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Dilek TAŞKIRAN[1]
Emin TAŞKIRAN[2]
Hakan ÖZSOY[2]
Veli LÖK[2]

Effects of Surgical Trauma on Articular Cartilage

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Departments of [1]Physiology,
[2]Orthopaedics, Faculty of Medicine,
Ege University Bornova, 35100 İzmir-Turkey

Abstract: This study was designed to evaulate the effects of reconstruction of anterior cruciate ligament on cartilage collagen and proteoglycan metabolism. Proteoglycan fragments (PF) and matrix metalloproteinase-1 (MMP-1) levels were assessed in synovial fluids aspirated from 17 patients preoperatively and at 1st month postoperatively. Synovial fluids of 10 healthy knee served as controls. Both MMP-1 and proteoglycan fragments were found significantly elevated in pathological knees preoperatively with compare to control. The postoperative levels of MMP-1 and proteoglycans were also significantly higher than preoperative and control values. These findings suggest that surgical trauma may have detrimental effects on cartilage matrix metabolism through increasing degradation of proteoglycan and collagen.

Key Words: Proteoglycans, matrix metalloproteinase, cartilage, anterior cruciate ligament, trauma.

Introduction

Homeostasis of articular cartilage in a healthy joint is influenced by several factors such as injury, synovitis, bleeding etc (1-4). Biochemical markers in synovial fluid have been shown to be elevated after injury involving ACL ligament (4, 5). In a recent study, Taşkıran et al. have clarified that proteoglycan metabolism is effected by surgical trauma and homeostasis of proteoglycan metabolism could not be provided until the end of 12th month with respect to control values (3). In our knowledge, affections of other components of articular cartilage such as collagen after surgical trauma has not been studied yet.

A main role in cartilage matrix degradation has been suggested for the metalloproteinase family. Metalloproteinases, such as collagenase and stromelysin, are produced by chondrocytes and synovial fibroblasts and considered to play a major role in the proteolytic degradation of extracellular matrix in joint and skeletal diseases. These enzymes are secreted in a proenzyme form and the destruction of articular cartilage is thought to be the result of the action of these potent enzymes (6-8).

Collagenase (EC 3.4.24.7, matrix metalloproteinase-1 (MMP-1)) cleaves the collagen triple helix at a single locus to yield characteristic 1-quarter-3-quarter products. The major secreted form of collagenase is a latent proenzyme of M_r 51, 929, which can be N-glycosylated to a minor form M_r 57,000. Procollagenase can be activated at least in vitro, by proteinases and mercurials (6, 9).

In the present study, we aim to demonstrate the effects of surgical trauma on degradative enzyme activity and the correlation between proteoglycan and collagen degradation in articular cartilage.

Material and Methods

Patients: Thirty anterior cruciate ligament (ACL) reconstructed knees comprised the study group. Average age was 27 (range: 18 to 42) and male to female ratio was 20:10. Synovial samples were obtained preoperatively and at the end of first month postoperatively. While proteoglycan fragments were measured in all samples (n=30), MMP-1 activity was measured only in randomly selected 17 knees of 30.

Controls: Ten asymptomatic opposite knees of any patient who underwent arthroscopic surgery rather than ACL reconstruction consisted of control group.

Collection of synovial fluid: Informed consent was obtained from all patients prior to investigation. Synovial

