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Araştırma Makalesi /Research Article

Effect Of Viscosupplementation with Sodium Hyaluronate (Orthovisc) on Nitric Oxide Production and Urinary Glycosaminoglycan Levels in Patients with Knee Osteoarthritis

Diz Osteoartriti Olan Hastalarda Sodyum Hiyaluronat (Orthovisc) ile Viskosuplementasyonun Nitrik Oksit Üretimi ve İdrar Glikozaminoglikan Düzeyleri Üzerine Etkisi

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Öz

Amaç: Osteoartrit (OA), patolojik olarak eklem kıkırdağının bozulması ve kaybı ile karakterize, inflamatuar olmayan bir hastalıktır. Bu çalışmanın amacı, eklem içi sodyum hiyalüronat (SH) enjeksiyonlarının serum nitrik oksit (NO) düzeyleri ve kıkırdak bozulmasının bir ürünü olan idrar glikozaminoglikanı (U-GAG) üzerindeki etkisini değerlendirmektir.

Gereç ve Yöntem: Çalışma grubumuz, 3 haftalık SH eklem içi enjeksiyonu ile çalışma süresince osteoporoz ve NSAI ilaçlar ile tedavisi gören, yaşları 58-73 arasında değişen 16 postmenopozal osteoporoz ve diz osteoartriti tanılı hasta ve 18 sağlıklı kontrolden oluşmuştu. Serum osteokalsin, alkalen fosfataz, NO düzeyleri ve idrar deoksipiridinolin, kalsiyum ve glikozaminoglikan (U-GAG) atılımları belirlendi.

Bulgular: Son tedaviden sonra, U-GAG seviyeleri başlangıç seviyelerinden daha yüksek (1.80mg/g ± 0.51, 1.27mg/g ± 0.47, $p<0.01$) ve kontrol grubu ile anlamlı bir fark yoktu. Serum nitrat ve nitrit konsantrasyonları ile idrar GAG seviyeleri arasında bir korelasyon yoktu. Diğer tüm kemik yapım – yıkım belirteçleri referans aralıklar içinde ölçüldü ve anlamlı bir fark gözlenmedi.

Sonuç: Sonuçlarımız, idrar GAG atılımlarındaki azalmanın NSAI ilaçlar ve osteoporoz tedavisinde kullanılan ilaçlarla ilişkili olabileceğini düşündürmektedir.

Anahtar Kelimeler: Sodyum hiyaluronat, Nitrik oksit, Glikozaminoglikan, Osteoartrit, Viskosuplementasyon

Abstract

Objective: Osteoarthritis (OA) is a non-inflammatory disease characterized pathologically by deterioration and loss of articular cartilage. The aim of this study was to evaluate the effect of intraarticular injections of sodium hyaluronate (SH) on serum nitric oxide (NO) levels and urinary glycosaminoglycan (U-GAG), which is a product of cartilage degradation.

Materials-Methods: Our study group was 16 postmenopausal patients with osteoporosis and knee osteoarthritis between the ages of 58-73 who were under treatment for osteoporosis and NSAI drugs during the study period of 3 courses of weekly intra-articular injections of SH and 18 healthy controls. Serum levels of osteocalcin, alkaline phosphates, NO and urinary deoxypyridinoline, calcium, and glycosaminoglycan (U-GAG) excretions were determined.

Results: After the last treatment, U-GAG levels were higher than the beginning levels (1.80mg/d ± 0.51, 1.27mg/d ± 0.47, $p<0.01$) and no significant differences with controls. There was no correlation between serum nitrate and nitrite concentrations and urinary GAG levels. All other bone turnover parameters were measured between the reference interval and no significant differences were observed.

Conclusion: Our results suggested that decreased levels of GAG excretions could be related to the NSAI drugs and drugs used for the treatment of osteoporosis.

