INTRODUCTION: The incidence of the injury to the knee ligaments has been steadily increased as a result of growing popularity of sport activities. Although all the knee ligaments are somehow involved in knee injuries, anterior and posterior cruciate ligaments (ACL and PCL) are the most important. Because of their poor healing potential, they usually end up in complex reconstruction surgeries [1]. Most clinicians agree that patients with reconstructed ACL or PCL do not possess normal knee functions [2]. With understanding the molecular events involved in the ligament injury, there would be a chance to find a medicine to help the injured ligament in its healing process, reducing the need of ACL or PCL reconstructions.

It is revealed that ligament fibroblasts produce many MMPs, which may be largely involved in processes such as remodeling, repair and degradation of the extracellular matrix [3]. Our previous studies have shown that the Matrix Metalloproteinase-1 (MMP-1), MMP-2 and MMP-9, enzymes effective in digesting the collagen fibers of the ligament, are increased following injury to the ligaments. This increase will digest the remaining collagen fibers of the ligaments, changing a sprain to a complete rupture.

In order to find a way to eliminate the MMPs detrimental effects, the following research was designed on the PCL ligament. Four in vitro experiments were done to evaluate separate cellular phenomena: 1) The MMP-2 and MMP-9 activities in constant PCL fibroblast stretch. 2) The effect of different signal pathway inhibitors on the MMP-2 and MMP-9 expression. 3) The MMP-1 expression after PCL injury. 4) The effect of different signal pathway inhibitors on the MMP-1 expression.

METHODS: Cell culture: PCL fibroblast was obtained from human explants provided by the San Diego Human Tissue Bank. Tissues were cleaned by trypsin-PBS and subjected to collagenase series digestions, followed by suspension in culture medium (DMEM system). Media was changed every two days. At 85% confluency, cells (primary to passage 2) were trypsinized and seeded onto culture flasks or stretch chambers with complete DMEM. The cells were allowed an additional 24 hours to seed and equilibrate. In vitro injury: PCL fibroblasts (40,000) were seeded on silicone membrane of equi-biaxial stretch chamber and were exposed to 12% stretch for 4, 12, 24, and 48 hours. The media was collected overtime for Zymography. Signal pathway inhibitors: After 24 hours the culture media was replaced by fresh inhibitor-containing media and cells were immediately subjected to 12% stretch for 4, 12, 24, and 48 hours. The media was collected overtime for Zymography. The signal pathway inhibitors used were G protein (pertussis toxin, 10 nM), p38 (SB203580, 3 M), ERK (PD98059, 50 M), AP-1 (curcumin, 50 M), and NF-κB (BAY 11-7082, 10 M). MMP-1 ELISA: MMP-1 ELISA was performed with Oncogene kits, according to the manufacturer’s protocol. 100 μl of each sample were used, and after spectrophotometry, the standard curve was made by the MMP-1 protein provided by the kit. The samples used were from the stretch control, NF-κB, ERK, AP-1 and p38 media, after 48 hours of constant stretch.

RESULTS: Stretch injury increased Pro-MMP-2 and Pro-MMP-9 activities: Zymography results showed that stretching PCL fibroblasts at a pathologic level (12%) increased the activity of pro-MMP-2 and pro-MMP-9 and was increased in the course of time (Fig. 1).

DISCUSSION AND CONCLUSION: The first study showed that the MMP-2 and MMP-9 were released into the media immediately after stretching and the expression was increased with time. This may explain why PCL repair process is not the same as many other ligaments with the potential of spontaneous repair.

The next study showed that different signal pathways are effective in regulation of MMP-2 and MMP-9 expression. In particular, MMP-2 production levels in PCL increased when the p38 and NF-κB pathways were inhibited. These results show that PCL fibroblast characteristics are totally different from ACL ones. Our previous study showed that in ACL, NF-κB, AP-1 and G protein were effective in the MMP-2 expression but ERK and p38 were not. This difference indicates that multiple pathways that regulate the MMP expression in ACL are different from PCL. The last study showed that in MMP-1 expression, like MMP-2 and MMP-9, different pathways are effective.

By inhibiting the signal pathways effective in MMPs production and by inhibiting the inflammatory factors inducing them, authors believe that it would be possible to make a cocktail to prevent the digestion of the remaining injured ligament, changing the treatment from a challenging ligament reconstruction to a simple cocktail injection.


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