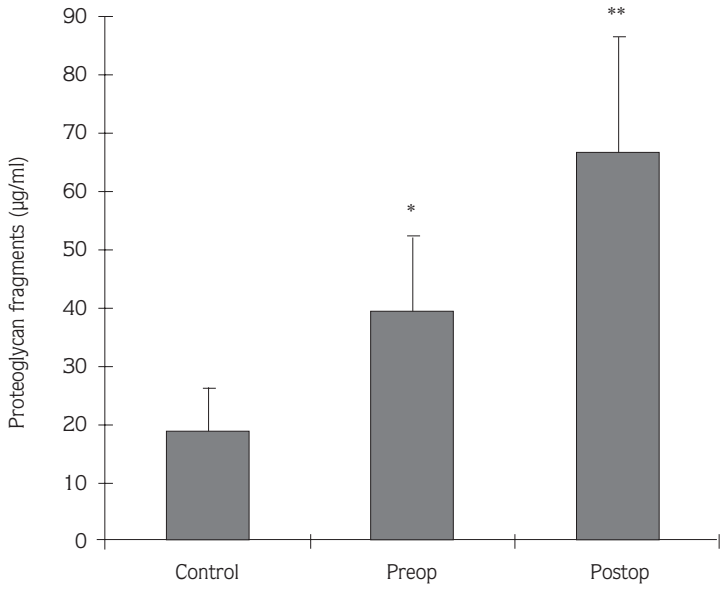

Figure 1. Proteoglycan fragments (µg/ml). Bars represent mean value±SD.* different from control ($p<0.01$), **different from preoperative values ($p<0.05$).

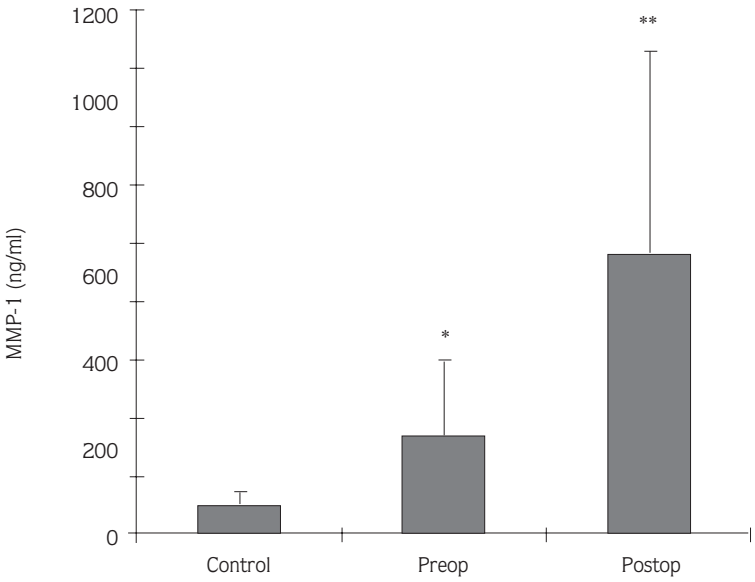

Figure 2. MMP-1 levels (ng/ml). Bars represent mean value±SD.*different from control ($p<0.05$), **different from preoperative values ($p<0.05$).

fluid samples were aspirated preoperatively and at 1st month postoperatively. Aspiration procedure was performed in the operating room under sterile conditions. Firstly, 20 ml of physiological serum was injected into the knee joint in all cases. Then, twenty cycles of passive motion were applied prior to aspiration. Synovial fluids were centrifuged to remove the cells and debris and the supernatants were stored at -30˚C until the assay.

Proteoglycan assay: Cartilage breakdown was determined by the amount of proteoglycans released into knee joint and proteoglycan fragments were measured using dimethylmethylene blue (DMMB) assay (10). Before the assay, aliquots of synovial (500 µl) were added to 500 µl 20 mM of sodium phosphate buffer (pH 6.8) containing 1 mM EDTA, 2 mM dithiothreitol, 4 mM N-acetylcysteine and 150 µg papain. All samples were then mixed and incubated for two hours at 65˚C. Digestion was stopped adding 10 mM of iodoacetic acid and then 250 µl of the sample was mixed with dimethylmethylene blue as described previously (10). The assay was

calibrated with shark chondroitin sulphate and suitable blanks. It was found to be linear from 2.5 to 50 µgr/ml. Results were given mean values obtained from three assays.

Collagenase (MMP-1) assay: Collagenase was assayed using double-antibody sandwich ELISA kit which is commercially available from Amersham™. The synovial fluids were diluted 1:10 and 1:20 and assays were performed at 1:10 dilutions when levels wereoo low to measure at 1:20 and assays were performed at 1:10 dilutions when levels were too low to measure 1:20 dilution. The standard curve was obtained between the 6,25-100 ng/ml.

