The use of topically-applied ceragenins to reduce *Pseudomonas aeruginosa* infections in a models of musculoskeletal trauma

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INTRODUCTION: Infections are a major complication associated with traumatic, high-energy extremity injuries on the battlefield. Open fractures, such as Gustilo Anderson Type IIIB fractures, which involve large amount of soft tissue loss and requires a flap for coverage, are the most prone to infection with rates near 75%. The mechanism of injury and the large trauma areas leave the wound open to the environment and inevitable contamination. Even with adequate, rapid surgical care, local infection is still a concern. With the anticipation of prolonged combat casualty care, the risk for musculoskeletal infection is only expected to increase. Current guidelines recommend copious irrigation, removal of foreign objects, clean bandaging, and broad-spectrum antibiotics. Often, high, potentially toxic, doses of antibiotics are required to reach therapeutics levels in the mangled tissues. A topical, antimicrobial treatment could reduce the risk of wound infections and maintain tissue viability during the prolonged transport to surgical care. Antimicrobial peptides (AMPs) play a central role in infection defense and are produced constitutively in normal tissues. However, in severe, contaminated wounds, the native AMPs, along with host immunity, are not enough to prevent infection. Ceragenins, non-peptide mimics of AMPs, could provide local antimicrobial support. In short, ceragenins are not subject to proteases like synthetic AMPs and can be synthesized in few quick steps. Ceragenins display broad-spectrum antimicrobial activity against both Gram-positive and -negative bacteria, including drug-resistant organisms, and pathogenic fungi. Herein, we examine the utility of topical ceragenins for reducing *Pseudomonas aeruginosa* infections in an animal models of musculoskeletal injury.

METHODS: Research was conducted in compliance with Animal Welfare Act, the implementing Animal Welfare regulations, and the principles of the Guide for the Care and Use of Laboratory Animals. The Institutional Animal Care and Use Committee approved all research conducted in this study. The facility where this research was conducted is fully accredited by the AAALAC International. All procedures were performed under general anesthesia and appropriate pain management and animals were assessed twice daily following the procedures and received additional analgesia as needed. An established rat model of musculoskeletal infection and a goat model of complex musculoskeletal injury were used to determine treatment efficacy when the ceragenins application was delayed after injury. For the rat study, briefly, Sprague-Dawley rats received a segmental defect in the right femur which was stabilized with a radiolucent fixation plate. Each defect was inoculated with ~10^5 CFU of *P. aeruginosa* in saline. The animals were recovered for 6 hours, when they were again anesthetized, the wound opened, and randomized to receive a ceragenin gel placed into the defect and wound pocket. The gels contained either 0.1 or 0.5% ceragenin or nothing as vehicle control. Following closure, animals were administered moxifloxacin (5mg/kg), which was repeated once daily for another 3 days. On the fourth day, animals were anesthetized and euthanized with an overdose of pentobarbital. Bone and implants were aseptically harvested and assessed for bacterial burden. For the goat study, briefly, castrated male Spanish Boer goats received a complex injury, which included a unicortical tibial defect, frank muscle loss, thermal (burn and freeze), and a skin excision, in the left hindlimb. The injuries were inoculated with a bioluminescent strain of *P. aeruginosa* (10^8 CFU) and covered. The animals were recovered for 6 hours, when they were again anesthetized, the dressing removed, and initial bioluminescent images acquired. The wounds were irrigated with 1L saline and scrub debridement before a second bioluminescent image and ceragenins, or empty as control, were applied. IV moxifloxacin was also given to two of the groups as a battlefield standard of care. Minimal irrigation and scrub debridement were used to better replicate battlefield medic procedures. 48h after initial inoculation, the animals are anesthetized and euthanized with an overdose of pentobarbital. A final image is acquired and tissue is harvested for quantitative microbiology.

RESULTS SECTION: Overall, There is a decrease in bacterial burden within wounds when they were treated 6h after initial P. aeruginosa inoculation. For the rat study, within the vehicle control, 4 of 5 animals had CFU>10^3 with a median $\log_{10}$ of 4.59 (3.2-4.7 IQR) (Fig 1). When treated with either the 0.1% or 0.5% ceragenins, 2 of 5 animals had CFU>10^3 with median $\log_{10}$ of 2.91 (0.0-4.3 IQR) and 2.7 (2.7-4.4 IQR), respectively. Blood was also collected at euthanasia and showed no significant findings. Of note, hemolysis, tissue necrosis, or a decrease in viability was not observed at the time of application or euthanasia/ tissue collection. Within the goat study, there was indications of ceragenin effectivity with a lower bacteria rebound, via bioluminescence, following wound irrigation (Fig 2). The quantitative microbiology supports the bioluminescent imaging showing a reduction in *P. aeruginosa* in ceragenin treated compared to empty or empty with moxifloxacin with ~1-log reduction of bacteria recovered.

DISCUSSION: Topical treatments are promising, easily applied adjuncts to systemic antibiotics. Ceragenins offer an opportunity to combat tissue infections without increasing the risk of antimicrobial resistance. Here, we present the utility of ceragenins to treat musculoskeletal infections caused by *P. aeruginosa*. It appears that ceragenins may have a use as a topical treatment to highly contaminated wounds, such as those sustained in combat. Battlefield injuries to the extremities have resulted in, amongst a cohort of 2,210 service members with deployment-related trauma infection, 45% skin and soft-tissue infections and 7% osteomyelitis. Additionally, among a cohort of 1,044 personnel who sustained an open extremity fracture, 30% acquired an infection even with the use of prophylactic antibiotics. It is critical to identify a solution to reduce the number of wound infections. Ceragenins have displayed antifungal, antiprotozoal, and antibacterial activity, even against multi-drug resistant organisms. Ceragenins have shown *in vitro* activity against a number of clinical strains of *P. aeruginosa* with minimum inhibitory concentrations comparable to standard antibiotics. Ceragenins already have a use in veterinarian medicine as a wound wash and are currently being evaluated in a Clinical Trial as an endotracheal tube coating to reduce Ventilator Associated Pneumonia. The information gained here provides an opening to the potential use of ceragenins to reduce infection associated with musculoskeletal battlefield injuries. Further investigation could provide more evidence for its utility on the battlefield.

SIGNIFICANCE/CLINICAL RELEVANCE: Extremity injuries are the most prevent injuries on the battlefield. These injuries continue to be at a high risk for infection. Topical ceragenins could provide antimicrobial support to systemic antibiotics to reduce infection and improve outcomes.

Fig. 1

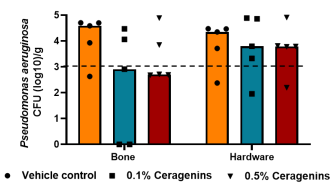

Fig. 2

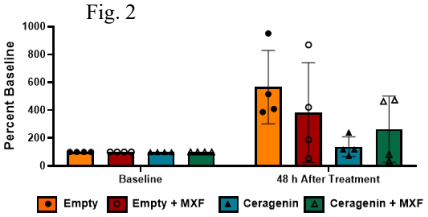

Figure 1: Quantitative microbiology of P. aeruginosa recovered from bone tissue and implant hardware.

Figure 2: Percent of baseline bioluminescence. Baseline was acquired immediately after irrigation and scrub debridement.