The effect of arthroscopic surgery and intraarticular drug injection to the antioxidation system and lipid peroxidation at osteoarthritis of knee

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ABSTRACT

الأهداف: يعتبر الالتهاب العظمي المفصلي من أكثر أمراض المفاصل التنكسية شيوعاً. الهدف من هذه الدراسة هو من أجل توضيح آثار تنظير المفاصل والعلاجات داخل المفصل على نشاط الأنزيم المضاد للتأكسد ودهون فوق الأكسدة.

الطريقة: أجريت هذه الدراسة في الفترة ما بين يناير 2005م ومايو 2005م. شملت هذه الدراسة إجمالي عدد 60 من المرضى الذين يعانون من ألم في الركبة والذين شخصت حالتهم بالتهاب عظمي مفصلي في الركبة وفقاً لتصنيف الكلية الأمريكية لأمراض الروماتيزم (ACR) وتم تقسيمهم بشكل عشوائي وبالتساوي الى أربعة مجموعات. تلقت المجموعة الأولى المعالجة بعقار هيلان G-F 20 داخل المفصل لمدة ثلاثة أسابيع. تلقت المجموعة الثانية المعالجة بعقار هيلان G-F 20 بالإضافة الى فيتامين Eعبر الفم لثلاث أسابيع. أجريت المعالجة بالمنظار للمفاصل فقط للمجموعة الثالثة(مجموعة التحكم). تلقت المجموعة الرابعة المعالجة بعقار هيالوروينت – ان أيه لمدة خمسة أسابيع. تم اخذ عينات الدم والسائل الزليلي من جميع المرضى لإجراء التحليل الكيميائي الحيوي وتم إجراء القياسات التالية: سوبرأوكسيد ديسموتيس (SOD)، كاتاليس (CAT)، جلوتاثون بيروكسيديس (GPx) ومالونديالديهيد (MDA).

النتائج: لم يكن هنالك تغير ملحوظ في مستويات (SOD) و(CAT) و(GPx) في الدم ومستويات(SOD) و(CAT) و(GPx) في السائل الزليلي لدى المجموعة التي تمت معالجتها بعقار هيالورونان عند المقارنة مع مجموعة التحكم. ولكن كان هنالك تغير ملحوظ في عينات مستويات (MDA) في السائل الزليلي للمجموعة الأولي والمجموعة الثانية والمجموعة الرابعة عند المقارنة مع مجموعة التحكم.

خاتمة: قد تقلل المعالجة بالهيالورونان للالتهاب العظمي المفصلي عقب تنظير المفاصل الدهون فوق الأكسدة في السائل الزليلي.

Objective: To elucidate the effects of arthroscopy and intraarticular hyaluronan therapies on antioxidant enzyme activity and lipid peroxidation.

Methods: This study was conducted between January and May 2005, at the Department of Orthopedics and Traumatology, Suleyman Demirel University, Isparta, Turkey. A total of 60 patients with knee pain who were diagnosed as knee osteoarthritis according to the American College of Rheumatology (ACR) criteria were included in this study and randomly and equally divided into 4 groups. Intraarticular Hylan G-F 20 treatment was given to group 1 for 3 weeks. Intraarticular Hylan G-F 20 treatment plus oral vitamin E were administered to group 2 for 3 weeks. Only arthroscopy treatment was applied to the control group (group 3). Intraarticular Na-hyaluronate treatment was given to group 4 for 5 weeks. Blood and synovial fluid samples were taken from all the patients for biochemical analysis, and the following parameters were measured: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and malondialdehyde (MDA).

Results: There was no significant difference in blood SOD, CAT, GPx levels, and synovial SOD and GPx levels in groups treated with hyaluronan, when compared with the controls. However, there was a significant change in MDA levels in synovial fluid samples of group 1, group 2, and group 4, when compared with the controls.

Conclusion: In knee osteoarthritis, intraarticular hyaluronan therapy following arthroscopy may diminish lipid peroxidation in synovial fluid.

