Comparing Therapeutic Effects of Intraarticular Ozone and Hyaluronic Acid in the Rat Model of Experimental Knee Osteoarthritis

Sıçan Diz Ekleminde Oluşturulan Deneysel Osteoartrit Modelinde İntraartiküler Ozon ve Hyalüronik Asitin Terapötik Etkilerinin Karşılaştırılması

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ABSTRACT

Purpose: In this study, we examined therapeutic effects of intraarticular ozone when compared with hyaluronic acid (HA) in the osteoarthritis model of rat. We investigated possible effects of those substances on inflammatory and nociceptive processes, both macroscobic and histological changes with differences on collagen structure in knee joints.

Methods: Intraarticular Kaolin-Carrageenan (CG) mixture was used to form osteoarthritis. In this study, 36 male Wistar albino rats were randomly divided into 6 groups including CG, CG+saline, CG+ozone, CG+HA, CG+ozone+HA and ozone-only. All therapeutic applications were performed to posterior right knee joints of rats and their posterior left knee joints were considered as their controls. As therapeutic protocols, 4 days after CG injections, three consecutive intraarticular applications were performed separately to the right knee joints by 4 days of intervals. Hot plate test was used for determination of antinociceptive effect and joint girths with knee diameters had been measured in a day before the beginning of experiments and the 16th day of this study. All rats were sacrified at 16th day and their posterior knee joints were dissected for histopathological examination.

Results: A statistically significant antinociceptive effect was observed in the ozone, HA and combination of Ozone+HA applications. Improvement of joint measurements was apparent with the ozone applications compared with HA group. When saline was applied to CG administered the joints, neither effect for reducing inflammation was observed in the arthritis. On the other hand, the ozone strongly reversed inflammation on the knee joints with carrageenan-induced arthritis. The histopathological appearance of joints obtained from both the ozone-applied and the control group were nearly similar to each other.

Conclusion: Ozone therapy has significant beneficial effects in terms of histopathological improvement, the antinociceptive effectiveness and knee joint measurements in osteoarthritis. These results suggested that both ozone alone and/or combination treatment have better therapeutic effects from HA alone.

Key Words: Osteoarthritis, Ozone, Hyaluronic acid, Rat

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ÖZET

Amaç: Bu çalışmada, sıçan osteoartrit modelinde eklem içi ozonun hyaluronik asit (HA) ile karşılaştırıldığında terapötik etkilerini inceledik. Bu maddelerin inflamatuar ve nosiseptif süreçler üzerindeki olası etkilerini, hem makroskobik hem de histolojik değişikliklerle birlikte diz eklemlerindeki kolajen yapısındaki farklılıkları araştırdık.

Yöntem: Osteoartrit oluşturmak için intraartiküler Kaolin-Carrageenan (CG) karışımı kullanıldı. Bu çalışmada 36 adet erkek Wistar albino rat rastgele CG, CG+salin, CG+ozon, CG+HA, CG+ozon+HA ve sadece ozon olmak üzere 6 gruba ayrıldı. Tüm tedavi edici uygulamalar ratların sağ diz arka eklemlerine uygulandı ve sağ diz arka eklemleri kontrol olarak kabul edildi. Terapötik protokol olarak CG enjeksiyonlarından 4 gün sonra sağ diz eklemlerine 4 gün ara ile ayrı ayrı art arda üç intraartiküler uygulama yapıldı. Antinosiseptif etkinin belirlenmesi için sıcak plaka testi kullanılmış ve deneylerin başlamasından bir gün önce ve çalışmanın 16. gününde diz çapları ile eklem çevreleri ölçülmüştür. Tüm ratlar 16. günde sakrifiye edildi ve histopatolojik inceleme için arka diz eklemleri diseke edildi.

Bulgular: Ozon, HA ve Ozon+HA kombinasyonlarında istatistiksel olarak anlamlı bir antinosiseptif etki gözlendi. HA grubu ile karşılaştırıldığında, ozon uygulamaları ile eklem ölçümlerinde iyileşme belirgindi. Eklemlere uygulanan CG'ye salin uygulandığında, artritte inflamasyonu azaltan hiçbir etki gözlenmedi. Öte yandan, ozon, karagenan kaynaklı artritli diz eklemlerindeki iltihabı güçlü bir şekilde tersine çevirdi. Hem ozon uygulanan hem de kontrol grubundan elde edilen eklemlerin histopatolojik görünümleri birbirine yakındı.

Sonuç: Osteoartritte ozon tedavisinin histopatolojik düzelme, antinosiseptif etkinlik ve diz eklemi ölçümleri açısından önemli yararlı etkileri vardır. Bu sonuçlar, hem tek başına ozonun veya kombinasyon tedavisinin sadece HA'dan daha iyi terapötik etkilere sahip olduğunu göstermiştir.

Anahtar Sözcükler: Osteoartrit, Ozon, Hyaluronik asit, Sıçan

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INTRODUCTION

Osteoarthritis is a common degenerative joint disease, which can be considered as a major factor of disability among elderly population. Due to the chronic inflammation, a progressive degeneration of articular structures and synovial membrane can lead to narrowing of effected joints, osteophyte formations, subchondral sclerosis and synovitis (1, 2). In the early stages of osteoarthritis, a loss in amount of chondral proteoglycans and type II collagen take place then the cartilage becomes softener and looses its structurel resistance. Necrosis and microfractures may occur in tissular level. Pain, stiffness, diminished range of motion (ROM) and deformities of affected joints can be observed as a result of those as cardinal symptoms (3).

