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Delayed administration of recombinant human parathyroid hormone improves early biomechanical strength in a rat rotator cuff repair model

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Background: Despite advances in intraoperative techniques, rotator cuff repairs frequently do not heal. Recombinant human parathyroid hormone (rhPTH) has been shown to improve healing at the tendon-to-bone interface in an established acute rat rotator cuff repair model. We hypothesized that administration of rhPTH beginning on postoperative day 7 would result in improved early load to failure after acute rotator cuff repair in an established rat model.

Methods: Acute rotator cuff repairs were performed in 108 male Sprague-Dawley rats. Fifty-four rats received daily injections of rhPTH beginning on postoperative day 7 until euthanasia or a maximum of 12 weeks postoperatively. The remaining 54 rats received no injections and served as the control group. Animals were euthanized at 2 and 16 weeks postoperatively and evaluated by gross inspection, biomechanical testing, and histologic analysis.

Results: At 2 weeks postoperatively, rats treated with rhPTH demonstrated significantly higher load to failure than controls (10.9 vs. 5.2 N; $P = .003$). No difference in load to failure was found between the 2 groups at 16 weeks postoperatively, although control repairs more frequently failed at the tendon-to-bone interface (45.5% vs. 22.7%; $P = .111$). Blood vessel density appeared equivalent between the 2 groups at both time points, but increased intracellular and extracellular vascular endothelial growth factor expression was noted in the rhPTH-treated group at 2 weeks.

Conclusions: Delayed daily administration of rhPTH resulted in increased early load to failure and equivalent blood vessel density in an acute rotator cuff repair model.

Level of evidence: Basic Science Study; Biomechanics

Keywords: Rotator cuff repair; parathyroid hormone; tendon healing; biomechanics; rat; rhPTH

The University of Iowa Animal Care and Use Committee reviewed and approved all portions of this work (Animal Protocol #1211240, The University of Iowa—Public Health Service Assurance No. A3021-01).

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More than 270,000 rotator cuff repairs are performed annually in the United States, with a continued increase in rates during the last decade.[9,18,36] Despite advances in intraoperative techniques and postoperative rehabilitation protocols,[22-24,34] rotator cuff repairs frequently do not heal.[5,12,25] Patients with intact rotator cuffs demonstrate increased strength with forward elevation and external rotation compared with repairs that do not heal.[5,32] Thus, decreasing the relatively high structural failure rate after rotator cuff repair should be a priority for treating surgeons.

To improve healing of rotator cuff repairs, biologic augmentation with various local and systemic agents has been explored, including platelet-rich plasma, collagen matrices, recombinant human bone morphogenetic protein-12 (rhBMP-12), and a variety of other osteoinductive factors. However, these treatments have demonstrated inconsistent biomechanical, histologic, and clinical results.[3,6,15,26,29-31,33]

Recombinant human parathyroid hormone (rhPTH) is a biologic agent that has been shown to improve fracture healing[2,17,38] through a proposed chondrogenic pathway.[20,28] The chondrogenic effects of rhPTH are particularly relevant to rotator cuff repair, because the collagenous tendon and calcified bony matrix within the native enthesis are bridged by a combination of fibrocartilage and mineralized fibrocartilage.

A previous study using a rat rotator cuff repair model revealed increased fibrocartilage formation, more type I procollagen–producing cells, and increased vascularity within the rotator cuff insertion out to 8 weeks after daily rhPTH administration.[15] By 8 weeks postoperatively, the load to failure and mechanical stiffness in the group receiving daily rhPTH was increased compared with the untreated control group. However, the same study found load to failure and mechanical stiffness were decreased at early time points after administration of rhPTH, with excessive angiogenesis presumed to cause decreased mechanical strength. Administration of rhPTH during the acute inflammatory phase after rotator cuff repair was suspected to be responsible for the excessive angiogenesis.[15]

The present study investigated the effects of delayed daily rhPTH administration on the biomechanical strength and histologic structure of the tendon-to-bone interface in an established rat rotator cuff repair model. We hypothesized that delaying administration of rhPTH until after the resolution of the acute inflammatory phase postoperatively would reduce the previously noted excessive angiogenesis, resulting in improved biomechanical properties at both early and late time points.

Materials and methods

Animal care and use in this study followed the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health Publication No. 85-23, revised 2010).

Study design

An a priori power analysis was performed using previously published data[15] with a difference in load to failure of at least 25% considered clinically significant. Power analysis revealed that 19 specimens per group dedicated to biomechanical testing would provide appropriate power. Using this information, 108 male Sprague-Dawley rats were obtained to account for attrition, histologic analysis, and mechanical testing of untreated control and rhPTH-treated experimental groups at 2 time points. Half of the rats within the control and experimental groups were euthanized at 2 weeks, and the remaining rats were euthanized at 16 weeks. All 108 rats underwent acute rotator cuff detachment and repair before allocation to the untreated control group (n = 54) or the rhPTH group (n = 54).

The rhPTH group received daily subcutaneous injections of 10 μg/kg of rhPTH (Forteo; Eli Lilly, Indianapolis, IN, USA) based on previous rat studies,[1,27,28,37] with injections beginning on postoperative day 7 and continuing until euthanasia at 2 weeks or until week 12. The untreated control group did not receive injections based on preliminary work that revealed no effect of normal saline injections on rotator cuff healing before establishment of the current acute rat rotator cuff repair model.[8,15]

Postoperatively, all rats were housed in pairs and allowed immediate ad lib activity. At each time point, 23 rats from each group underwent dissection and preparation for biomechanical testing, and 4 rats from each group were dissected and immersed in 10% neutral buffered formalin for histologic analysis.

Surgical technique

Rats were induced in a rodent induction chamber using 3% isoflurane in 100% oxygen. General anesthesia was maintained using isoflurane throughout the duration of the procedure. Respiratory rate, effort, and pedal reflex were monitored to ensure adequate depth of anesthesia. The right forelimb served as the operative extremity. Sterile technique was used. A deltoid-splitting incision was made, and the acromioclavicular joint divided to visualize the underlying rotator cuff, as previously described.[4,15,16] The supraspinatus tendon was isolated using blunt dissection and a curved clamped passed under the tendon. The supraspinatus was then sharply dissected off its insertion on the greater tuberosity. The tuberosity was débrided of all soft tissues and decorticated before rotator cuff repair.

A 3-0 Ethibond suture (Ethicon, Somerville, NJ, USA) was passed through the free end of the supraspinatus tendon in Mason-Allen fashion. Bone tunnels were drilled through the greater tuberosity using a 21-gauge needle. The suture ends were passed through the bone tunnels and tied over the humeral cortex, allowing for anatomic repair at the supraspinatus footprint. The deltoid split was reapproximated using 3-0 Ethibond suture, followed by closure of the subcutaneous tissues with 3-0 Vicryl (Ethicon), and lastly, skin with 3-0 Ethilon (Ethicon) nylon suture. Rats were awoken from general anesthesia and observed by veterinary staff before being placed in paired housing units.

Specimen preparation

Rats were euthanized at 2 and 16 weeks. Rats allocated for biomechanical testing were placed intact into a −20°C freezer and stored whole until biomechanical testing was performed. Rats allocated for histologic analysis underwent immediate dissection of the operative extremity, which included disarticulation of the humerus at the elbow and shoulder. The supraspinatus tendon insertion at the proximal humerus was retained by dissecting the muscle from the supraspinatus fossa. Specimens were placed in 10% neutral buffered formalin before further preparation for histologic analysis.