MMP-2 EXPRESSION OF THE INJURED, SIGNAL TRANSDUCTION PATHWAY MEDIATED ACL IN EX VIVO & IN VIVO

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INTRODUCTION: The anterior cruciate ligament (ACL) is a major ligament contributing to the stability of the knee joint. However, the ACL has very poor healing potential, compared to injured medical collateral ligament (MCL) [1]. Re-operations of the reconstructed ACL are frequently reported [2]. 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]. Authors tried to evaluate the expression of MMP-2 in the injured ACL and to find out the possible signal transduction pathways involved in tissue remodeling process of the ligaments via ex vivo ACL stretch in the rats.

METHODS: Experimental groups and Animal experiment: First, the femur-ACL-tibia complexes were harvested from 20 wistar rats (300 to 400 g) and were divided in 2 groups as below. (1) Group I: stretched by 5 and 12N for 10, 30, 60 minutes separately. (2) Group II: 15N for 30 minutes and various signal pathway inhibitors were added to the supernatants. Reagents: Pertussis toxin is a G protein inhibitor and SP600125 is a JNK inhibitor. PD98059 is an ERK inhibitor and KT5720 is an inhibitor of PKA. Curcumin is an AP-1 inhibitor and BAY11-7082 is a NF-κB inhibitor. The final concentration used are as following: Pertussis toxin, 2 u/ul; SP600125, 1.5 u/ul; PD98059, 1.5 u/ul; KT5720, 1.5 u/ul; Curcumin, 2 ul; Bay11-7082, 2 ul. Pertussis toxin, Curcumin and Bay11-7082 reagents were first prepared as 150X and SP 600125, PD 98059 and KT 5720 were prepared as 200X in appropriate solution.

Sample collection: After completion of stretching injury, the ACL were carefully isolated and transferred into 96-well plate with 300 ul 10% FBS-DMEM media. The samples were incubated at 37°C and 100 ul supernatant sample was separately collected at 6 and 12 hours in the group I and 24 and 48 hours of incubation in the group II. After collection of supernatant, the ligaments were removed from media and minced into small fragments, mixed with 300 ul Laemml sample buffer and grinded until homogeneity was reached. MMP-2 activity assay: Pro- and active MMP-2 activity of the samples were evaluated using 0.05% gelatin zymography. Samples were separated in 10% SDS-PAGE gel and washed with 2.5% Triton-X-100 for 1.5 hours. The gel was incubated in proteolysis buffer (50 mM CaCl2, 0.5 M NaCl, 50 mM Tris, pH 7.8) for 15 hours. Then, the gel was stained with 0.25% Coomassie Brilliant Blue and destained for visualization of proteolytic bands.

RESULTS:
1. Increased MMP-2 release after Rat ACL injury. After rat ACL was injured, very high amount (about 10 fold higher) of MMP-2 was released compared with control (Fig 1).
2. Group I: Pro-MMP-2 expression was increased in proportion to the stretching time and magnitude in all samples and the changes were much more remarkable in 12 hour collection sample. Active form of MMP-2 was not observed (Fig 2).
3. Group II: Compared with the stretch group, MMP-2 expressions were slightly decreased in SP600125- and PD98059-added groups and significantly decreased in curcumin- and Bay11-7082-added groups in 24 hours collection samples. In pertussin toxin- and KT5720-added groups, the signals were slightly increased rather than decreased. In 48 hours collections samples, the MMP-2 expressions was generally decreased, compared with the stretch group. Among them, the expressions in PD98059- and curcumin-added groups were much more decreased than other groups (Fig 3).
4. ACL tissue study In the ligament tissue analysis, pro- and active MMP-2 was definitely decreased in SP600125-, PD98059-, curcumin- and Bay11-7082-added groups, compared with the stretch group, while the expression in pertussin toxin- and KT5720-added groups was increased reversely (Fig 4).

DISCUSSION AND CONCLUSION: MMP-2 was released promptly into the media after ACL injury and it was increased correspondingly in proportion to the stretching duration and magnitude. As for the changes of the MMP-2-related to the signal transduction pathways, JNK, ERK, AP-1 and NF-κB pathways were involved in the modulation of MMP-2. The inhibition of their pathways caused the expression to be decreased. However, G protein and PKA pathways did not affect MMP-2 expression.


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