Etiopathogenesis of osteoarthritis is not clearly understood but, besides genetical factors, chronic joint degeneration with predisposing elements such as aging, obesity and trauma may lead to arthritis. Today, no effectively stringent treatment modalities have been revealed for osteoarthritis (4, 5). The aim of current therapeutic approaches e.g., physiotherapy, pharmacotherapy and interventional techniques can be considered as minimizing the perception of pain and to secure ROM of affected joints (4, 6).

Intra articular injection of hyaluronic acid is known to be a common treatment option at the early stages of osteoarthritis. The viscoelastic property of hyaluronic acid increases the ability of articular movement in synovial joints but its lack of competence as a solitary therapeutic agent has been verified in many studies (7, 8). According to recent studies, another treatment modality as the intra articular injection of medical ozone seems to be effective and useful for chronic painful knee and shoulder disorders (9).

The ozone has been found effective and safe for medical purposes in many experimental and clinical trials (10-12). Muto et al. found that the intra-discal injection of oxygene/ozone combination may be useful for patients with lumbar disc herniation (13). In a recent study which has focused on the therapeutic effect of ozone and the role of ozone in regulating the level of TNF-α (tumor necrosis factor), TNF-R1 (tumor necrosis factor receptor 1), and TNF-R2 in rats with rheumatoid arthritis (RA), it has been shown that the intra-articular injection of ozone can effectively suppress the joint swelling caused by RA (14). Inflammation and formation of new blood vessels are integrated mechanisms in osteoarthritis and they may affect disease progression and pain. It has been noticed that inflammation can exacerbate cartilage degradation in osteoarthritis with increased TNF-α and IL-1 levels by stimulating chondrocytes that may produce metalloproteinases (MMP) and plasminogen activator, which degrade matrix proteoglycans and collagen (15). Stimulation of angiogenesis by synovial inflammatory processes may also contribute to progressive joint damage in osteoarthritis. Inhibition of inflammation and angiogenesis may provide an effective therapeutic option by improving symptoms and retarding joint damage (15-16).

In this study, we focused on therapeutic effects of intraarticular ozone and hyaluronic acid in the rat model of experimental carrageenan-induced arthritis in order to investigate nociceptive processes and changes of knee joints in the manner of macroscobic, structural and ultrastructural (alterations on synovial epithelium and fibroblasts) with type III and IV collagen immunoassay procedures.

METHODS

Animal Care

All experiments were carried out in compliance with The National Institute of Health *Guide for the Care and Use of Laboratory Animals.* The experimental procedure was approved by the Local Ethical Committee of Animal Care and Use of Gazi University (B.30./183-18833). In this study, 36 male Wistar albino rats (200-230 gr) were accommodated in an air-conditioned room (22±1°C) under a 12-hour light/dark cycle with adaptive feeding of standart chow diet and water ad libitum for a week. Those conditions were preserved at the end of experimental procedures.

Experimental protocols and groups

All rats were randomly divided into 6 groups including CG, CG+saline, CG+ozone, CG+HA, CG+ozone+HA and ozone-only. Rats were anaesthetized with an intraperitoneal injection of ketamine (80 mg/kg) + xylazine (10 mg/kg) during measurement and interventional procedures.

In 100 µL intraarticular volume, Kaolin (1%) as a stabilizer with CG (1%) mixture was applied into posterior right knee joints to form arthritis and their posterior left knee joints were considered as their controls.

In CG group, after intraarticular CG injection, rats were given 15 days of standby period. As therapeutic applications, 4 days after CG injections, three consecutive intraarticular applications were performed separately to the right knee joints by 4 days of intervals. Intraarticular saline (100 µL), ozone (1 µg/100 µL), HA (50 µg/100 µL) and ozone (0.5 µg/50 µL) + HA (25 µg/50 µL) combination were performed. In order to observe the effects of ozone (1 µg/100 µL) alone, it was applied intraarticularly without CG injections.

Hot plate (AHP 0603, Commat, Turkey) test was used for determination of antinociceptive effect. The reaction time was taken as the interval from the instant animal reached the hot plate until the moment animal licked its feet or jumped out. A cut off time of 40 sec was determined to avoid any thermal injury to paws. The reaction time of all rats had been examined and recorded by using hot plate a day before the beginning of experiments and at the 16th day. For determination of maximum possible effect of antinociception in percentages (MPE%) hot-plate test formulated by Schmauss and Yaksh was preferred (17).

$$\mathrm{MPE\%} = \frac{(T_1 - T_0)}{(T_2 - T_0)} \times 100$$

To: Baseline reaction time of rats before experiments
T_1: Baseline reaction time of rats after experiments
T_2: Cut off time

In all groups, before experiments and after the functional study protocols in the 16th day, the joint girths and knee diameters of anesthetized rat had been measured and recorded by using tapeline and caliper compass in order to determine joint girth index (JGI) by formulating the difference between day 0 (G_0) and 16th day (G_1).

$$\mathrm{JGI\%} = \frac{(100 \times G_1)}{(G_0)} - 100$$

G0: Joint girth of rats before experiments
G1: Joint girth of rats after experiments
All rats were sacrified by using over dosage thiopenthal at 16th day and their posterior knee joints were dissected for histopathological examination to obtain inflammation scores.