Statistical analysis: To find the statistically differences between control, preoperative and postoperative values Wilcoxon matched paired test was performed. Spearsman's correlation test was used to evaluate the correlation between PF and MMP-1 levels. All data were expressed as means±SD and the limit of statistical significance was set up at $p<0.05$.

Results

MMP-1 levels were significantly higher in pathological knees (234.73±178.07 ng/ml) preoperatively than those in controls (69.41±41.73 ng/ml, $p<0.05$). MMP-1 level at the end of the first month (685.18±487.97 ng/ml) was significantly different when it was compared with preoperative values ($p<0.05$) and control values ($p<0.01$) (Figure 1).

Proteoglycan fragment levels were found significantly higher in pathological knees (38.55±13.05 µg/ml) preoperatively with respect to controls (18.26±7.33 µg/ml, $p<0.01$) and the values obtained at 1st month were significantly higher than control ($p<0.01$) and preoperative values similar to MMP-1 levels (65.83±19.74 µg/ml, $p<0.05$), (Figure 2).

There was no significant correlation between the pre and postoperative PF and MMP-1 levels ($r=0.449$, $p=0.062$).

Discussion

Matrix metalloproteinases are critical in maintaining the proper biochemical composition and physical characteristics of all tissues particularly in cartilage matrix. At the transcriptional level, MMP expression is precisely controlled by various cytokines acting through positive or negative regulatory elements of its genes (11). Moreover, MMP activity is post-transcriptionally regulated by proteolytic activation of the latent proenzymes and by interaction with specific tissue inhibitors of metalloproteinases (TMPs) (12). Under normal physiological conditions, constitutive expression of the MMPs is low. However, under pathologic conditions such as inflammation, injury, osteoarthritis, rheumatoid arthritis MMP expression in cartilage is disregulated (13, 14).

Recently, Lohmander et al. have indicated increased levels of proteoglycan fragments, stromelysin-1 and collagenase in knee synovial fluids of ACL or meniscus injured patients (15). In our study, preoperative levels of MMP-1 and PF levels showed similar pattern and they were found significantly higher in preoperative knees than the normal knees. The proteolytic breakdown of the matrix molecules seems to be due to a change in biomechanics of the injured knee.

In the present study, both postoperative PF and MMP-1 levels were found elevated with respect to preoperative values. These findings reflect some significant changes in the collagen and proteoglycan metabolism after the surgery. It is possible to say hemarthrosis, and joint debridement may have some activational effects on cartilage catabolism during the surgery. Furthermore, since synovium is one of the tissues which produce proteinases, synovial changes after ACL reconstruction (16) may cause activation of metalloproteinases and degradation of proteoglycans and collagen.

Although levels of PF and MMP-1 were found elevated pre and postoperatively, there was no significant correlation between them. In accordance with this data, Wolfe et al. have demonstrated that the regulation of collagenase is distinct from stromelysin which is mainly responsible for proteoglycan degradation and these two metalloproteinases have different roles in normal and abnormal cartilage (14). In a recent study, Kozaci et al. have showed a correlation between the MMP activity and type II collagen, but not proteoglycan, in cartilage cultures (17). These observations reveal that collagen and proteoglycan have different rate of metabolism during cartilage matrix turnover in normal and disease.

In an injury model, La Fleur et al. investigated the roles of matrix metalloproteinases and TIMP-1 and observed both of MMPs and their inhibitors were induced after tissue injury and expression of mRNAs of MMPs and TIMP-1 were regulated by cytokines in vivo (18). In another study, Moses et al. assessed the involvement of MMPs and their inhibitors in normal wound healing by measuring MMP-1, MMP-2 and MMP-9. They detected the presence of MMP-1 in the wound fluid during days 1-

6 after wounding and the time course activity of matrix metalloproteinase inhibitors (19). These findings clearly indicate that MMPs can be activated during the injury and both MMPs and their inhibitors have importance in remodelling of extracellular matrix.

From our data we can conclude that surgical trauma has some detrimental effects on the articular cartilage and it is probably due to action of metalloproteinases. Our results may provide very important clues about the postoperative status of cartilage but more detailed experimental studies are required.

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