ORIGINAL ARTICLE

Radiographic Evaluation and Comparison of Chondroprotective Effects of Hyaluronic Acid and Triamcinolone in a Rat Model of Osteoarthritis

Noaman Ishaq[1], Muhammad Bilal Shahid[2], Samia Yasin[3], Nausheen Ata[4], Muhammad Waqar Aslam Khan[5], Malik Sikandar Mehmood[6]

ABSTRACT

Objective: To evaluate and compare the chondroprotective effects of Hyaluronic acid and triamcinolone at radiographic level in rat model of osteoarthritis.

Study Design: Laboratory based Randomized control trial.

Place and Duration of Study: This study was conducted in Pharmacology Department, Army Medical College, Rawalpindi, from May to July 2019.

Materials and Methods: Osteoarthritis was induced by medial meniscus and anterior cruciate ligament resection in right knee joints of twenty-four rats. They were divided in three groups with eight rats in each. Group I, II and III were treated with intra articular saline, hyaluronic acid, and triamcinolone once weekly for four weeks respectively. After one week, radiographs of corresponding knee joint of anesthetized rats were taken.

Results: Collective comparison of radiographs of control, hyaluronic acid and triamcinolone groups exhibited a *p* value of 0.001 While intergroup comparison of group I and II, group I and III and group II and III depicted *p* value of 0.05, 0.01 and 0.01 respectively.

Conclusion: Intra articular administration of hyaluronic acid and triamcinolone exhibited chondroprotective effects at radiological level in a rat model of osteoarthritis. On comparison of treatment groups, it was concluded that hyaluronic acid has better chondroprotective effects as compared to triamcinolone.

Key Words: *Chondroprotective Effects, Hyaluronic Acid, Osteoarthritis, Rat Model, Triamcinolone.*

Introduction

Osteoarthritis (OA) is the most common joint disease of old age, heterogeneous in character, affecting commonly joints of the hand, knee, hip and spine.[1] It is one of the leading causes of long term pain and disabilities. Overall old age population is increasing with improvement in health service in modern era. So with increase in old age people, Incidence and prevalence of OA is progressively increasing. It affects around 240 million people all around the world. Approximately 10% males and 18% females aged over 60 years are suffering from OA all around the globe. Basis of OA is degradation and chronic inflammation of the connective tissue of the joint, including the cartilage. Due to long term damage to chondroblasts, chondrocytes and extra cellular matrix caused by oxidative stress, as well as inflammatory factors and mitochondrial dysfunction that cause DNA damage are the leading factors of initiation and progression of OA. Currently few options are available for treatment and prevention of OA and many drugs are in the phase of investigation.[2,3,4] Drug treatment of OA in modern era is still limited to viscosupplement substances, non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids.[5]

Hyaluronic acid (HA), a macro molecule of repeating unit of D glucuronic acid and D acetyl glucosamine, is major constituent of synovial fluid which helps to facilitate lubrication and shock absorption in joints. It is used in OA for viscosupplementation. It is a naturally occurring substance present in synovial fluid. It restores the viscoelasticity of the synovial

[1]*Department of Pharmacology*
Bakhtawar Amin Medical and Dental College, Multan
[2]*Department of Surgery*
Mayo hospital Lahore
[3]*BHU Chak 60 GB Jaranwala, Primary and secondary health department, Punjab*
[4,6]*Department of Pharmacology*
Army Medical College, Rawalpindi
[5]*Department of Pharmacology*
CMH Kharian Medical College, Kharian

Correspondence:
Dr. Noaman Ishaq
E-mail: noamanishaq@yahoo.com

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fluid, may have anti-inflammatory and anti-nociceptive properties. It also stimulates denovo HA synthesis.[6,7] Triamcinolone, an intermediate acting synthetic corticosteroid, is quite effective and is one of the most common drugs used to relieve symptoms in OA. It shows anti-inflammatory activity via inhibition of gene expression of prostaglandins and other inflammatory substances. So, it not only relieves pain but shows functional improvement and chondroprotective effects. It also influences macrophage activation and eventually decreases osteophytosis and cartilage degeneration.[8,9]

Definite cure of OA is not currently available. HA, triamcinolone, and many other drugs are investigational and are used to reduce symptoms and delay the progression of disease. Aim of this study is to evaluate and compare the chondroprotective effects of HA and triamcinolone at radiographic level in a rat model of OA.

