

Title

Estrogen stimulates phagocytosis by macrophages in both *in vitro* and *ex vivo* models of age-related impaired healing via the estrogen-receptor alpha.

Names

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Key words

Macrophage, Inflammation, Estrogen, Phagocytosis, Chronic wounds.

Abstract

Annual expenditure for the treatment of chronic wounds in the elderly exceeds \$9 billion. Chronic wounds are frequently colonised by opportunistic pathogens such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*, and declining levels of estrogen with increasing age delays healing. This study investigated the effect of hormonal aging (estrogen deprivation) on the clearance of methicillin-resistant *S. aureus* (MRSA) and *P. aeruginosa* by macrophages derived from U937 and human primary CD14⁺ monocytes. Concentrations of 17 β -estradiol were used to model estrogen levels found in the elderly (estrogen deprivation: absolute absence and 1x10⁻⁹M), young adults (1x10⁻⁸M) and following exogenous supplementation (1x10⁻⁷M). The estrogen receptor (ER) isoform(s) involved in bacterial clearance were determined using selective ER modulators.

Estrogen at concentrations typical of youth or supraphysiological levels significantly ($P < 0.05$; $n = 24$) increased the phagocytosis of MRSA and *P. aeruginosa* in a concentration-dependent manner compared to estrogen deprivation. Confocal and scanning electron microscopy confirmed estrogen increases co-localisation of fluorescent GFP-*S. aureus* or mCherry-*P. aeruginosa* with macrophages and promotes bacterial internalisation. ER-alpha (ER α) activation mirrored the stimulatory effect of estrogen on phagocytosis whilst ER α antagonism completely blocked the effect of estrogen. In contrast, activation or antagonism of ER-beta (ER β) had no effect on phagocytosis, confirming estrogen mediates bacterial clearance via ER α alone.

These findings suggest estrogen promotes the resolution of wound bacteria during youth but this protection is lost as estrogen levels decline with increasing age. Novel dressings that provide estrogen supplementation or selective activation of ER α may be an effective treatment option for colonised wounds in the elderly.