development of chronic wounds in postmenopausal women [3, 4].

The presence of the oestrogen (estrogen) receptor (ER) in normal skin fibroblasts, as well as in wound fibroblasts and inflammatory cells of both young and aged males and females, suggests that local oestrogen levels may influence cutaneous physiology, including the wound healing process. The positive effect of topical oestrogen has been shown in elderly women and men, reflecting the marked decrease in local oestrogenic activity as a result of reduced ovarian activity in women, a decline in the adrenal oestrogenic precursor dehydroepiandrosterone in men and women, and altered aromatisation of systemic precursors to local active oestrogen [5].

In aging females, although cutaneous wound healing is delayed, there is an improvement in the quality of scarring; studies have shown that this is associated with reduced levels of transforming growth factor-β1 (TGF-β1) [6]. Exogenous addition of oestrogen accelerates wound healing and involves oestrogen-induced increases in TGF-β1 secretion by dermal fibroblasts, leading to increased scarring. Exogenous

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addition of oestrogen reverses the reduced inflammatory response and increases matrix deposition observed in elderly wounds by down-regulating macrophage migration inhibitory factor [1].

Androgens also have important functions in the skin. For example, the male genotype is a strong positive risk factor for impaired healing in the elderly [7] and studies have revealed that castration of male mice results in improved cutaneous wound healing, associated with dampened inflammatory response and increased matrix deposition [8]. The maintenance of levels of androgen that inhibit wound healing in conjunction with reduced local and systemic oestrogen may contribute to impaired healing in elderly males.

Nuclear hormone receptors

Oestrogen acts through binding to the ER. Interaction of ER, liganded with oestrogen, primarily leads to transcriptional activation of genes via recruitment of coactivators and other transcription factors, although extranuclear signalling events can also occur [9]. There are two nuclear ERs, ERα and ERβ. These can form heterodimers and homodimers which exhibit distinct transcriptional properties [10]. Both are expressed in human skin; ERα is generally restricted to fibroblasts whereas ERβ is expressed in fibroblasts and in keratinocytes throughout the epidermis [11, 12]. Our studies show that ERα and ERβ expression is differentially affected by increasing concentrations of oestrogen in skin fibroblasts in culture (Figure 1).

Additionally, while ERα is predominantly found in the nucleus in untreated and oestrogen treated fibroblasts, ERβ translocates from the cytoplasm into the nucleus when treated with oestrogen (Figure 2). It is within the nucleus that the ERs can regulate gene expression by binding to oestrogen response elements in the promoters of various genes.

Many genes important in wound healing are regulated by ER via oestrogen response regions in their promoter which bind ER directly or bind a complex containing ER and an intermediary transcription factor, typically Sp1. These include genes involved in regulation of the cell cycle (cyclin D1), cell growth (EGF receptor, TGF-α, VEGF, c-fos) and cell maintenance (IGF-I, keratin) [13, 14]. Notably, IGF-I, EGF receptor and c-fos are known to be important in wound healing [13, 15, 16].

Androgens act through binding to the nuclear androgen receptor (AR), a member of the same nuclear receptor family as ER [17], to control androgen-dependent gene expression. In wound healing, AR is upregulated in the skin, at which time it is also expressed in inflammatory cells [8]. Unliganded AR is an inactive oligomer complexed to heat shock proteins (e.g. Hsp90, Hsp70) and located in the cytoplasm. The oligomeric complex dissociates on ligand binding, and undergoes a conformational change before translocating into the nucleus to bind as a homodimer to DNA. Androgen-bound AR binds to androgen response elements to regulate the expression

Figure 1. Effect of oestrogen on ER expression in skin fibroblasts.
Skin fibroblasts were cultured in DMEM and treated with increasing concentrations of β-estradiol (0, 1 pg/ml, 1 ng/ml, 1μg/ml, 1mg/ml) for 24 hours. Protein was extracted from the cells and 10μg loaded per lane onto polyacrylamide gels and run for 1 hour at 100V. Proteins were then transferred onto nitrocellulose and incubated with antibodies specific to ERα and ERβ. Bands were visualised using enhanced chemiluminescence. Increasing expression of ERα is observed with increasing concentration of β-estradiol. Decreasing expression of ERβ is observed with increasing doses of β-estradiol.

