Estrogen Promotes Macrophage-Mediated Clearance of Biofilms in an *in vitro* Model of an Infected Diabetic Foot Ulcer.

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Abstract

Background:

Diabetic foot ulcer (DFU) infections are common due to defective phagocytic activity of innate immune cells and/or one or more invasive bacterial species evading host responses. DFU infections are the main cause of diabetes-related hospitalisation and are a major cause of diabetes-related amputation, often persisting in protective biofilm arrangements. Alleviating biofilm growth is often one of the primary aims of DFU infection management. With antibiotic resistance now commonplace, there is an urgent need to reduce the reliance on antibiotics and develop novel treatments to combat wound infections. One novel strategy is to stimulate natural innate immune responses through increased phagocyte activity. The endogenous hormone estrogen is reported to be a master regulator of innate inflammatory responses, with declining levels of estrogen with increasing age leading to impaired wound healing. However, the effect of estrogen supplementation on innate host responses in DFU infections, particularly those involving biofilms, remains largely unknown. Thus, the aim of this study was to investigate the potential use of estrogen to promote macrophage-mediated clearance of two nosocomial wound-associated pathogens, methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* (PA), under diabetic (hyperglycaemic) conditions using an *in vitro* biofilm model of an infected DFU.

Materials/methods:

In vitro host-pathogen interaction assays were used to determine the effect of estrogen (17-β estradiol) on the clearance of MRSA and PA biofilms (n=12) by U937-derived M1 macrophages cultured under increasingly hyperglycaemic (>11mM glucose) conditions. To confirm bacterial uptake and biofilm clearance by the estrogen-stimulated macrophages, the phagocytic activity of monocyte-derived macrophages was visualised by confocal and scanning electron microscopy (SEM).

Results:

Hyperglycaemia significantly ($P<0.05$; n=12) impaired M1 macrophage-mediated clearance of both MRSA and PA biofilms in a concentration-dependent manner, suggesting poorly controlled hyperglycaemia in diabetics may dampen phagocyte activity. Intriguingly, estrogen ($1x10^{-7}M$) significantly ($P<0.05$; n=12) reversed the detrimental effects of hyperglycaemia on both MRSA and PA biofilm clearance. Pronounced interaction and uptake of bacteria by estrogen-treated M1 macrophages was visualised by confocal microscopy and SEM, confirming enhanced biofilm clearance compared with untreated controls.

Conclusions:

The findings suggest estrogen can reverse the detrimental effects of hyperglycaemia on M1 macrophage-mediated phagocytosis of key wound-associated bacteria, with estrogen enhancing the clearance of established biofilms. This highlights the possibility that topical estrogen application or localised estrogen receptor activation at the wound site might be effective therapeutic strategies to tackle the protective biofilm environments of DFU infections.