Histopathological Examination of Joints

Isolated joints were examined under both light and electron microscopic evaluation to obtain inflammation scores (18).

Light microscopic prosedures

Joints were decalcified and fixed with EDTA, disodium salt of 5.5 g, distilled water of 90 mL, formaldehyde (37-40 %) of 10 mL. Tissue samples were incubated for 15 days in this mixture and the routin light microscopic methods were applied, then embedded in paraffin. After that, 5 µm-long sections were obtained from each paraffin blocks and stained with haematoxylin and eosin (H&E). Histological analysis was performed using a light microscope (Leica DM 4000, Germany).

Synovial membrane thickness and inflammation scoring

The maximal thickness was scored semiquantitatively (0:1–2; 1:3–4; 2:5–6; 3:≥7 cell layers).
0: Normal synovial intimal membrane no inflammation, normal thickness 1-2 cells
1: Mild inflammation a few inflammatory cells, intimal thickness 3-4 cells
2: Mild inflammation many inflammatory cells and clusters of small lymphocytes, intimal thickness 5-6 cells
3: Intense inflammation, intense inflammatory cells and large, dense perivascular infiltration of lymphocytes, ≥7 cells or more of intimal thickness (19, 20).

Electron microscopic procedures

Tissue samples were decalcified in 1000 mL phosphate buffer, 100 gr of EDTA, 65 gr of sucrose and 25 mL of gluteraldehyde and then they were washed overnight with phosphate buffer. Samples were divided into small pieces as 1 mm^3.

Tissues were fixed by 2.5 % gluteraldehyde solution with 1/15M phosphate buffer, pH 7.4 at 4 °C, and then they were washed for four times in phosphate buffer. All samples were once again fixed in 1% osmium tetraoxide (OsO_4) with 1/15M phosphate buffer in +4 ºC. Afterv that procedures, specimens were dehydrated through graded alcohol series. Samples were purified with water and incubated in 2.5 mL Araldyte (Araldyte CY212) and 2.5 mL DDS (dodecenylsuccinic Anhydride) composition overnight prior to embedding material. They were placed in the original embedding material (2,5 mL Araldyte and 2,5 mL DDSA 0,2 mL BDMA (benzyl dimetil amine)). In this mixture, samples were first left at room temperature for 2 hours and then they were incubated for 24 hours in 40 ºC and 48 hours in 60 ºC. Thin sections (60-90nm) were obtained with ultramicrotome (Leica) and stained with uranyl acetate and lead citrate, examined by tramission electronmicroscope (Carl Zeiss Libra 120; Jena, Germany) and digitally photographed. Synovial epithelium and fibroblasts were examined as ultrastructurally (21).

Immunohistochemical procedures

Immunohistochemistry procedures for Collagen III and IV were performed. Antibodies used for this study were as followed: a rabbit polyclonal anti-Collagen III antibody (Abcam, ab7778) at a dilution of 1/100 and a goat polyclonal anti-Collagen IV antibody (Santa Cruz, sc-18178,) at a dilution of 1/100. Goat Immunocruz Staining System (sc-2050, Santa Cruz Biotechnology, CA, USA) was used as a seconder antibody for Collagen III and IV antibodies.

Sections were incubated at 60˚C overnight then dewaxed in xylene for 30 minutes. After rehydration through a decreasing series of ethanols, all sections were washed in distilled water for 10 minutes. They were treated with 10 mM citrate buffer (Labvision, AP-9003-125) at 95 ˚C for 5 minutes, to unmask antigens by heat treatment. Slides were kept in the cool in buffer for 20 minutes afterwards they were washed in deionized water for three times in 2 minutes. Pap pen (Thermo Scientific) was used for delineating and then, samples were incubated in a solution of 3 % H_2O_2 for 5 minutes to inhibit endogenous peroxidase activity. Afterwards the following incubation steps were carried out; incubation with normal serum blocking solution for 30 minutes and humid chamber for 18 h at +4 ˚C with antibodies to: Collagen III and IV. After this step, incubation with biotinylated IgG was implemented, then for 30 minutes streptavidin conjugation to horseradish peroxidase, which were all prepared according to kit instructions, was provided. They were finally incubated with 3,30 diaminobenzidine hydrochloride (DAB), which was also prepared according to kit instructions, and nuclei were counterstained with Mayer's hematoxylin. Phosphate buffered saline (PBS) which had the feature of pH 7.4 was used for all dilutions and irrigation solutions. Sections were dehydrated through a graded ethanol series, cleared in xylene, mounted in Entellan. Cross-sections were evaluated under a light microscope (Leica DM 4000 Germany) using a computer-supported imaging system to take images with the Leica Q Vin 3 program. All the results of control and experimental groups were compared particularly. Immunohistochemical evaluations were performed for 5 serial sections from each sample. Evaluations of type III collagen were performed on the larger capillaries. For all sections, equal amount of basement membranes of all capillaries were chosen for the analysing of evaluation on type IV collagen. Immunostaining intensity on a semiquantitative scale ranging from no expression (–), moderate (+) and strong (++) was provided by two independent observers' consideration. The concordance between the grading of both observers was 83 %. In the remaining cases agreement was reached after joint inspection and reevaluation of the slides (22).