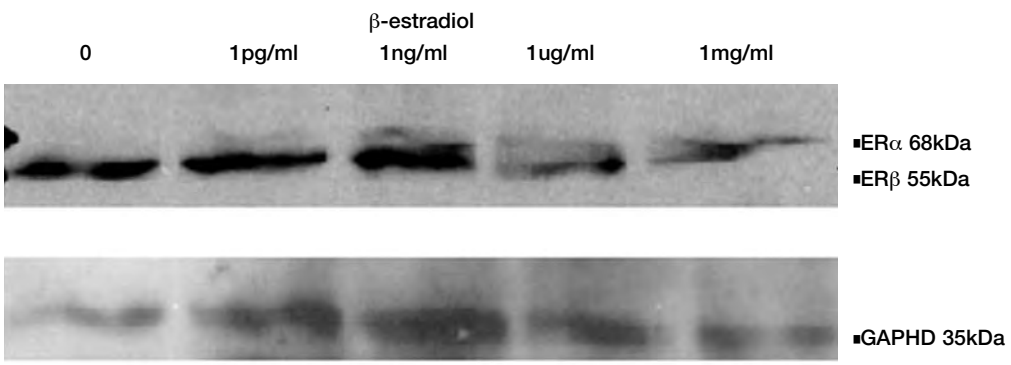
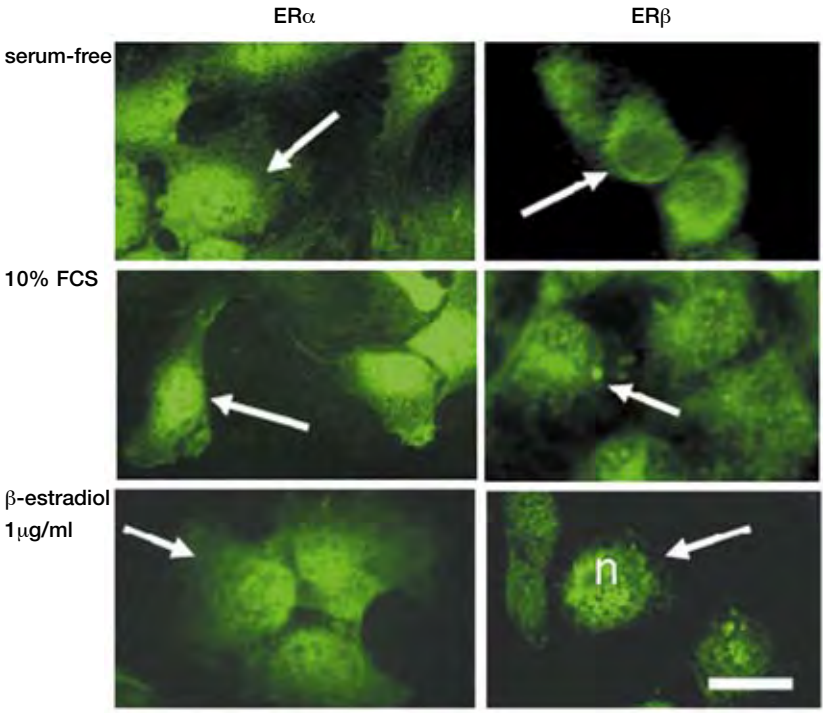


Figure 2. Expression of ERs in human skin fibroblasts.
Skin fibroblasts grown in culture were treated with 10% fetal bovine serum (FBS) or β-estradiol (1μg/ml) for 24 hours and the cellular localisation of oestrogen receptor α and β determined using immunocytochemistry. ERα was observed throughout the cell but predominantly within the nucleus with treatment with FBS and β-estradiol. Translocation of ERβ from the cytoplasm into the nucleus was observed with treatment of β-estradiol. Magnification bar=100μm.



of various target genes, including prostate specific antigen, Probasin, KGF and p21 [18].

Sex hormones in chronic wound healing

In venous ulceration, continual leukocyte recruitment and activation causes chronic tissue injury due to the release of destructive inflammatory mediators. Around 70% of all cases of chronic wounds are venous ulcers and they affect 1% of the elderly population [19].

