Evaluation of Bioburden on the Development of Heterotopic Ossification in an Established Rat Model

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Introduction: Heterotopic ossification (HO) forms in the majority of severe combat-related amputations, particularly when the amputation occurs within the zone of injury. Clinically, HO formation can result in persistent pain, skin breakdown and difficulty with prosthetic wear leading to a delayed operation for surgical excision. Current means of prophylaxis for HO in the combat setting are logistically complicated. Additionally, these wounds are often heavily contaminated and require multiple debridements. Subsequent infection remains one of the common and significant complications following blast-related severe fracture and amputation with \textit{Acinetobacter Baumannii} and \textit{Methicillin Resistant Staphylococcus Aureus} (MRSA) being the most common isolate from combat wounds. Whether potentiation of HO in this setting is a result of the heightened prolonged local and systemic inflammatory response secondary to blast trauma and/or concomitant bacterial colonization is under much study. Expanding on a rat model for the development of post-traumatic HO, we sought to evaluate the influence of wound colonization on the formation of HO.

Methods: We exposed forty-three adult male Sprague-Dawley rats (350-400g) to 120±7 kPa blast over pressure using a shock tube followed by femur fracture, thigh crush injury and transfemoral amputation done within the zone of injury. Each wound was inoculated beneath the myodesis with 1.0 10\textsuperscript{6} CFUs of a highly virulent strain of either \textit{Acinetobacter Baumannii} or \textit{Methicillin Resistant Staphylococcus Aureus} (MRSA) which were isolated from combat wounds. A control group was injured as described above, but did not undergo inoculation. Rats were followed for visual evidence of wound infection requiring irrigation and debridement and euthanized if three serial debridements performed at least 24 hours apart did not result in clinical improvement. We performed microCT (mCT) imaging weekly for the first month and at 8 and 12 weeks post-operatively in order to measure HO volume. Samples of muscle tissue adjacent to the amputation site and bone marrow from the residual femur were harvested to determine persistence of infection. Representative animals in each treatment group underwent en bloc resection of the femur and surrounding musculature for histologic analysis.

Results: All rats in both experimental groups as well as the control group developed HO. Survival rate among treatment groups was less in the MRSA experimental group (14/20, 70\%) in comparison to the Acinetobacter experimental (16/18, 89\%) and control (5/5, 100\%) groups. Specifically, 6 of 20 rats were euthanized in the MRSA group, chiefly for overwhelming infection persistent after three debridements,
which occurred between the 4th and 5th week postoperatively. Whereas two rats inoculated with 
Acinetobacter were euthanized during week 2 and week 4 for weight loss of greater than 10% of 
preoperative weight. At 12 weeks, we observed significantly more robust HO on mCT volumetric analysis 
in animals infected with MRSA (122.32 mm$^3$ ± 29.22) when compared to Acinetobacter (15.04 mm$^3$ ± 
2.43; p<0.05) and controls (11.22 mm$^3$ ± 2.77; p<0.05). There was no significant difference shown in this 
measure when comparing Acinetobacter with surgical controls not inoculated with bacteria. Sample 
tissue from a cohort of five rats inoculated with MRSA during initial surgery still had culture positive 
results in the tissue of three rats, and the bone marrow of two rats. All rats inoculated with 
Acinetobacter *Baumannii* tested negative for the inoculated bacteria, however (2/14) bone marrow 
samples and (6/14) muscle tissue cultures demonstrated growth of other bacteria.

**Discussion:** Our findings suggest that persistent MRSA infection, refractory to antibiotic treatment, may 
result in chronic local inflammation leading to increased HO formation. Osteomyelitis or chronic local 
tissue infection particularly when colonized by MRSA and Acinetobacter strains used for this experiment 
may be associated with more robust HO formation. The presence of other bacteria in the *Acinetobacter 
Baumannii* inoculated group may indicate co-colonization and is deserving of further study.

**Significance:** This work emphasizes the importance of the initial wound debridements in addition to the 
ystemic and perhaps local antimicrobial therapies geared towards decreasing bioburden in combat 
wounds.

![Figure 1](image-url)