Chemicals

Carrageenan (λ, 22049) and Kaolin (K7375) were purchased from Sigma Aldrich (St. Louis, MO, USA). The ozone (Ozonosan®, Germany), Hyaluronic acid (Orthovisc® 2 mL, Anika, USA), all other chemicals were obtain from local companies in Turkey.

Statistical Analysis

Values are expressed as mean ± SEM. Statistical analyses were performed using the SigmaPlot (Systat Software Inc., USA) version 11 for Windows. One-way ANOVA have been performed for the data acquired among groups as a statistical significance tests. In the analysis of varience when there were a difference among groups and variences were homogeneous as a result of Barlett test, Tukey's multiple comparisons test had been performed for post hoc comparison of groups.

Also for statistical analysis in histopathological data, Statistical Package for the Social Sciences for Windows (version 21.0; SPSS Inc., Chicago, IL, USA) was used. The immunohistochemistry results for significance between groups were analyzed with Kruskal-Wallis and Mann-Whitney U tests. Values were considered significantly different when p<0.05.

RESULTS

Evaluation of antinociceptive activity in experimental groups with hot-plate test;

A significant increase of nociceptive activity was observed according to the evaluation of % MPE of antinociception results with the hot plate test in rats with carrageenan-induced arthritis. A statistically significant reversal of hyperalgesia and a production of antinociception with the ozone, HA and the combination of Ozone+HA applications were also observed in this study (P<0.05) (Fig 1).

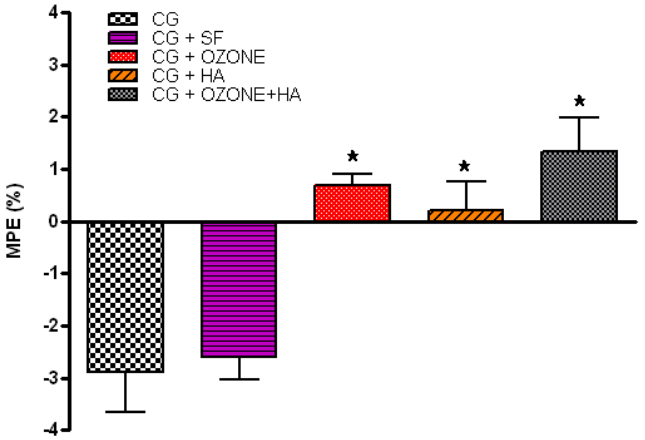

Figure 1. Effects of ozone, HA and the combination of Ozone+HA at carrageenan induced arthritis in nociceptive hot-plate model for rats**.** MPE; maximum possible antinociception, SF; saline, CG; carrageenan, HA: hyaluronic acid. *Different than CG group (P<0.05) (n=6).

Changes in Joint Girth Indexes

There is no statistically significant difference in JGI results of joints with carrageenan-induced arthritis by saline applications. But there is a statistically significant difference between JGI values of joints with carrageenan-induced arthritis and the other therapeutical application including the ozone, HA and the combination groups (Fig 2). Also a statistically significant reduce is shown in the JGI values of joints with the ozone applications when compared with HA-applied group (P<0.05).

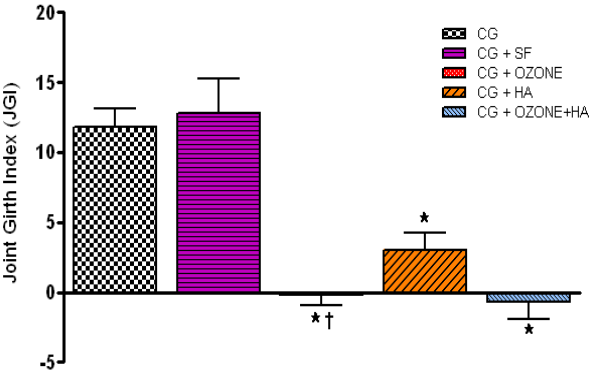

Figure 2. Comparison of JGI between the CG and therapeutic groups. Values were given as mean ± SEM (n=6). JGI; The difference in joint girths between day 0 and day 16 in percentage, CG; carrageenan, SF; saline, HA: hyaluronic acid. *Different than CG group, †Different than CG+HA group (p<0.05) (n=6).

Histological changes

In the groups of CG and CG+Saline, epithelial layer was thick and cellular debris was observed on epithelial surface. Also, edema was determined in intracytoplasmic space and extracellular area. In these two groups, vascularization was increased in connective tissue under the synovial membrane (Fig 3A, D).

In the ozone-only group, epithelial and subepithelial structures were in normal histological features (Fig 3E). Likewise, in the groups of CG+Ozone and CG+Ozone+HA, epithelial and subepithelial findings were in normal histological features (Fig 3B, F). In the group of CG+HA, epithelial thickness was similar with the CG and CG+Saline group. Also, in same regions, intercellular junctional degenerations were observed. On the other hand, in this group, epithelial and subepithelial edema was lower compared to group CG and CG+Saline (Fig 3C).