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Osteoarthritis is a non-inflammatory and chronic disease of the joints characterized with pain, stiffness, activity restriction, cartilage damage, and new bone formation on joint surfaces. It leads to deterioration in locomotor system functions. Reactive oxygen species (ROS) are hazardous compounds that can be formed in many physiologic and pathologic reactions. They can initiate some reaction cascades, which in turn can destroy proteins, lipids, and nucleic acids. The importance of ROS involvement in pathophysiology of several cases has been revealed in recent past. Thus, the role of ROS in cartilage damage is notable in this context. The molecular weight and concentration of hyaluronic acid, which is an important compound of synovial fluid, decreases in osteoarthritis, leading to a decrement in viscosity and elasticity of the joint fluid. In the recent past, new aspects and methods have emerged in the treatment of knee osteoarthritis. Intraarticular hyaluronate injection is one of these methods. After intraarticular hyaluronic acid therapy, viscosity, and level of hyaluronic acid significantly increase. It is assumed that this injection therapy stimulates the production of hyaluronic acid and accelerates the regeneration process in joints. In recent studies, statistically significant results have been reported. Arthroscopy provides us new perspectives in diagnosis and treatment of especially knee joint disorders. The old-fashioned 'wait-and-see' method was replaced with today's new 'see-and-apply' approach. In current practice, arthroscopy is being used effectively in knee osteoarthritis surgical treatment. There are few studies in the literature regarding the effect of ROS on the pathogenesis of osteoarthritis or the effect of treatment on ROS. The aim of this study is to reveal whether post-arthroscopical viscosupplementation therapy has an effect on oxidative stress in osteoarthritis by measuring the levels of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and malondialdehyde (MDA) in blood and synovial fluid.

Methods. A total of 60 patients (24 men and 36 women) diagnosed as having knee osteoarthritis according to the criteria of the American College of Rheumatology (ACR) were included in this prospective study and were separated randomly and equally into 4 groups, each of which consisted of 15 patients as follows: group 1 (5 men, 10 women), group 2 (6 men, 9 women), group 3 (7 men, 8 women) and group 4 (6 men, 9 women). This study was conducted between January and May 2005 at the Department of Orthopedics and Traumatology, Suleyman Demirel University, Isparta, Turkey. Before the treatment procedure, written consent was provided from the participants. Ethical approval was provided from the Ethical Committee of Suleyman Demirel University School of Medicine. Patients with the following conditions were excluded from the study: pregnancy, drug allergy, or hypersensitivity, severe systemic disorders, infective cases that can cause arthritic complications, underwent intraarticular therapy in the last 3 months, or arthroscopy treatment in the last 3 years. Physical examination and history of all the patients were recorded. Hemogram, blood biochemistry, erythrocyte sedimentation rate, C-reactive protein level, rheumatoid factor level, blood group, hemostasis parameters, and urine close examination analysis were performed and directional knee radiographs were taken for all patients before arthroscopy. Participants were randomly and equally divided into 4 groups, each of which consisted of 15 patients. Intraarticular hylan G-F 20 treatment was given to group 1 for 3 weeks after arthroscopy (2 ml per week). Hylan G-F 20 plus oral vitamin E at a dose of 400 UI/day were given daily to group 2 for 3 weeks. Only arthroscopic surgery was applied to group 3 (controls). Intraarticular Na-Hyaluronate at a dose of 2 ml weekly to group 4 for 5 weeks (**Table 1**). On biochemical analysis, blood SOD, CAT, GSH-Px and synovial SOD, and GPx levels were measured before the first injection and one week after the last injection. Also, malondialdehyde (MDA) levels were measured to determine lipid peroxidation. All arthroscopic applications were performed in the operating room under spinal or general anesthesia conditions. Arthroscopic debridement and microfracture procedure by Kirchner wire were applied to all patients. Cartilage lesions were leveled with Outher Bridge. The first dose of Na-hyaluronate or hylan G-F 20 injection was given after the arthroscopy, except in group 3. Superoxide dismutase activity was assayed by determining the ability of the enzyme to inhibit the superoxide anion-mediated reduction of nitro blue tetrazolium (25 μmol/L) to formazan, according to the method of Sun et al.[1] The latter was determined spectrophotometrically at 560 nm. The superoxide anion required for this reaction is generated by xanthine (0.1 mmol/L) and xanthine oxidase (200 U/L). GPx activity was measured via the method described by Roveri et al.[2] In this method, spectrophotometric absorbance is measured at 340 nm. The test mixture contained GSH (1 mM), DTPA (1 mM), glutathione disulfide reductase (0.6 unit/ml), and NADPH (0.1 mM) in 0.1 M sodium phosphate, pH 7.3. GPx samples were added to the test mixture at room temperature, and the NADPH oxidation rate was recorded for 1 min. The reaction was started by the addition of tert-butyl hydroperoxide (1.2 mM). Activity was calculated from the rate of NADPH oxidation. Carboxymethylation of the selenol in selenocysteine of GPx was carried out according to Roveri et al,[2] 5 μM GPx was incubated with 2 mM iodoacetate and 3 mM GSH for 10 min at 37 °C and then dialyzed. The Aebi et al[3] method was used to measure catalase activity. For determination of lipid peroxidation, MDA, which is a