Key Words: Sodium hyaluronate, Nitric oxide, Glycosaminoglycan, Osteoarthritis, Viscosupplementation

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INTRODUCTION

Osteoarthritis (OA) is a non-inflammatory common disease, which is characterized pathologically by deterioration and loss of articular cartilage. OA and osteoporosis are both common conditions in the elderly age. Some of the authors mentioned an inverse relationship between osteoporosis and OA. Although OA is a non-inflammatory disease some studies have shown evidence of mild inflammatory changes in OA synovium. The pathophysiology of OA involves a complex interplay of mechanical stress, biochemical mediators, and inflammatory processes, ultimately resulting in cartilage breakdown and joint dysfunction. Among the mediators of OA pathology, nitric oxide (NO) plays a pivotal role due to its involvement in inflammatory cascades and chondrocyte apoptosis. Concurrently, alterations in glycosaminoglycan (GAG) metabolism reflect changes in cartilage integrity.

Many types of treatment have a role in the management of pain in patients with OA. Viscosupplementation is one of the treatment types for knee osteoarthritis. The intra-articular injection of hyaluronic acid (HA)-based products, aims to restore synovial fluid viscoelasticity, reduce inflammation, and improve joint function. Orthovisc, a high-molecular-weight sodium hyaluronate preparation, has shown promise in symptom relief and functional improvement in knee OA. However, its effects on biochemical markers such as NO and urinary GAGs remain underexplored.

The aim of this study was to evaluate the effect of intraarticular injections of sodium hyaluronate (SH) on urinary glycosaminoglycan (GAG), which is a product of cartilage degradation and serum nitric oxide (NO) levels that are known to be an important biological molecule that may have pro and anti-inflammatory effects and essential for the normal function of a number of diverse biological processes.

MATERIALS AND METHODS

Our study included 16 postmenopausal patients with osteoporosis and knee osteoarthritis between the ages of 58-73 who were under treatment for osteoporosis and NSAI drugs during the study period. These patients were treated with 3 courses of weekly intra-articular injections of SH. 24-hour urine and 12-hour fasting blood samples were collected before and 1 week after the last treatment.

Excess dietary nitrite/nitrate intake was excluded. NO production was determined in serum by the measurement of nitrate and nitrite release using the Griess reaction[1-3], compared with standard solutions of sodium nitrite.

The following biochemical markers were measured in a group of 16 patients and in 18 healthy controls: for bone formation, serum osteocalcin (Diagnostic Products Corporation, DPC.Immulite 1000 otoanalyzer) and alkaline phosphates (AEROSET*System (Abbott otoanalyzer), for bone resorption urinary deoxypyridinoline (İDS, lot no:40880, RIA and Berthold 2111 gamma counter) and calcium; for cartilage, urinary glycosaminoglycan (U-GAG) excretions. U-GAG excretions were analyzed by modified Whiteman method by spectrophotometer, and other routine parameters (U-urea, U-creatinin, U-Ca, U-Na, U-K, U-Cl) were analyzed by AEROSET*System (Abbott otoanalyzer)[4-7,9,10,12].

Statistical analysis was performed using the statistical package for social sciences. Data in tables are expressed as the mean, Mann-Whitney U-tests and Students t-tests were used as appropriate for comparisons.

RESULTS

Biochemical markers of the patients before and after the treatment were compared to healthy controls. There were no significant differences in serum nitrate and nitrite levels before and after the treatment (5.2μmol/L ± 1.20, 6.7μmol/L ±1.10, $p > 0.05$), but levels were higher than the control group (3.98 μmol/L ± 0.78, $p < 0.05$). SH did not modify nitric oxide production. 24-h U-GAG excretions of the controls were (1.91± 0.55) significantly higher than the 24-h U-GAG excretions of the OA patients (1.27± 0.47 $p<0.01$, -35%). After the last treatment, U-GAG levels were higher than the before treatment levels (1.80 mg/d ± 0.51, 1.27mg/d±0.47, $p<0.01$, respectively) and no significant differences with controls. There was no correlation between serum nitrate and nitrite concentrations and U-GAG levels. All other bone turnover parameters were measured between the reference intervals, and no significant differences were observed. The

variation of the biochemical parameters of the treatment with sodium hyaluronate on patients with knee osteoarthritis compared to healthy controls was presented in Table 1.