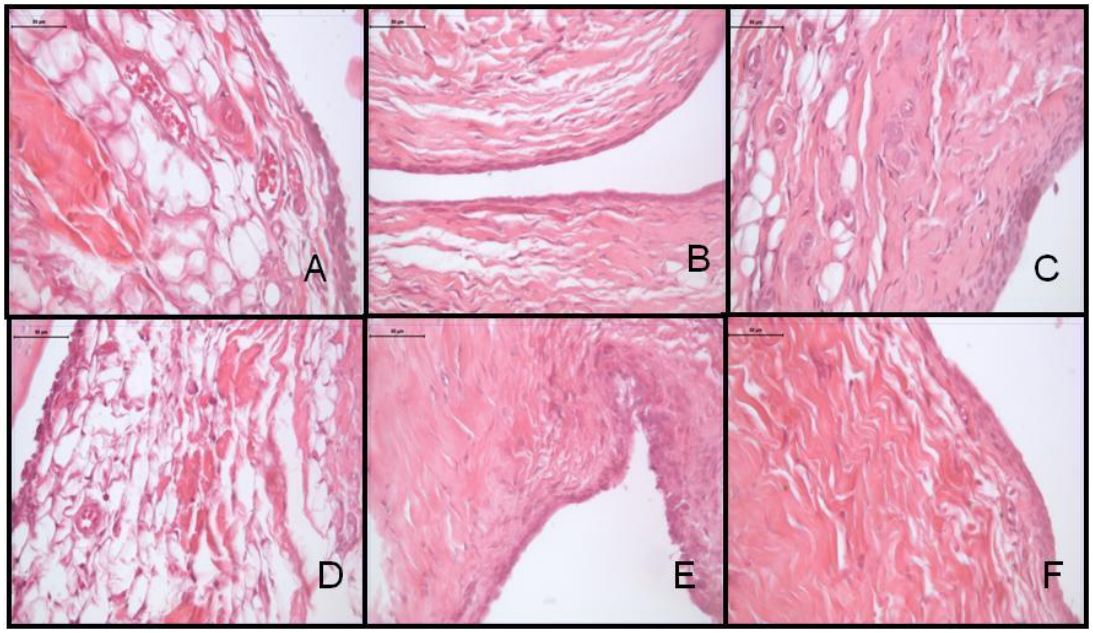

Figure 3. Light microscopy. The edematous epithelial and subepithelial region in CG and CG+SF groups, irregular collagen fibers, respectively (A, D), Normal morphological appearance in CG+Ozone, Ozone and CG+Ozone+HA groups (B, E, F), The epithelial and subepithelial structures in the CG+HA group properties like in the CG and CG+SF groups, mild degeneration was evident (C), With H&E.

Inflammation scores

According to the data obtained from the examination of joints with saline application and joints with carrageenan-induced arthritis, there is no statistically significant difference between the inflammation scores of those groups. On the other hand, a statistically significant reduction in the other therapeutical application groups including the ozone, HA and the combination groups is shown when compared with the inflammation scores of joints with carrageenan-induced arthritis. Also there is a much more reduction in the inflammation scores of the ozone and combination groups when compared with HA group ($P<0.05$) (Fig 4).

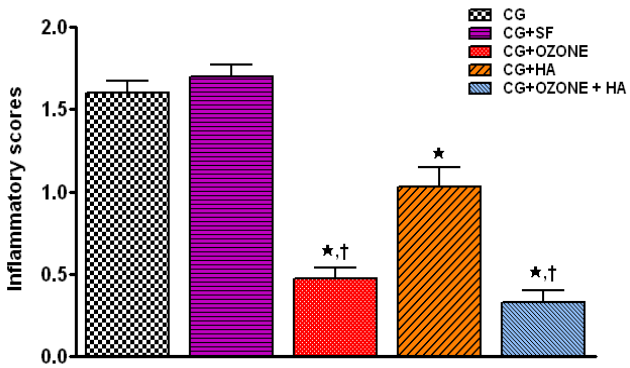

Figure 4. Comparison of inflammation scores in rat knee joints. Values were given as Mean ± SEM (n=6). CG; carrageenan, SF; saline, HA: hyaluronic acid. *Different from CG group, †Different from CG+HA group ($P<0.05$, n=6).

Ultrastructural changes

When synovial epithelium was examined as ultrastructurally, the epithelial continuity was found impaired due to junctional degenerations in CG and CG+Saline groups. It was thought that the reason was the debris caused by intercellular junctional degenerations as observed in light microscopy. Numerous cells with apoptotic and necrotic nuclei were differentiated. In another region, epithelial cells with degenerative vacoules, intracytoplasmic edema and mitochondrial crystalysis were observed. In addition, rough endoplasmic reticulum sisternas were dilated in these groups. The extracellular edema was evident in the subepithelial connective tissue. The increase in epithelial thickness, as in light microscopy, would be due to intracytoplasmic edema with junctional degeneration and lined up cell nuclei at different levels. There was much less intercellular junctional degeneration in epithelium and it was composed of fewer layers in CG group when compared to CG+SF group (Fig 5A, D). Cells of synovial membrane were in normal ultrastructure in intact knee and saline administrated groups (data not shown). Also cytoplasmic properties and all organels of epithelial cells were normal in CG+Ozone group (Fig 5B). The synovial epithelial integrity was preserved and ultrastructural composition of epithelial cells was normal as it was observed in the ozone-only group (Fig 5E). Likewise, in the group of CG+ozone+HA, epithelial integrity was preserved and epithel cells and their organels were observed to be normal (Fig 5F). Junctional units were preserved in the CG+HA group. Structure of intercellular junctional units exhibited smaller degenerative apertures in CG+HA group than CG group. Also, swelling in mitochondrias and cytoplasmic vacuoles of epithelial cells of CG+HA group were observed apparent (Fig 5C).