Material and Methods

It was a Laboratory based randomized control trial that was carried out in department of Pharmacology and Therapeutics, Army Medical College (AMC), Rawalpindi in collaboration with National Institute of Health (NIH), Islamabad. Ethical approval certificate was endeavored from ethical review committee of "Centre for Research in Experimental and Applied Medicine (CREAM)", AMC. Tenure of rat's intervention was two months from May 2019 to July 2019. They were kept and nurtured in animal house of NIH during the complete study period. Preliminary twenty-four (24) adult male or non-pregnant female rats of Sprague Dawley breed, approximately 10 weeks old and weighing about 500 grams were selected through nonprobability convenient sampling. They were randomly assigned in three (03) groups with eight (08) rats in each group. Group I, II and III were labelled as Control, HA and triamcinolone groups respectively. Rats were kept in standard environment with temperature ranged 25±5˚C, adequate humidity, and 12 hours day night cycle. Free excess to clean drinking water and standard rodent diet *ad libitum* was ensured during the whole study period. Surgical procedure was performed to induce OA in right knee joint of all rats. Before surgery rats were anesthetized with intraperitoneal injection of 5% xylazine and 1% ketamine.[10] Skin of the joint was shaved in a sterilized environment. Then a para patellar incision was made on medial side for complete exposure of the joint. Anterior cruciate ligament and medial meniscus were identified and transected. Aseptic closure of wound with surgical stapler was done after the completion of the surgery. Animals were allowed to move freely in the cage for two weeks thereafter.[11] Then intra articular drugs were administered in the corresponding joint of the rats. Rats of control, HA and triamcinolone groups were injected with 0.2 ml of Normal saline, 0.2 ml HA, and 70 µl (1.4 mg/ml) triamcinolone respectively once weekly for 04 weeks. [12,13,14] Thereafter Animals were anesthetized with intraperitoneal injection of xylazine 10% and ketamine 1%, transported to Radiology department of a private institute of Rawalpindi where their knee joints were radiographed. 500mA digital X ray machine China operated at 220 V with a 0.3 sec exposure time was used for radiography. Kellgren and Lawrence system was considered to grade the severity of the OA. According to Kellgren and Lawrence grading system grade 0 characterizes no radiographic features of OA, grade 1 represents doubtful joint space narrowing (JSN) and possible osteophyte lipping, grade 2 portrays definite osteophytes and possible JSN on anteroposterior weight-bearing radiograph, grade 3 depicts multiple osteophytes, definite JSN, sclerosis, possible bony deformity while grade 4 describes large osteophytes, marked JSN, severe sclerosis and definite bony deformity. [15,16] After radiographic grading with Kellgren and Lawrence system, animals were euthanized with toxic dosage of chloroform. Obtained data was analyzed using IBM SPSS version 23. Groups were analyzed through *kruskul wallis test* followed by *Post Hoc Tukey HSD test*. The differences between two interpretations were considered statistically significant if the *p* value was equal to or less than 0.05 ($p \leq 0.05$)

Results

Two radiographs (25%) of group I (control group) depicted severe changes of OA and graded as grade 04, while four (50%) radiographs depicted moderate, and two (25%) radiographs depicted minimal changes of OA and they were graded as grade 03 and grade 02 respectively. Figure 01 is an X-ray of a rat of control group that has characteristic features of sclerosis and possible joint deformity. Grade of this

X-ray is 03. Most of the radiographs of group I feature osteophytes, JSN and bone deformity.

