ANALYSIS OF COLLAGENS SYNTHESIZED BY CELLS HARVESTED FROM MCL IN THE EARLY STAGES OF HEALING

Introduction: Following complete rupture, the medial collateral ligament (MCL) heals, but does not fully recover its original biomechanical, morphological, or biochemical properties even after two years (1,2). With an influx of various types of cells, type III collagen synthesis is increased and accumulates in the healing tissue (2). Specifically, the high content of type III collagen is thought to result in the formation of smaller collagen fibrils (3). Recently however, type V collagen was also shown to be elevated in healing (4). Therefore, the objective of this study was to examine changes in types III and V collagens, in relation to type I collagen, by cells harvested from scar tissues in the early stages of MCL healing. It was hypothesized that cells isolated from the healing ligament will exhibit an increase in type III and V collagen synthesis when compared to cells isolated from normal MCL.

Materials & Methods: Sixteen skeletally mature (12 months old) New Zealand White Rabbits underwent a mop-end tear of the MCL of the left leg. The animals were equally divided into 4 experimental groups, sacrificed at 0, 3, 7, and 14 days after surgery, and the MCL’s were harvested. From the healing groups, each MCL scar was minced, and the explants were grown to 3, 7, and 14 days after surgery, and the MCL’s were harvested. From the time 0 group was confluenct in Ham’s F-12 nutrient mixture supplemented with 10% fetal healing groups, each MCL scar was minced, and the explants were grown to 3, 7, and 14 days after surgery, and the MCL’s were harvested. From the time 0 group was confluenct in Ham’s F-12 nutrient mixture supplemented with 10% fetal bovine serum and 1% antibiotics at 37°C in 5% CO2. The time 0 group was treated in the same way to obtain cells. Once confluent, the cells were trypsinized and seeded into 6 well plates, in duplicates, at a concentration of 1x10^5 cells/well. After 48 hours, cells were washed twice with Gey’s Balanced Salt Solution and then cultured in serumless medium supplemented with 50µg/ml L-ascorbic acid for 24 hours. Twenty-four hours later, cells were transferred into a serumless medium supplemented with 50µg/ml L-ascorbic acid, 50µg/ml β-APN, and 10µCi/well of [3H]-proline. After 16 hours, cell lysates and the medium of each well were combined and treated with pepsin (100µg/ml) in 0.5M acetic acid for 4 hours. The pepsin resistant proteins were recovered by centrifugation, and protein pellets were suspended in 1ml of 0.05M NH4HCO3 and dialyzed against the same buffer for 4 days. Samples were subsequently freeze dried and analyzed by SDS-PAGE under delayed reducing conditions followed by autoradiography. Purified collagens type I, III, and V were electrophoresed in conjunction with experimental samples and served as standards (4). Autoradiographs were scanned, and the areas under the peaks for α 1(III) and type I and V collagen α chains were used to determine the collagen type ratios.

Statistical analysis was performed to determine differences in collagen ratios between experimental groups using an unpaired Student’s t-test with significance set at p<0.05.

Results: Cells recovered from normal MCL synthesized approximately equal amounts of type III and type V collagens. At 3 days after rupture, cells harvested from the healing MCL tissue showed an increase in type III and type V collagen synthesis as indicated by a higher ratio of these collagens (by 13 and 13.6% respectively) to type I collagen (fig. 1,2). Cells recovered from MCL 7 days after rupture showed a significant decrease in the collagen type III:1 ratio, while the ratio of type V:1 remained elevated (fig. 1,2). By 14 days after rupture, the cells harvested from the healing tissue still showed a decrease in the collagen type III:1 ratio compared to that of the 3 days group, but the ratio of type V:1 remained elevated.

Discussion: In the early phase of MCL healing, there is a rapid, and increased, synthesis and deposition of collagen types III and V as compared to normal MCL. We have shown in the present study however, that in rabbits, this initial increase in collagen type III in relation to type I may be limited only to the first 3 days of healing. In contrast, type V collagen is consistently synthesized at elevated levels throughout the first two weeks of healing. These data are in agreement with the previous analysis of tissues which demonstrated that healing MCL contained elevated levels of type V collagen up to 1 year after MCL injury (4). Healing ligaments have been shown to contain smaller collagen fibrils than normal ligaments (5). The present data suggests that both type III and V collagens may contribute to the smaller collagen fibrils observed in healing MCL. The data also suggest that therapeutic interventions to improve ligament healing should involve suppression of type III and V collagens.

Fig. 1: Ratios of collagen type III:I synthesized by cells at different times after MCL injury. * significant difference between time-points (p<0.05)

Fig. 2: Ratios of collagen type V:I synthesized by cells at different times after MCL injury. * significant difference between time-points (p<0.05)

References:

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