Apoptosis in the Torn Rotator Cuff Tendon

Department of Orthopedic Surgery, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea

Yang-Soo Kim, M.D. · Jung-Man Kim, M.D. · Yun-Kyoung Lee, M.S. · Jae-Young Lee, M.D.

Introduction

The pathogenesis of rotator cuff tendon tear is one of the most debating issues in the shoulder. The mechanical impingement, vascular factors, inflammation, tendon degeneration by aging, and apoptosis in the tendon have been known as contributing factors for development of rotator cuff tendon tear. Recently, the role of apoptosis, or programmed cell death, in the tendon has come under close scrutiny. There were, however, limited reports about the presence and characteristics of apoptotic cells in the rotator cuff tendon. Especially, there is no study, to our best knowledge, on the spatial distribution of apoptosis within the rotator cuff tendon. The purpose of this study was to elucidate the spatial distribution of apoptosis within the supraspinatus tendon and compare it to normal. This study will be helpful to understand the cellular and molecular bases of tendon degeneration.

Materials and Methods

Caspase-3/7, 8, and 9 activities were quantified at each tissue samples for elucidating intracellular apoptosis pathway using caspase-Glo 3/7, 8, 9 activity assay (luminescence spectrometer SL50B). Apoptotic cells were determined by an in situ terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end-labeling (TUNEL) stain and immunohistochemistry was used to detect the caspase-3/7, 8, 9 within the tendon. Statistical analysis was performed with student-t test and Mann-Whitney U test.

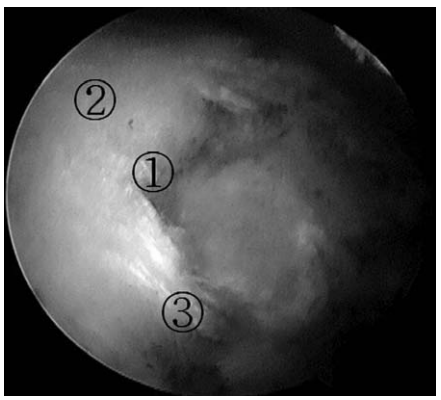

Fig. 1.

Results

Table 1.

	Caspase-8	Caspase-3/7	Caspase-9
Control	1243±58.077	1045±58.095	1900±85.44
1	2118±140.388	2136±185.829	4218±218.635
2	1866±172.874	2021±279.873	3847±315.561
3	1803±75.159	1676±50.084	3655±294.457

Table 1 and graph showed the average activities(RLU) of capase-3/7, 8, 9 quantified by activity assay. The apoptotic activities of sample ①, ②, ③ were significantly increased than controls. There were, however, no significant differences among three samples sites. A similar trend was observed with caspase-3/7, 8, 9 immunohistochemistry.

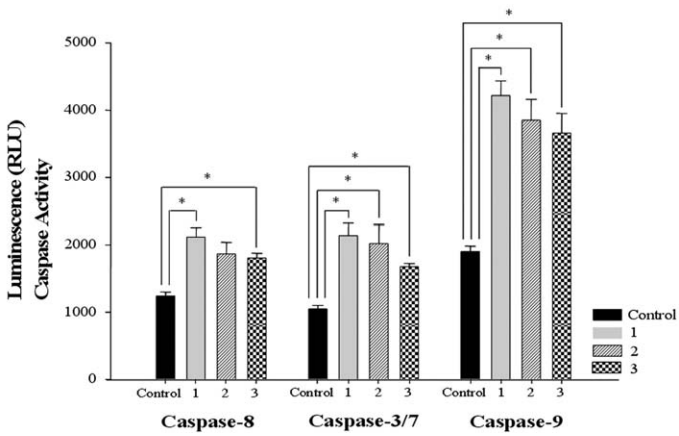

Fig. 2.

Figure shows the significantly increased activities of caspase 3/7, 8, 9 in tissue sample ①, ②, ③ compared to controls. Especially, the caspase 9 activity sharply incresase in all tissue samples. In situ TUNEL assay showed a large number of positive staining cells were observed in the sample ①, ②, ③, whereas few apoptotic cells were observed in control tendons.

Discussion & Conclusion

Apoptosis is a physiological process leading to a highly regulated, programmed cellular suicide mechanism which is controlled by numerous proteins, including a family of cysteine proteases known as caspases. Caspases regulate apoptosis by cleaving cellular proteins at several steps within a proteolytic cascade. Caspase 8, 9 are known as 'initiator' since they are

usually coupled to upstream pro-apoptotic signals. Once activated, these initiators triggered 'executioner' caspases including caspase 3, 7 which operate the final cell death processes.

Distinct findings of this study are threefold. First, the torn suprapinatus tendon showed the significantly increased apoptotic activity compared to control tendons. This finding is agreed with the previous studies and confirmed that the degeneration is closely related with rotator cuff tendon tear. Second. this study newly elucidate that the intracellular apoptotic pathway is not only through the cell membrane receptor, but also via mitochondria cascase since the activation of caspase 8 is initiated from the cell surface death receptor and caspase 9 is triggered by changes in mitochondria integrity. Especially, the upregulated caspase 9 indicated that initiation of apoptosis is caused mainly by intrinsic intracellular stress rather than extracellular stress. Third, the apoptosis of three sites showed no significant differences. This result demonstrates that the degeneration and apoptosis occurred at the same time regardless location in the tendon. That is, the degeneration starts not only at the insertion site, but also at all area simultaneously within the tendon. And once tear occur at lateral insertion site by another factors like mechanical impingement, the tear propagate into any direction. We sometimes observed that re-tear of repaired rotator cuff tendon occurred at the very medial site of sutured point of tendon, not avulsion of insertion site. This study would help to understand the spatial distribution pattern of apoptosis and it's influence on tear morphology in the rotator cuff tendon.