Radiographic changes of group II (HA group) that received IA injection of HA described no radiographic changes of OA in two radiographs (25%), doubtful changes in four (50%) and minimal changes in two (25%) radiographs and they were graded as grade 0, grade 01, and grade 02 respectively. Figure 02 is an X-ray of a rat of HA group that depicts the feature of minimal changes of OA. Grade of this X-ray is 02. No or doubtful changes of OA were the feature of radiographs of this group. Radiographic changes of group III (triamcinolone group) that received IA injection of triamcinolone depicted as moderate radiographic changes of OA in four (50%), minimal changes in three (37.5%) and doubtful changes in one (12.5%) radiograph and were graded as grade 03, grade 02 and grade 01 respectively. Figure 04 is an X-ray of rat of triamcinolone group that depicts moderate changes of OA. Grade of this X-ray is 03. Most of the radiographs showed features of osteophytes and JSN in this group. When *Kruskul wallis test* was applied between all the three groups, *p* value was 0.001 that was statistically significant thus claiming the chondroprotective effects of hyaluronic acid and triamcinolone at radiographic levels. Intergroup comparison of groups via *post hoc tukey HSD test* depicted *p* values as showed in Table 01. Comparison of HA group and triamcinolone group exhibited *p* value of 0.01 that confirmed HA has superior chondroprotective effects as compared to triamcinolone.

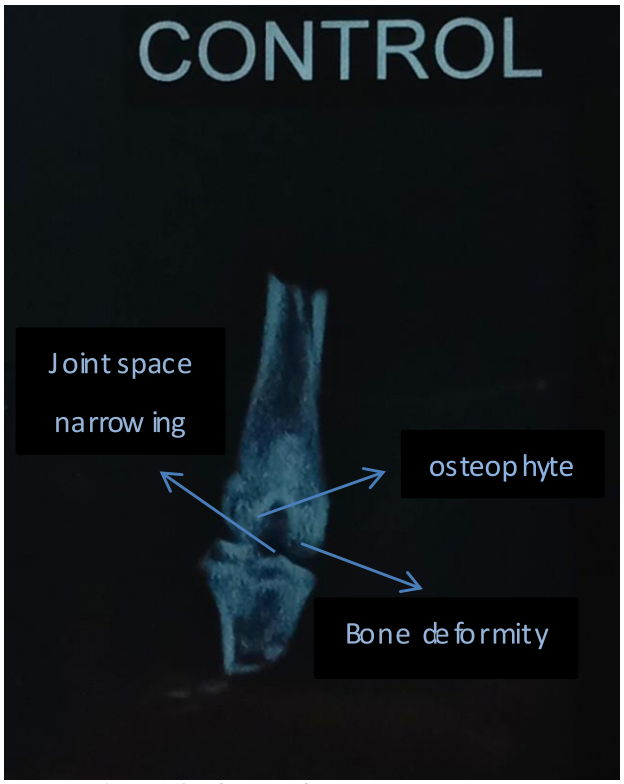


Fig 1: Radiograph of a rat of group I (Control group)

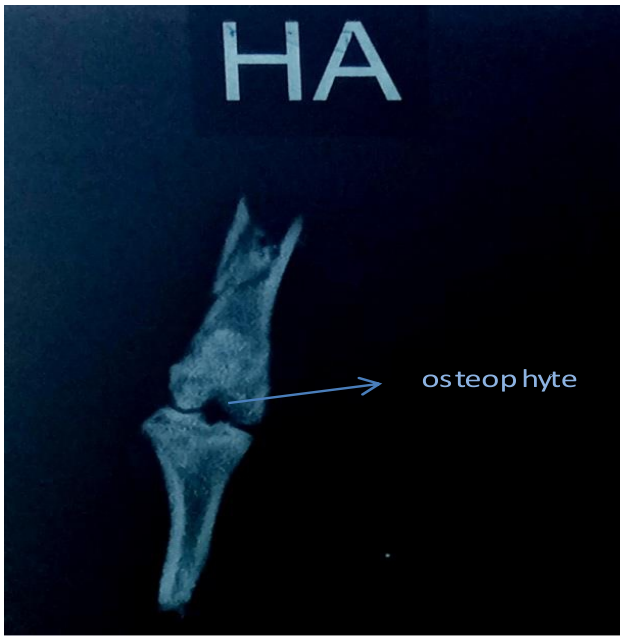


Fig 2: Radiograph of a rat of group II (Hyaluronic Acid group)