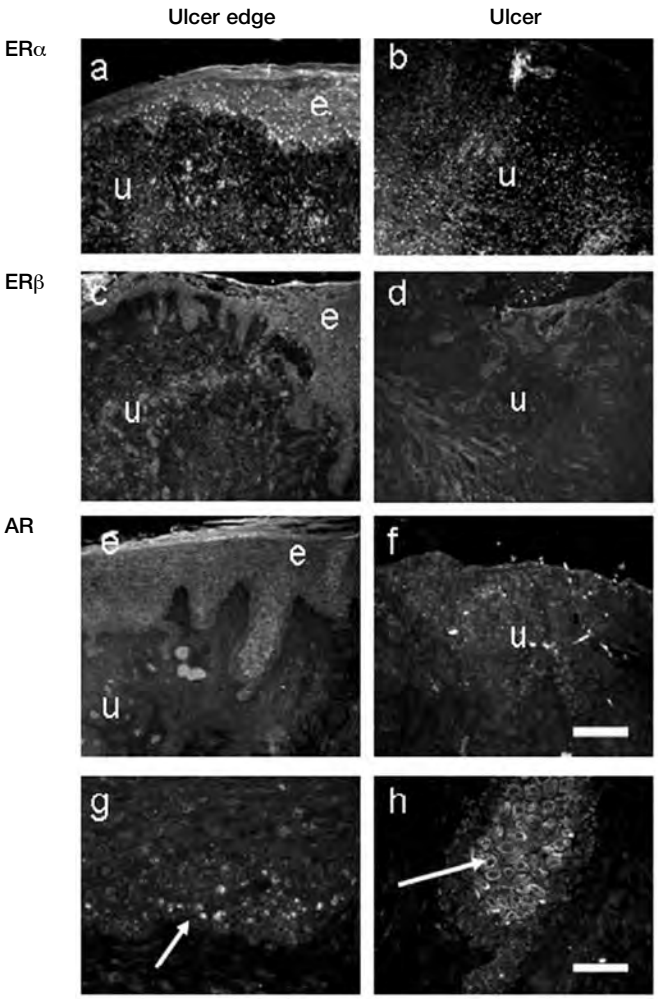
Recent evidence suggests HRT prevents the development of chronic wounds in postmenopausal women [3]. Direct involvement of oestrogen in the progression of chronic wounds has also been found, with ERβ regulating inflammation in chronic wounds [19]. A specific ERβ variant has now been identified which predisposes elderly individuals to venous ulceration [19].

Our studies reveal that ERα expression is observed within the proliferative, basal layer of the epidermis adjacent to the wound edge (Figure 3 – a & b). ERα is also highly expressed in fibroblasts within the ulcer and in the adjacent tissue at the wound edge. In contrast, little ERβ expression is observed in the epidermis at the wound edge or within fibroblasts of the wound (Figure 3 – c & d).

It has been suggested that reduced ERβ expression may result in elevated levels of TNFα at the wound site. Therefore the observed reduction in ERβ expression in chronic wounds may affect the prolonged inflammatory response which is characteristic of these wounds and, as ERβ is more potent than ERα in mediating oestrogen-induced repression of TNFα [20], the reduction in ERβ may be more significant than the presence of ERα in the wound.

In normal human skin, AR is expressed in keratinocytes and fibroblasts [8] where it is implicated in regulating collagen levels [21]. In chronic wounds, ARs are observed in fibroblasts and suprabasal keratinocytes, suggesting roles in collagen synthesis and epidermal differentiation (Figure 3 – e & f). The presence of ARs in chronic wounds also suggests that androgen-bound AR may bind to androgen response elements and regulate the expression of various target genes, including KGF [14], an important growth factor involved in keratinocyte proliferation and differentiation.

Figure 3. Expression of sex hormone receptors in chronic wounds. Biopsies taken through chronic venous leg ulcers were stained for ERα, ERβ and AR using immunohistochemistry. Representative images are shown. a & b – ERα staining of edge of ulcer and ulcer respectively. c & d – ERβ staining of edge of ulcer and ulcer respectively e & f – AR staining of edge of ulcer and ulcer respectively. g – Higher magnification view of ERα staining of epidermis adjacent to ulcer wound. h – Higher magnification view of AR staining of epidermis adjacent to ulcer wound. Magnification bar in (F) refers to (A-F) and =50μm. Magnification bar in (H) refers to (G-H) and =75μm.



Conclusion

In summary, steroid sex hormones and their receptors are important mediators of wound repair. Age related changes in their levels contributes to the impaired healing and increased prevalence of chronic non-healing wounds observed in the elderly. Manipulation of hormone levels and strategies aimed at altering receptor expression and activity may provide beneficial therapeutic advances for the treatment of wounds in the young and aged population.

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