lipid peroxidation product, was detected with Draper and Hadley's thiobarbituric acid reactivity method.[4]

Windows (12.0) software was used for statistical analysis SPSS. Wilcoxon test was performed to compare each group before and after the treatment, and a *p*-value less than 0.05 was considered statistically significant.

Results. The youngest patient was 40 years old, the oldest 68, and the mean age was 52.53 years. The average age of each group was given in **Table 1**. There was no statistically significant difference between the groups by means of age ($p>0.05$). Cartilage lesions of patients were graded by the Outerbridge Scale. Grade one lesion was assigned to 3 females and 2 male patients, grade 2 lesion to 13 females and 8 male patients, grade 3 lesion to 16 females and 10 male patients, grade 4 lesion to 14 female and 4 male patients. When we examined the distribution of cartilage lesions, we observed the medial condyle of femur as the most affected part from the disease, followed by trochlea of femur and patella (**Table 2**). The levels of blood CAT, MDA, SOD, and GPx were measured before and after the treatment. There was no statistically significant difference between the groups by means of these biochemical parameters ($p>0.05$) (**Table 3**). However, there was a statistically significant difference between the group comparisons by means of synovial MDA levels ($p<0.05$), although, no difference was found in synovial SOD and GPx levels between groups before and after the treatment ($p>0.05$) (**Table 4**).

Table 1 - Average age for each group, numbers of male, female and total patient applied treatment.

Groups	Applied drug or treatment	Male patient	Female patient	Average age	Total
Group 1	Hyalan G-F 20	5	10	53.26	**15**
Group 2	Hyalan G-F 20 and oral Vitamin E	6	9	52.93	**15**
Group 3	Arthroscopy	6	9	51.8	**15**
Group 4	Na-Hyaluronate	7	8	52.13	**15**

Table 2 - Distribution of cartilage lesions according to knee joint structures.

Effected region of knee	Grade 1	Grade 2	Grade 3	Grade 4
Patella	4	28	35	11
Medial condyle of femur	3	41	44	14
Lateral condyle of femur	2	11	10	3
Trochlea of femur	4	38	38	4
Medial condyle of tibia	5	25	28	3
Lateral condyle of tibia	2	10	11	2

Table 3 - The average levels of blood catalase, malondialdehyde, superoxide dismutase, and glutathione peroxidase before (B) and after (A) the treatment.

Groups	CAT B	CAT A	MDA B	MDA A	SOD B	SOD A	GPx B	GPx A
	U/g		µmol/L		U/g			
Group 1	9.144	9.128	35.95	35.96	1761.9	1761.92	74.85	74.82
Group 2	10.854	10.861	33.48	33.43	2174.33	2174.33	87.43	87.44
Group 3	8.436	8.397	32.48	32.85	1691.8	1691.85	85.03	85.04
Group 4	12.92	12.11	34.42	34.52	2147.45	2146.8	112.91	112.25

Table 4 - Mean synovial fluid malondialdehyde, superoxide dismutase and glutathione peroxidase levels before (B) and after (A) the treatment.