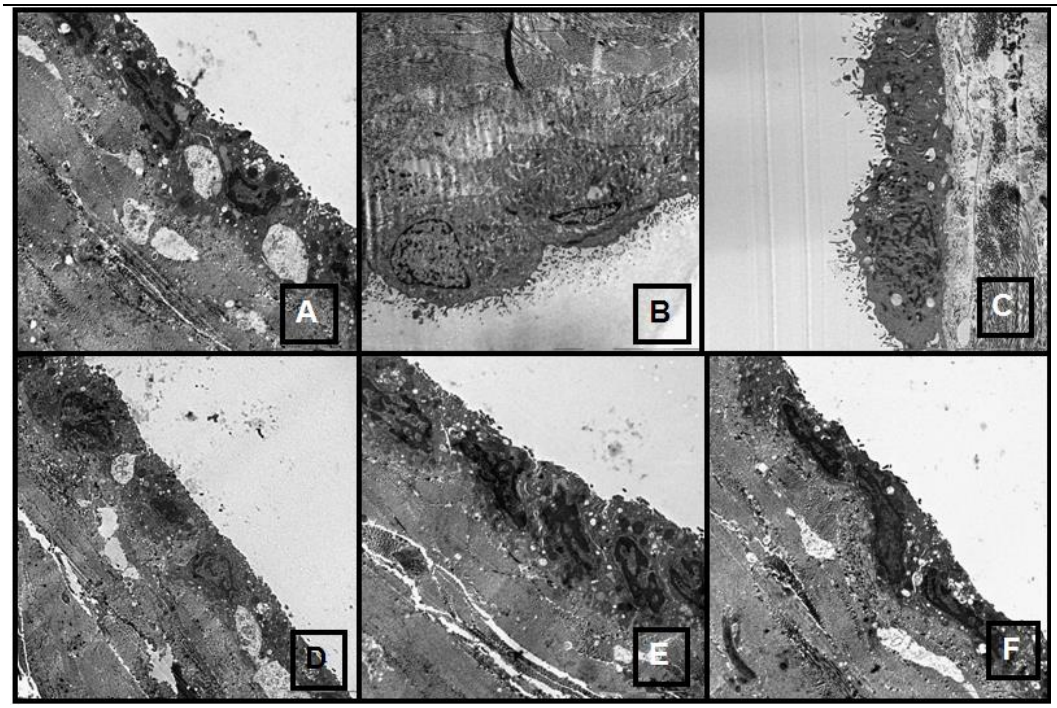

Figure 5. Transmission electron microscopy. Epithelial junctional degeneration, intracytoplasmic vacoules and subepithelial edema in CG and CG+SF groups (A, D). Normal ultrastructural appearance in epithelial cells and subepithelial region in CG+Ozone, only Ozone and CG+Ozone+HA groups (B, E, F). Intercellular vacuoles in epithelial cells in CG+HA groups (C). Uranyl acetate-Lead citrate.

Fibroblasts, known as the responsible cells for collagen synthesis and extracellular matrix, were observed in subepithelial connective tissue. In CG group, intracytoplasmic edema was cumulated in fibroblasts. Because of edema formation, these cells showed pale staining during electron microscopic examination. It was also detected that these cells have dilated endoplasmic reticulum sisternas and mitochondrial cristalysis. Additionally, necrotic nuclei were observed in some of those cells (Fig 6A). Fibroblasts of the CG+Saline group were similar to those in the CG group and had numerous necrotic cells (Fig 6D). In CG+Ozone, Ozone-only and CG+Ozone+HA groups, active fibroblasts with endoplasmic reticulum sisternas were observed related to the production of collagen. Cells and related organels were also, ultrastructurally normal (Fig 6B, E, F). In contrast, fibroblasts were electron pale, inactive and their mitochondrias were swollen in CG+HA group (Fig 6C).

