Title:

Estrogen reverses the age-related impaired clearance of wound bacteria by human macrophages via activation of estrogen-receptor alpha and actin cytoskeleton reorganisation.

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Background:

Chronic wounds often become colonised by opportunistic pathogens such as *Staphylococcus aureus* and *Pseudomonas* aeruginosa. Estrogen deprivation with increasing age delays healing but the effect on wound infections is largely unknown.

Objectives:

To investigate the effect of hormonal aging (estrogen deprivation) on host clearance of methicillin-resistant *S. aureus* (MRSA) and *P. aeruginosa*.

Methods:

U937- and human peripheral blood monocyte-derived macrophages were used in phagocytosis assays (n=24) to determine bacterial clearance following treatment with estrogen at levels found in elderly ($<1\times10^{-9}$M) and young adults (1×10^{-8}M), and following exogenous supplementation (1×10^{-7}M). The mechanisms by which estrogen mediates phagocytosis were interrogated using estrogen receptor (ER) agonists/antagonists followed by immunoblotting to assess downstream effects on mediators of the actin cytoskeleton.

Results:

Estrogen-deprivation significantly ($P<0.05$) inhibited the clearance of MRSA and *P. aeruginosa* compared to physiological (typical of youth) or supraphysiological levels. Confocal microscopy confirmed estrogen deprivation reduces macrophage uptake of fluorescent GFP-*S. aureus* and mCherry-*P. aeruginosa*. Stimulation of phagocytosis following ER-alpha (ER-α) activation was completely reversed by ER-α antagonism ($P<0.05$), whereas ER-beta (ER-β) activation had no effect on bacterial clearance. Scanning electron microscopy confirmed estrogen induces bacterial internalisation through dynamic formation of pseudopodia by phagocytes and this was mirrored by elevated levels of focal adhesion kinase (FAK), Rac-1, Cdc-42 and Rho-G but reduced levels of Rho-A. These findings suggest estrogen deprivation with increasing age leads to impaired bacterial clearance and that novel dressings that provide estrogen supplementation or selective activation of ERα may be effective treatment options for colonised wounds in the elderly.