Table 1: Effects of the Treatment with Sodium Hyaluronate on Patients with Knee Osteoarthritis Compared to Healthy Controls

Parameters	Control (n=18)	OA (n=16) Before Treatment	After Treatment
Age	65.27 ± 6.27	68.58 ± 6.77	
U-GAG mg/day	1.91 ± 0.55*	1.27 ± 0.47*	1.80 ± 0.51*
Nitrite+nitrate (µmol/l)	3.98 ± 0.78**	5.20 ± 1.20	6.70 ± 1.10
Osteocalcin ng/ml (R.I. 3.10-13.7ng/ml)	8.04 ± 3.29	7.84 ± 4.26	8.01 ± 4.01
ALF U/L (R.I.30-165 U/L)	140.72 ± 38.05	180.38 ± 68.1	170.56 ± 54.56
DPY nM/mM (R.I. 2.5-5.5 nM/mM)	3.4 ± 1.08	3.63 ± 1.58	2.49 ± 1.04
U-urea g/day (R.I. 10-35g/day)	22.74 ±10.49	16.15 ± 5.09	15.72 ± 4.99
U-crea g/day (R.I. 0.8-1.8 g/day)	1.30 ± 0.64**	0.92 ± 0.49	0.77 ± 0.31
U-Ca mg/day (R.I. 50-300 mg/day)	137.56 ± 107.14	101.93 ± 61.43	109.35 ± 80.08
U-Na mmol/day (R.I. 50-225 mmol/day)	137.0 ± 89.06	132.28 ± 65.83	126.91 ± 70.80
U-K mmol/day (R.I. 25-125 mmol/day)	67.30 ± 39.39	50.67 ± 23.39	48.66 ± 18.23
U-Cl mmol/day (R.I.110-250 mmol/day)	149.64 ± 85.37	138.64 ± 74.14	130.51 ± 72.81
*** p< 0.01 **p<0.05**			

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DISCUSSION

Osteoarthritis is not generally considered to be an inflammatory disease however some studies have shown evidence of mild to moderate inflammatory changes in OA synovium. In OA, hyaluronic acid (HA) concentration and molecular weight are significantly reduced, impairing joint lubrication and shock absorption. HA is a key component of synovial fluid, contributing to its viscoelastic properties. Sodium hyaluronate preparation was effective for the treatment of OA as previous double-blind control studies suggested[8,11]. The clinical and *in vitro* data showed that the possible mechanisms of SH preparation could be chondroprotective. Viscosupplementation with HA products, such as Orthovisc, seeks to restore synovial fluid viscoelasticity, modulate inflammatory pathways, and promote chondroprotection and cartilage repair. Orthovisc's high molecular weight and prolonged residence time in the joint cavity enhances its therapeutic potential. We evaluated the oxidative stress related to reactive nitrogen and oxygen intermediates, measuring NO_2 as a stable end–product of nitric oxide generation. NO is a critical mediator in OA pathogenesis. Elevated NO levels are associated with increased matrix metalloproteinase activity, leading to collagen and proteoglycan degradation, inhibition of ECM synthesis by chondrocytes and promotion of synovial inflammation and pain perception. Our results suggested that Orthovisc did not have definite effects on although several studies suggest that tissue injury in inflammation involves NO production in patients with knee osteoarthritis. Viscosupplementation may reduce NO production by downregulating pro-inflammatory cytokines and iNOS expression, thereby mitigating cartilage damage and inflammation.

Key biochemical changes of knee osteoarthritis include increased NO production that NO is synthesized by inducible nitric oxide synthase (iNOS) in response to pro-inflammatory cytokines such as interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α). Excessive NO contributes to cartilage matrix degradation, chondrocyte apoptosis, and oxidative stress.

Urinary GAG levels serve as a non-invasive biomarker for cartilage metabolism. Elevated levels reflect heightened cartilage degradation and are correlated with OA severity. Monitoring urinary GAG levels can provide valuable insights into the efficacy of therapeutic interventions, including viscosupplementation. Increased urinary GAG levels indicate enhanced cartilage breakdown and ECM turnover.

CONCLUSION

Our results suggested that decreased levels of GAG excretions could be related to the NSAI drugs and drugs used for the treatment of osteoporosis.

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