Local Delivery of Yoda1 Improves Bone Quality After Fracture Under Mechanical Unloading in Polytrauma

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INTRODUCTION: Patients with a major long bone fracture as a component of polytrauma are often subjected to delayed or altered weight bearing due to their critical illness and/or injury burden. Rodent models for studying unloading and bone homeostasis in space flight show that the lack of mechanical loading leads to bone resorption and poor fracture healing. Clinically, polytrauma has been identified as an independent risk factor for nonunion in long bone fractures with likely a multi-factorial etiology. Our lab has demonstrated in a murine polytrauma model that the polytrauma environment elicits a systemic and local inflammatory cascade and dysregulated immune response that results in impaired fracture healing (1, 2). How delayed return to weightbearing intersects with these responses affects fracture healing remains unknown. Piezo1 is a ubiquitous mechanosensitive calcium channel present in diverse cell types and active in essential physiologic processes including embryologic development, the baroreceptor response, epithelial proliferation, and neuronal growth. Piezo1 expression in mesenchymal stem cells (MSCs) is correlated with osteoblast differentiation, upregulation of osteogenic markers, and concurrent downregulation of adipogenesis. In response to the application of mechanical loading (3). The molecule Yoda 1 is a selective agonist of the Piezo1 channel that has been shown to induce Piezo1 channel response even in the absence of mechanical stimulation. We hypothesized that in a mouse model of polytrauma, the treatment of long bone fractures with Yoda 1 during postoperative immobilization would result in an improved fracture healing response.

METHODS: Animal surgeries and experimental protocols were authorized by Institutional Animal Care and Use Committee (IACUC) and performed in the Department of Orthopedic Surgery at UC Davis in compliance with ARRIVE guidelines. Twelve male Sprague Dawley rats were used in this study. A standardized murine polytrauma model was used that included blunt chest trauma and a left femur diaphyseal fracture. Six-hole plates were applied to the mid-diaphysis followed by creation of 1 mm diaphyseal defects under the center of the plate with a gigli saw (**Figure 1A**). There were three treatment groups and one control group, with four animals each. The control group consisted of uninjured animals that were unloaded. Hydrogels were delivered by injection locally at the fracture site (**Figure 1B, C**). The treatment groups were 1) loaded (weight bearing as tolerated) with hyaluronic acid-based hydrogel (loaded HA) 2) unloaded with HA and 3) unloaded with HA and Yoda1. We used an internally validated murine hindlimb unloading protocol (**Figure 1D**). The unloaded groups were suspended for seven days under standardized conditions allowing free movement initiated by the forelimbs. The loaded group was allowed to weight bear as tolerated for this period. All animals were then returned to full weight bearing for an additional three weeks. At four weeks, the animals were sacrificed and the left femurs harvested. We quantified the fracture healing and remodeling response to unloading with micro computed tomography (μCT). Histology and immunohistochemistry (IHC) are currently in process. All analyses were performed in GraphPad Prism 8 (GraphPad Software Company, San Diego, CA). Significant differences were presented as *P < 0.05 and **P < 0.01,***P < 0.001 and ****P < 0.0001.

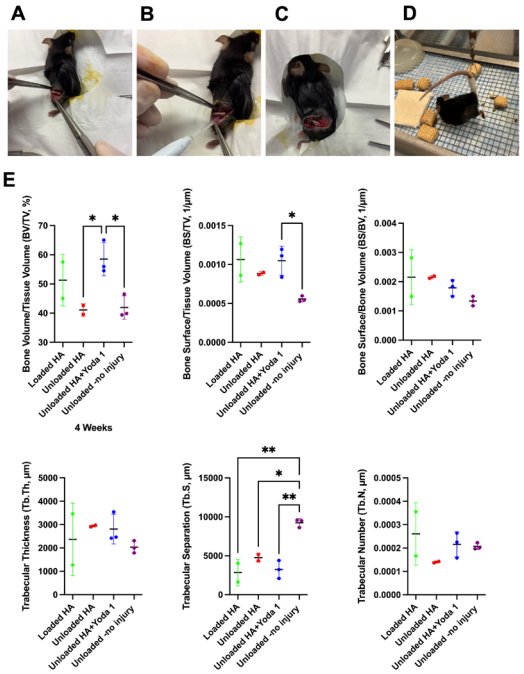

Figure 1. Experimental design and fracture healing outcomes. (A–D) Schematic of fracture fixation, hydrogel/Yoda1 delivery, and hindlimb unloading protocol. (E) μCT analysis showing bone volume fraction, bone surface density, and trabecular separation across groups. Significant differences indicated (*P < 0.05, **P < 0.01).

RESULTS: MicroCT (μCT) analysis demonstrated significantly greater bone volume fraction (BV/VT) in the Yoda 1 group compared to the unloaded HA group and the unloaded control group (P<0.05) (**Figure 1E**). The Yoda 1 group also demonstrated a significantly greater bone surface density (BS/TV) compared to the unloaded control group (P<0.05). Additionally, trabecular separation (Tb/S) was significantly lower in the Yoda 1 group than in the unloaded control group (P<0.01). Trabecular separation was also significantly lower in the both the loaded HA and unloaded HA groups when compared to unloaded controls (P<0.01 and P<0.05, respectively). There were no significant differences between any of the groups with respect to specific bone surface (BS/SV), trabecular thickness, (Tb.Th) and trabecular number (Tb.N).

DISCUSSION: This study demonstrates that local fracture treatment with Yoda1 is associated with superior bone quality at four weeks when compared to treatment with a hydrogel carrier alone during immobilization in a murine polytrauma model. The Yoda 1 group displayed greater bone volume fraction when compared to the hydrogel alone group and the unloaded uninured group. The Yoda 1 group showed superior trabecular morphometric indices including bone surface density and decreased trabecular separation compared to not only the unloaded hydrogel but also the unloaded uninjured group, suggesting that treatment with Yoda 1 not only results in improved fracture healing, but also may be protective against the deleterious effects of decreased weightbearing in the absence of injury.

SIGNIFICANCE:

Impaired fracture healing and disuse-mediated osteopenia can be lasting and devastating complications of immobilization in polytrauma that currently lack effective preventive therapies. Our results demonstrate that fracture healing and bone quality can be improved in the setting of decreased weight-bearing in polytrauma by chemically modulating a mechanotransductive pathway. Our results also demonstrate the therapeutic potential of locally delivered Yoda 1 in improving fracture healing in polytrauma.

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