Targeted Stimulation of Retinoic Acid Receptor Signaling Mitigates the Formation of Heterotopic Ossification Formation in an Established Blast-Related Traumatic Injury Model

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Background/Purpose: Heterotopic ossification (HO), which is the abnormal development of bone in nonosseous tissue, has been shown to be prevalent in 65% of combat wounds. Complications can be devastating, including skin ulceration, pain management crises, and prosthetic wear intolerance, all of which deteriorate rehabilitation and often lead to additional surgeries that can be wrought with complications. Furthermore, commonly used civilian prophylactic measures are generally contraindicated in the setting of combat and blast-induced trauma. Recently, our group has developed a traumatic small animal model that incorporates a combination of critical physiologic injury patterns sustained by combat casualties including blast exposure, extremity fracture, quadriceps crush injury followed by limb amputation through the zone of injury, and contamination of the myodesis with a combat wound isolate of methicillin-resistant Staphylococcus aureus (MRSA). Using this model we histologically characterized the timing of chondrogenesis to begin at about 10 days following injury. Knowing the proper timing of prophylactic administration, we now have a well-characterized model of HO whereby the efficacy of prophylactic therapeutics can be rigorously evaluated in a clinically relevant animal model. One such candidate drug retinoic acid receptor gamma (RAR-γ) agonist has been shown to mitigate a bone morphogenetic protein (BMP)-2-induced ectopic endochondral bone formation and inhibit cartilage vascularization in a non-trauma injury model in mice. Expanding on these findings, we sought to evaluate the efficacy and applicability of RAR-γ agonist in attenuating ectopic bone formation in our blast-trauma model.

Methods: We exposed 72 adult male Sprague-Dawley rats to 120 ± 7 kPa blast overpressure, followed by femur fracture, quadriceps crush injury, transfemoral amputation through the zone of injury, and bacterial inoculation with MRSA. Rats were either started on enteral gavage administration of RAR-γ agonist (1 μg/g of Palovarotene [Roche Pharmaceuticals] every other day for 14 days) or corn oil/DMSO (vehicle control) beginning on either postoperative day (POD) 1 or 5. Rats were monitored for 12 weeks for evidence of wound dehiscence and ectopic bone formation using micro computed tomography (CT) imaging to quantitate ectopic bone volume.

Results: Vehicle control-treated rats infected with MRSA gavaged on POD 1 and POD 5 had a mean ectopic bone volume of 42.8 ± 10.4 mm3 and 38.9 ± 8.5 mm3 respectively, compared to rats that received RAR-γ agonist on POD 1 (15.6 ± 4.1 mm3; P = 0.04) and POD 5 (20.7 ± 7.4 mm3; P =.09). The statistically significant attenuation of ectopic bone in rats adminis-

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tered with RAR-γ agonist beginning at our earliest time point (POD 1) correlates with our early histologic findings in which mature chondrocytes and vascularized hyaline cartilage appear by 10 to 14 days postinjury.

Conclusion: Given our early findings, RAR-γ agonist administered shortly following trauma may act to preclude the proliferation and further differentiation of chondrocytes during early endochondral ossification. This intervention may therefore represent a promising prophylactic therapy against ectopic bone formation. To our knowledge, this is the first study to demonstrate the applicability of a drug that can mitigate the formation of ectopic bone in a trauma-induced injury model without interfering with wound healing reparative processes.

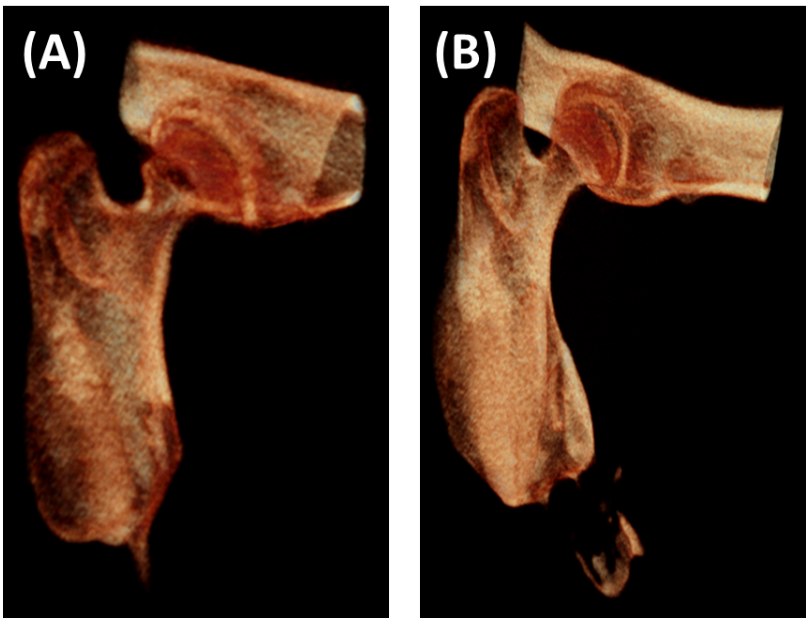

Figure 1. shows a 3D reconstruction of a 12-week post-injury MicroCT of a trauma-induced rat model infected with MRSA that was gavaged with (a) RAR-γ on POD1 (b) corn oil/DMSO beginning on POD1.