Groups	MDA B	MDA A	SOD B	SOD A	GPx B	GPx A
	µmol/L		U/g			
Group 1	0.3108	0.1066	0.914	0.946	228.21	228.2
Group 2	0.4928	0.334	0.913	0.875	273.39	273.44
Group 3	0.9302	0.928	2.607	2.581	278.7	278.26
Group 4	1.9308	1.6813	2.063	2.56	259.56	258.88

Discussion. Osteoarthritis is a degenerative joint disease and most commonly seen in the knee. In pathogenesis, there is a progressive focal degradation of joints, which leads to chronic pain and loss of function. The incidence of knee osteoarthritis increases with age and is more common in women. The rate of female patients in this study is also in accordance with the literature.[5] In knee osteoarthritis, the mechanism of cartilage matrix degradation is not clearly understood, but it is thought that ROS may cause the condition.[6] The ROS are more in synovial fluid of a joint with osteoarthritis than in a normal joint. There are lots of theories on the production of ROS. As there is an increased extracellular exuda fluid production in OA, this can lead to an increased pressure by motion, which in turn can cause transient ischemia and reperfusion damage on the surface of the synovial membrane.[7] This may be explained in a way that the joint surface is sensitive to ischemia and oxygen products, which are formed by dehydrogenase enzymes. In vitro studies showed that oxygen found in synovial fluid is formed by NADPH oxidase enzyme activity. The ROS are the molecules that can catch electrons from other molecules easily, thus spoiling the molecules, which loose their electrons. In living organisms, most important radicals are the ones derived from oxygen. Superoxide radicals are the earliest formed radicals, but they change to OH- and H_20_2 radicals, which are more harmful to tissues. In osteoarthritis, target molecules of oxygen radicals are collagen, hyaluronate, and proteoglycan components.[8] The damage formed by ROS gives rise to a decrement in the viscosity of synovial fluid. Although the responsible factor for this decrement is not clear, it is well known that the molecular weight of hyaluronan decreases when it is exposed to ROS.[9] In healthy individuals, hyaluronan prevents the formation of superoxide anions from polymorphonuclear leucocytes (PMNL) and this case results in inhibition of phagocytosis. In the case of OA, disturbance of hyaluronan by ROS results in more anion production by PMNL and spoiling of hyaluronan as a result.[10] The ROS are involved in the pathophysiological mechanisms of OA. Also, the modification of proteins by oxidation with ROS causes tissue adhesions that can lead to restricted joint activity. This modification increases the cross bindings in protein.[11] The ROS increase the lipid peroxidation in chondrocytes under in vitro conditions. This may be a sign that synovial fluid, which includes hyaluronan, has a protective function against oxidative damage. A healthy cartilage needs oxygen for stimulation of glycolysis, for the formation of adenosine triphosphate, and extracellular matrix.[12]

There are various antioxidant enzymes of the body such as SOD, CAT, and GSH-Px. In normal conditions, ROS are removed by these antioxidant enzymes. The SOD is an enzyme that catalyzes the transformation of superoxide to H_2O_2 and molecular oxygen. The physiological function of the enzyme is to protect the cells from the harmful effects of superoxide free radicals. Additionally, it inhibits lipid peroxidation. The SOD activity is high especially in the presence of oxygen utilization. During normal metabolism, superoxide production is in high level, whereas the extracellular activity of SOD is low.[13] The CAT protects cells from the deleterious effects of H_2O_2. The CAT exists in most aerobic cells. The reductive activity of catalase is effective on small molecules like H_2O_2. However, it does not affect the lipid hydroperoxides of large molecules. The GPx is responsible for the reduction of hydroperoxides. Under in vivo conditions, GPx and Ca+ metabolize oxygen by transforming H_2O_2 or OH-. The GPx protects the cell membrane against peroxidation when vitamin E is in restricted amount. Decrement of GPx activity leads to increase in hydrogen peroxide levels, and this may cause severe cellular damage. Synovial fluid includes all these enzymes. Vitamin E is an important non-enzymatic antioxidant. It is the first defense line against lipid peroxide radicals, thus, it breaks the lipid peroxidation chain.[14]