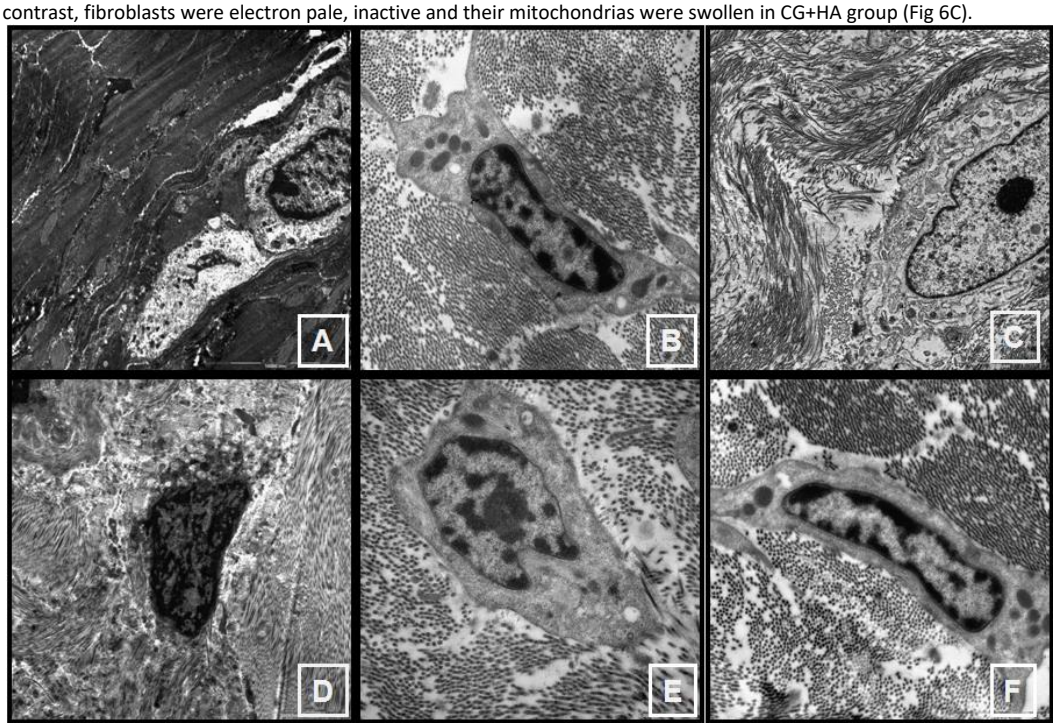

Figure 6. Fibroblastic activity of Synovia**.** Intracytoplasmic vacuoles, electron pale fibroblasts in CG group (A), Fibroblasts with necrotic nuclei in CG+SF group (D). Fibroblasts with normal ultrastructure in CG+Ozone, only Ozone and CG+Ozone+HA groups (B, E, F), Organels with normal histological structure but electron pale fibroblasts in a form of inactive cellular conditions in CG+HA group (C), Uranyl acetate-lead citrate.

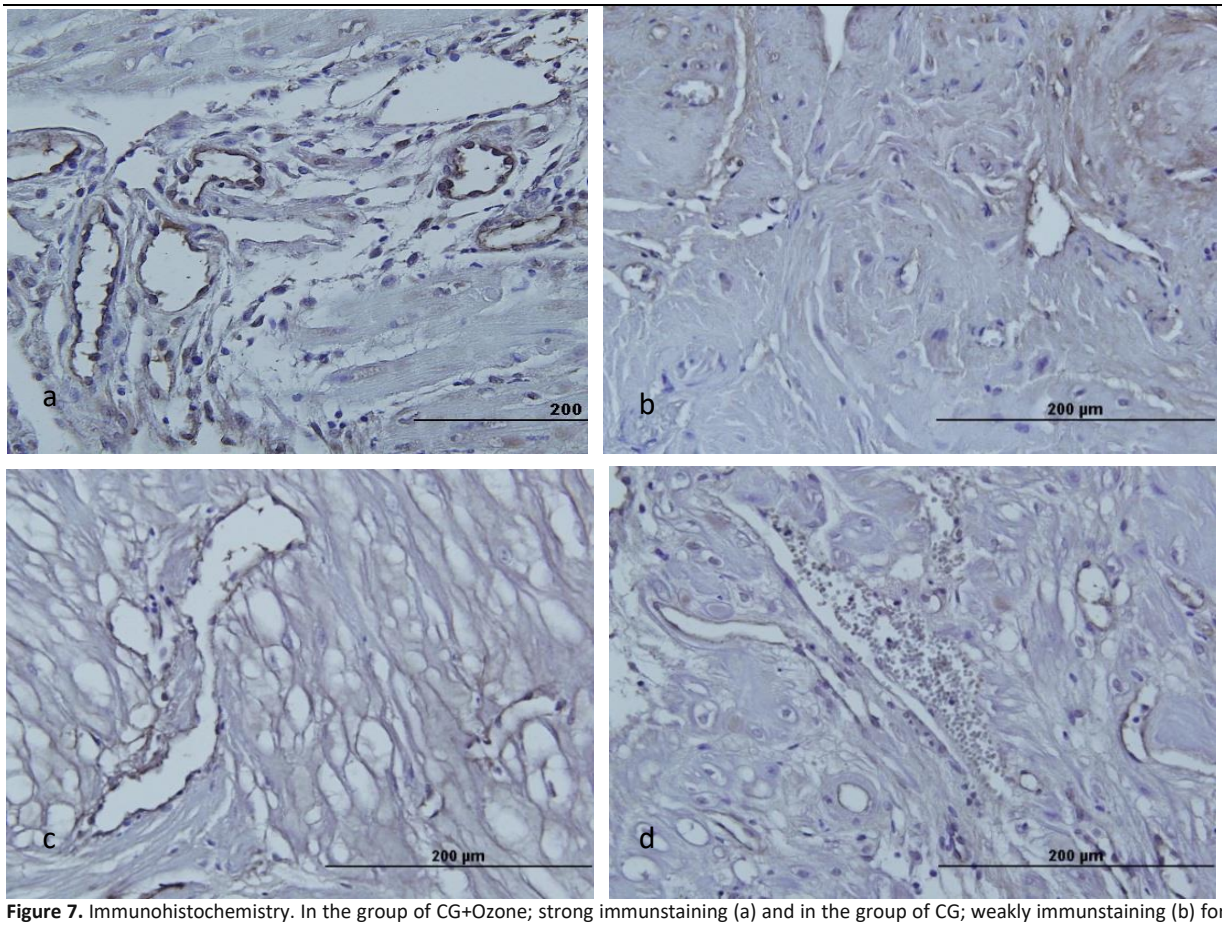

Figure 7. Immunohistochemistry. In the group of CG+Ozone; strong immunstaining (a) and in the group of CG; weakly immunstaining (b) for type III collagen; In the group of CG+Ozone; strong immunoassay (c) and in the group of CG; weakly immunreaction (d) for type IV collagen.

