Introduction: The healing potential of the ACL is extremely poor compared to the medial collateral ligament. The difference in healing capacity between ACL and MCL has been previously investigated, and many factors, including blood supply, anatomical location and intrinsic cellular properties, have been suggested[1-4]. Tissue resorption and remodeling is essential to the natural healing process after injury and the matrix metalloproteinases(MMPs) have been found to be closely involved in these processes. In order to further elucidate the relationship between MMP-2 and ACLs injury/healing mechanisms, we conducted experiments investigating MMP-2 expressions in ex vivo ACL stretch and the possible signal transduction pathways involved in tissue remodeling process of the ligaments.

Materials and Methods: Firstly, authors investigated whether MMP-2 was released from the ligament or not and if so, how long it was released. After the femur-ACL-tibia complex was harvested from 10 wis-tar rats, we stretched the ligament using 15N for 30 minutes and checked MMP-2 expression for 1 week. And then, the ligament complex was har-

Results: MMP-2 was continuously released from the ligament tissue for a week and the signal intensity was gradually increased with the lapse of time(Fig. 1). (1) Group I: In 5N-stretched group, only slight increases in pro-MMP-2 levels were observed in the 5 and 10 minute samples. Starting from the 10-minute sample of the 15N-stretched group, slight increases in expression level of MMP-2's pro-form were observed and the magnitude got bigger in response to the increase in stretch time(Fig. 2-A). In the ligament tissue sample, both pro- and active MMP-2 were observed and their levels augmented with increased stretch magnitude. However, for the 5-minute stretch duration, MMP-2 expressions of the 15N-stretch samples were significantly stronger compared to the barely visible MMP-2 bands of the 5N-stretch group, an outcome which was not observed in our evaluations of the supernatant samples(Fig. 2-B). (2) Group II: In the supernatant samples, we detected only pro-MMP-2 levels and the control and 5N-stretch groups were observed to have expressed relatively equal amounts of pro-MMP-2. In all other stretched groups pro-MMP-2 level has slightly increased in response to an increase in stretch magnitude. This was more evident in the 60-minute stretched group(Fig. 3-A). In the ligament tissue analysis, both pro- and active MMP-2 levels were observed and both levels increased with an increasing stretch magnitude. However, unlike the supernatant sample, an increase in MMP-2 levels was noticeable in 5N-stretch group compared to the control group(Fig. 3-B). (3) Signal transduction pathway: MMP-2 expressions were slightly decreased in SP600125- and PD98059-added groups and definitely decreased in curcumin- and Bay11-7082-added groups in 24 hours collection samples.

Discussion: Expression of MMP-2 level was increased in proportion to the magnitude of injury to the ACL. As for injury time, the increase in MMP-2 expression level started to accumulate after 5 minutes of injury in the 15N stretch whereas the increase first became visible after 10 minutes of injury in the 5N stretch. It is suggested that after a damaging force was applied to the ligament, 5-minute injury time was enough to release MMP-2. It took much more injury time to release MMP-2 in case of less injurious force. In addition, active MMP-2 in the ligament tissue sample was expressed earlier than in the supernatant sample. It might be related with the conversion time of pro-MMP-2 to active form. It is considered that once a ligament is injured, pro-MMP-2 is instantly released and it takes time to convert pro-form into active form in the ligament tissue and be released into the media. Excessive amount of MMP-2 may disrupt the delicate balance between removal of damaged matrix components and the deposition of newly synthesized matrix during the injured ACL remodeling processes. It is conceivable that the signal pathways which are affected by MMP-related factors will be able to modulate MMP-2 activities. In this experiment, when JNK pathway, ERK pathway, AP-1 pathway and the NF-κb pathway were inhibited, the MMP-2 expression levels were decreased in the injured ACL. However, G protein pathway and PKA pathway did not affect the MMP-2 expression level in the injured ACL.


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