Bovine Lactoferrin, A Novel Antimicrobial Adjunct For Use As An Antimicrobial Agent In Battlefield-relevant Open Fractures.

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INTRODUCTION: Up to 50% of type 3b compound fractures are complicated by biofilm infections [1]. Staphylococcus aureus (S. aureus), the most common cause of infection, resides within a protective biofilm. Lactoferrin (Lf), a glycoprotein with antimicrobial and immunomodulatory properties, is found naturally in human and animal milk [2]. Bovine Lf (bLf) is a potent stimulator of bone growth adding to its appeal as a treatment for compound fractures [3]. The goal of this study was to test the ability of bLf as an adjuvant to antibiotics in the context of compound fracture infections. Specifically, we aimed to: 1) Demonstrate the in vitro anti-biofilm properties of bLf. 2) Translate the in vitro antibiofilm activity of bLf to an in vivo periprosthetic, biofilm infected tibia rodent model.

METHODS: In vitro: S. aureus Xen-36 biofilm was generated in a standard bioreactor on metal coupons and tested against cefazolin (CEF) and flucloxacillin (FLU) with or without bLf. Viable counts of S. aureus were recovered and enumerated. In vivo: Surgery was performed on adult male rats (n = 40), where the craniomedial tibia was exposed, and a 1.5 mm defect created and inoculated with Ten µL (≈10^7 CFU) of S. aureus biofilm. A 2 mm length stainless steel pin was placed within the medullary cavity of the tibia, and the wound treated with STIMULAN® beads loaded according to 4 treatment groups: 1) Control (empty), 2) bLf 3) FLU, 4) bLf and FLU. All rats received subcutaneous FLU 200 mg/kg immediately prior to surgery. At day 7 post infection, rats were re-anaesthetised for bioluminescent and x-ray imaging, euthanized, and tibial explant tissue/metal pins collected for bacterial colony enumeration.

RESULTS SECTION: In vitro: bLf augments the anti-biofilm activity of antibiotics such as CEF and FLU in vitro (Figure 1A), and in combination with FLU significantly eradicates S. aureus biofilm infection in vivo when compared against controls (Figure 1B). In vivo imaging confirms correct placement of metalware and inoculum (Figure 1C).

DISCUSSION: This study has shown the promising in vitro and in vivo effects of bLf, one of the main limitations observed within this study was the prolonged setting time of STIMULAN® beads when loaded with bLf, taking at least 12 hours to fully set.

SIGNIFICANCE/CLINICAL RELEVANCE: bLf holds promise as an anti-infective in battlefield relevant open fractures, supported by the anti-biofilm properties of bLf highlighted within this research. Further work is required to translate these finding into clinical practice in military conflicts.


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IMAGES AND TABLES:

Figure 1: A) Viable counts (CFU) recovered from biofilm coupons treated with CEF or FLU in the presence (red) and absence (black) of bLf. One-way ANOVA with Sidak correction showed that the inclusion of bLf significantly improved the antibiofilm activity of CEF (p<0.001) and FLU (p<0.0001) B) One-way ANOVA with Tukey’s test demonstrated that FLU-Lf beads significantly eradicated S. aureus compared with controls (empty bead) (p<0.001). C) Bioluminescence and x-ray imaging of a rat, periprosthetic, biofilm infected tibia.