

Blebbistatin as a Method to Improve Outcomes Following Joint Capsule Release Surgery in a Rat Model

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INTRODUCTION: Elbow trauma can lead to joint contracture and reduced range of motion (ROM). Contracture is characterized by the thickening and stiffening of the joint capsule. Nonsurgical interventions such as physical therapy or splinting can improve ROM, but in some cases capsule release surgery is required¹. Although surgery can be beneficial, it often does not restore full ROM.² Improvements following surgery may also only be temporary. Thus, alternative or adjunctive therapies are needed. Activated myofibroblasts are commonly associated with fibrotic tissue. Myofibroblasts are one of the major drivers of fibrosis. They generate large traction forces and deposit collagen and other extracellular matrix (ECM) proteins at the site of injury³. Mechanical and biochemical cues drive myofibroblast ECM remodeling via a feedback loop that can result in either normal or pathological healing. We hypothesize that the drug blebbistatin can interrupt this feedback loop leading to better ROM. We have previously demonstrated that blebbistatin can safely inhibit myofibroblast contractile forces and reduce collagen synthesis in vitro^{3,4}. The purpose of this study was to assess its use in an animal model of elbow joint contracture.

METHODS: This protocol was approved by the University of Iowa IACUC. Flexion joint contracture was induced in 9 groups of 4 rats (n=36) using a procedure described previously.⁵ The left elbow of each rat was injured surgically and immobilized. The uninjured contralateral arms of each rat were used as a control group. After 6 weeks, 6 of 9 groups received capsule release surgery. In addition to surgery, some groups received blebbistatin via a 20 µL dose of poly(lactic-co-glycolic acid) microparticles. Others received daily injections of 5 mg/kg in a vehicle (or vehicle alone) made of 0.9% saline, dimethyl sulfoxide, and hydroxypropyl β-cyclodextrin. Treatments and timelines are outlined in Figure 1. At the end of the study, all arms were mechanically tested using a similar protocol to a previous study⁵. Briefly, the upper limb was disarticulated at the glenohumeral joint, and the paw was removed at the wrist. The arm was then mounted into a custom-built flexion-extension device. Arms were then cycled between 10 N-mm in flexion and extension. Max angle of extension was used as the primary mechanical outcome since it is the relevant part of ROM in the case of flexion contractures. It was compared for all groups via one-way ANOVA. Comparisons between groups were determined by Tukey's HSD.

RESULTS: Capsule release surgery alone significantly improved ($p<0.01$) the angle of maximum extension 1 week after surgery (Surgery + 1W Recovery) compared to the Injury Only group and was not significantly different from the Uninjured group. Three weeks after surgery (Surgery + 3W Recovery), the maximum extension angle decreased, indicating joint stiffening consistent with what is observed clinically. The addition of blebbistatin-loaded microparticles (Surgery w/ Blebb) did not improve ROM, but blebbistatin injections (Surgery + 3W Daily Blebb) led to better outcomes compared to surgery alone, although not significantly. Vehicle injections (Surgery + 3W Daily Vehicle) did not lead to outcomes significantly different from blebbistatin injections or surgery.

DISCUSSION: Capsule release surgery improved maximum extension angle, but on average the amount of extension decreased 3-weeks post-surgery from that at 1-week post-surgery, which suggests that contracture may be returning following surgery. Blebbistatin tended to mitigate the contracture, though not significantly. Future work will investigate via histology relationships among treatment, fibrotic tissue deposition, myofibroblast activity, and biomechanics in order to determine if blebbistatin (or another compound) is a useful adjunctive therapy together with surgical intervention for treating joint contracture.

SIGNIFICANCE/CLINICAL RELEVANCE: Approximately 8% of patients that undergo surgical treatment for elbow trauma develop joint contracture and current treatment methods provide limited benefit. Joint contracture of the elbow, or any other joint, and the resulting decreased ROM can severely impair an individual's quality of life. Development of new treatment strategies could lessen the disabling impact of joint contracture.

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IMAGES AND TABLES:



Figure 1. Timelines for the 9 treatment groups. Surgery + 1W Recovery represents the group of rats that received blebbistatin microparticles and the group that did not (2 total groups: Surgery w/ Blebb + 1W Recovery & Surgery + 1W Recovery). Surgery + 3W Recovery represents the groups that received microparticles, drug injections, vehicle injections, and the group that did not receive additional treatment (4 total groups: Surgery w/ Blebb + 3W Recovery, Surgery + 3W Daily Blebb, Surgery + 3W Daily Vehicle, & Surgery + 3W Recovery).

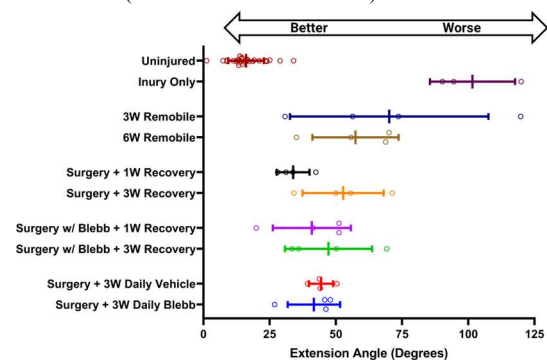


Figure 2. Extension angles for all treatment groups except the 3W Remobile group were significantly better than the Injury Only group ($p<0.01$). Extension angles for all treatment groups except the Surgery + 1W Recovery group were significantly worse than the uninjured controls ($p<0.01$).