

Effect of Relaxin-2 and its Receptor Antagonist on Cultured Patient Anterior Cruciate Ligament Cells

Li Yue¹, Matthew Mu², Ryan Lim², Brett D. Owens^{1,3}

¹Department of Orthopaedics at the Warren Alpert Medical School of Brown University and Rhode Island Hospital, Providence, RI, ²Department of Biology at Brown University, Providence, RI, ³University Orthopedics, East Providence, RI

Email of Presenting Author: li_yue@brown.edu

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INTRODUCTION: Female athletes have a disproportionately higher risk for developing anterior cruciate ligament (ACL) ruptures in the knee, which requires surgical reconstructions. High levels of relaxin-2 is associated with increased risk for ACL injuries, particularly in females. In female ACL cells, relaxin-2 increases the expression of matrix metalloproteinases (MMP)1, MMP3 and MMP13, which degraded type I and III collagens, the critical components of the ACL's structural integrity. We reported that the relaxin-2 receptor RXFP2 was present in ACL tissues from male and female patients with ACL reconstruction. However, it is unknown whether relaxin-2 and its receptor antagonist can be used as a prophylactic option for athletes at risk of ACL injury.

METHODS: Biopsy tissues of the ACL in the knee were collected from 20 patients with ACL reconstruction (ages ranging from 16 years to 61 years old, 10 males and 10 females). We preliminarily identified relaxin-2 antagonists and relaxin-2 receptor antagonists through computational screening of FDA-approved drug library. After computational screening, we chose to test two commonly used molecules-Folic Acid (FA) and NADH, whose safety is established. FA was identified as a relaxin-2 antagonist, and NADH was identified as a relaxin-2 receptor antagonist. Estrogen priming increases relaxin-induced MMPs expression, inhibits collagen generation in the fibrocartilaginous cells, and modulates expression of relaxin-2 receptors. ACL cells were isolated and treated with 100 ng/mL of relaxin-2, 130 nM of MMP3 inhibitor, 1 μ M of 17 β -estradiol in the absence or presence of 10 μ g/ml of FA or 100 μ g/ml of NADH. Expression of type I and III collagen, MMP1, MMP3 and MMP13 genes was measured by using quantitative real-time PCR. Protocol for patient enrollment was approved by the IRB committee at Rhode Island Hospital. GraphPad PRISM 8 software was used to perform all statistical analyses. Results were presented as mean \pm standard error of the mean (SEM) of different groups, and considered significant when $p < 0.05$. A t-test was used to compare any two groups. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs their individual control group. ## $p < 0.01$ vs their individual relaxin-2 group.

RESULTS: In ACL cells isolated from female patients, relaxin-2 treatment enhanced MMP3 and MMP13, and decreased collagen 1A gene expression, whereas these effects were not observed in cells from male patients. Priming treatment with estrogen increased MMP1, MMP3 and MMP13 levels and decreased collagen 1A and collagen 3A levels in relaxin-2-treated cells isolated from female patients. Male and female patient ACL cells primed with either FA or NADH led to a significant decrease in MMP expressions and an increase in collagen 1A levels. MMP3 inhibitor treatment resulted in a significant reduction in MMP3 expression in both male and female ACL patient cells.

CONCLUSION: Relaxin-2 and its receptor antagonists have a significant effect on both male and female patient ACL cells. Additionally, relaxin-2 and its receptor antagonists yielded similar effects in the MMP intracellular pathway within the ACL cells.

DISCUSSION: Although relaxin-2 treatment has no effect on male ACL cells, relaxin-2 antagonist and its receptor antagonist still regulate MMP levels and collagens expression in male cells. Further study is needed to investigate the mechanisms of these effects of FA and NADH in male ACL cells. In addition, further research should be performed to validate whether the effect of FA and NADH through RXFP2 signaling pathways using RXFP2 knock-down method in ACL cells.

SIGNIFICANCE/CLINICAL RELEVANCE: Our discovery provides a potential prophylactic option to prevent ligamentous injury by blocking relaxin-2 and its receptor using FA or NADH in individuals as high risk for ACL injury.