However, the role of exogenous vitamin E in the pathogenesis and treatment of OA has not been clearly defined yet. At cartilage cultures, the effect of ROS decreases when vitamin E is added to the medium.[15] However, in another study, it has been reported that vitamin E addition does not necessarily change the effect of ROS.[16] Oral vitamin E has no effect on the pathogenesis and progression of knee OA. In our study, we have also observed there was no significant increase in synovial SOD, CAT, GSH-Px, and MDA levels before and after the treatment. Furthermore, this synovial MDA level decreased significantly in treatment groups. However, this situation is explained by the cause of intraarticular hyaluronan therapy rather then

oral vitamin E. In synovial fluids of the patients with OA, it is expected that antioxidant levels are lower than in normal synovial fluid, because of the increment ROS levels. Sometimes, the antioxidant enzyme level is very low or does not exist. This is not an unexpected condition, because when CAT activity in extracellular fluid is very low, SOD and GPx activities are also very low. This is in parallel to the literature.

Intraarticular hyaluronan therapy is an alternative method in OA treatment. Na-hyaluronate and Hylan GF-20 are approved drugs by the Federal Drug Association in the treatment of OA that does not respond to acetaminophen administration or non-pharmacological therapy.[17] These 2 agents were used in our study. We could not find any related studies in the literature on intraarticular hyaluronan therapy and the effect of arthroscopy on oxidative stress. In another study, it was indicated that celecoxib and tenoxicam therapies do not affect the blood levels of SOD, GPx, and MDA.[18] In our study, arthroscopy, and intraarticular hyaluronan therapy, or the arthroscopic surgery alone did not affect the blood SOD, GPX, and MDA levels. However, synovial MDA levels were significantly lower in groups that were given both arthroscopy and intraarticular hyaluronan therapy than the controls. This result suggests that intraarticular hyaluronan therapy decreases lipid peroxidation. There was no significant difference between the Na-hyaluronate and Hylan GF-20 therapy groups by means of lipid peroxidation, and any other diverse clinical effect could not be demonstrated between the 2 applications.[19] In our study, MDA levels were significantly decreased in groups treated with intraarticular Na-hyaluronate and Hylan G-F 20 along with arthroscopy.

Although the mechanism by which intraarticular hyaluronan is involved has not been clearly explained, there are some suggested mechanisms on the inhibition of the inflammatory mediators such as cytokines and prostaglandins, stimulation of cartilage matrix synthesis, inhibition of cartilage degradation, and the protection effect on nociceptive nerve endings. The pain relieving effect of intraarticular hyaluronan therapy is significantly higher than intraarticular placebo injection and also, the pain relief effect of orally administered non-steroidal anti-inflammatory drugs (NSAIDs) is comparable with this. In addition, pain relief of hyaluronan is similar or much better than the glucocorticoid injection.[20]

In knee OA, arthroscopy is a safe and simple method in classification and treatment of the disease. It is also a diagnostic method used to evaluate the pathology of joint cartilage. The chondropathy level can be graded by the Outerbridge scale,[21] which has the highest validity scale and is the most frequently used one in literature.[22] The patients in our study were graded by this scale, and it was observed that 5 patients were in grade one, 21 in grade 2, 26 in grade 3, and 18 in grade 4. In knee OA, there is controversy on the role of arthroscopic treatment. Some studies showed that symptoms can be controlled by arthroscopy for up to 5 years.[23] There is an increasing amount of data supporting the therapeutic value of arthroscopy in OA. However, the most important factor that determines the success is the patient selection for arthroscopy. Arthroscopic surgery is more suitable for young patients who have mechanical symptoms like lockage of knee with pain and have light-middle level radiographic findings. Arthroscopic lavage and debridement is useful for this group of patients. Nevertheless, arthroscopic treatment of OA is not a curative method and the experience of the physician is also important. This treatment method is not suitable for patients who have severe mal alignment, or who do not have any mechanical complaint. The arthroscopic debridement provides good results in appropriately chosen patients and decreases morbidity.[24]

As a result, it can be suggested that arthroscopy accompanied by intraarticular hyaluronan therapy has significant effects on lipid peroxidation in a knee with OA, however, the antioxidant system activity is not affected from this therapy. Arthroscopic surgery alone has no effect on either the antioxidation system activity or lipid peroxidation. We could not find any related study in the literature about the effects of intraarticular hyaluronan therapy on antioxidation system in synovial fluid. For this reason, comprehensive, placebo controlled and blind-ended clinical studies including a larger number of patients are warranted to get more insight into this subject.

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