According to these results, ozone applications suppressed the inflammation of synovial membrane by preventing to form angiogenesis that is a parameter for inflammatory response in arthritis-induced knees.

DISCUSSION

Osteoarthritis also known as degenerative arthritis, is a clinical presentation of a group of mechanical abnormalities involving degradation of joints, including articular cartilage and subchondral bone with a loss of the liquid content of the cartilage as a result of a reduced proteoglycan content, thus causing the cartilage to be less resilient. Symptoms may include joint pain, tenderness, stiffness, locking, and sometimes an effusion. When bone surfaces become less well protected by cartilage, bone may be exposed and damaged. As a result of decreased movement secondary to pain, regional muscles may atrophy, and ligaments may become more lax that can be the cause for the decrease in Range of Motion of affected joints (2-5).

There is currently no proven treatment to stop or slow the progression of osteoarthritis and there are treatments to alleviate the pain and other associated symptoms, and for a group of pations the condition will not become debilitating. Intra-articular application of HA in the early stages of osteoarthritis is considered to be reliable by many reports (7-8, 8-9). But there are reservation about the effectiveness of HA application in the treatment of osteoarthritis. Karlsson et al. in 2002 suggested that there were no significant differences in outcome between any of the their study groups during the first 26 weeks and in direct comparison against placebo for weeks 0–52, neither HA treatment showed a significantly longer duration of clinical benefit than placebo (2-5).

Intra-articular application of medical ozone can be efficient in rheumatoid arthritis, gonarthrosis and traumatic joint disorders and it is reported in many studies that the intra-articular injection of ozone to shoulder and knee joints is effective and beneficial in acte and chronic painful musculoskeletal conditions (9-10, 21-107, 23-108).

Also it is reported that the acceleration of decongestion with the reabsorbtion of edema and a reduction in impairment of range of joint movement is retrieved by the medical ozone applications (24-98).

In this study, a modified inflammatory arthritis model of Lawand et al. in 2000 was performed by intra-articular injection of 1% Carrageenan and 1% Kaolin combination to rat knee joints. JGI - a similar parameter with Yun Cho et al. in 2002 for joint diameter measurement - and a modified hot-plate test of Hong et al. in 2006 for nociceptive evaluation were performed to compare results with SF applications. Also the histopathological examination of knee joints was performed by using the light and electron microscoby with further immunohistochemical staining for collagen stereotype analyses. According to all those parameters, there is a statistically significant difference between SF and CG applications (Fig.7). These results are consistent with the previous reports, confirmed the reliability of the model (25-116, 26-120, 27-121). There was no significant improvement for inflammation scores and JGI values in joints with carrageenan-induced arthritis after the intra-articular SF applications. Results were convenient with other relevant studies indicating that SF has no positive effect to inflammatory processes in joints with carrageenan-induced arthritis (28-112). There was a significant improvement for inflammation scores and JGI values in joints with carrageenan-induced arthritis after the intra-articular ozone applications. It is indicated that the ozone application has an intensive anti-inflammatory effect on affected rat knee joints. Those findings were similar with studies of Bochkov and Tamoto (29-117, 30-118, 31-119).

After the intra-articular HA applications, there was a significant improvement for inflammation scores and JGI values in joints with carrageenan-induced arthritis. Those findings were similar with studies of Carabba et al. in 1995 (32-66), but the improvement in inflammation scores with HA was found significantly lesser than the ozone alone and the combination application.

After the intra-articular combination applications, there was a significant improvement for inflammation scores in joints with carrageenan-induced arthritis.

It can be suggested that the combination application has an intensive anti-inflammatory effect on affected rat knee joints and it will be considered as a strong alternative therapeutic modality for the treatment of knee osteoarthritis.

According to the data obtained from hot-plate results at the beginning and the end of experiments, it can be referred that the hyperalgesia occurred with the CG injections, was reversed by the ozone and HA applications. Those findings were similar with studies of Carabba and Sakakibara indicating that HA has a positive effect on nociceptive processes in osteoarthritis (32-66, 33-122).

During experiments, all applications including the solitary intra-articular saline, ozone, hyaluronic acid and combination injections, were found innocent for triggering the inflammatory processes in rat knee joints (data not shown).

CONCLUSION

Intra-articular ozone therapy has significant beneficial effects in terms of histopathological improvement, the antinociceptive effectiveness and knee joint environmental measurements in joints with osteoarthritis. Also, the ozone alone and the combination treatment have better therapeutic effects from HA alone. Those results will indicate that the intra-articular application of medical ozone can be beneficial to conceive new treatment protocols for osteoarthritis.

Conflict of interest

No conflict of interest was declared by the authors.

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