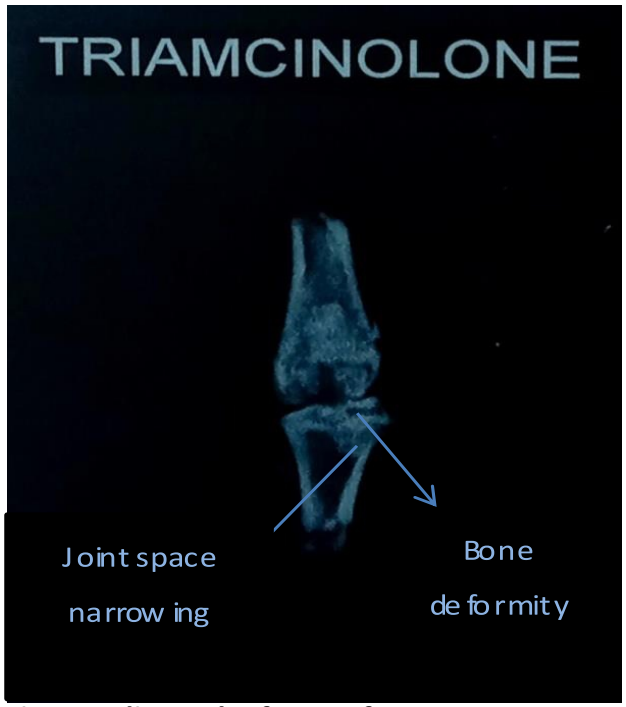
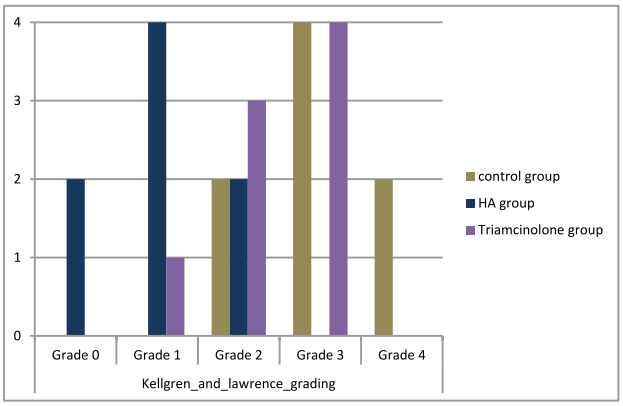


Fig 3: Radiograph of a rat of group III (Triamcinolone group)

Table I: Intergroup Comparison of Radiographs When Post Hoc Tukey Test HSD is Applied

Groups	P value
Group I and II	<0.05
Group I and III	<0.01
Group II and III	<0.01



Graph 1: Kellegren and Lawrence Grading of OA Knee Joints of Rats of all Groups

Discussion

Viscosupplement substances, nonsteroidal anti-inflammatory drugs (NSAIDs) and corticosteroids are group of drugs that are investigational but frequently used in the management of *osteoarthritis*.[17] HA, a viscosupplements, is a natural component of synovial fluid that helps to facilitate lubrication and shock absorption in joints. It is one of the many investigational molecules in the treatment of OA. Viscosupplementation with intra articular HA has positive outcome in pain alleviation and joint function improvement in OA.[18] Triamcinolone, a synthetic corticosteroid is being used in the management of OA for decades. Triamcinolone binds to intracellular glucocorticoid receptors and down regulates the expression of genes in prostaglandins synthesis and leukotrienes release. Moreover, it also enhances lipocortin expression that modulates anti-inflammatory effects. These factors ultimately lessen the inflammation of synovium and other articular structures in OA.[19,20]

This animal study is planned to evaluate and compare the chondroprotective effects of HA and Triamcinolone. Human admissible dosage of these drugs that was proficiently effective in rat model was selected by substantial search and literature review. OA was induced by medial meniscus and anterior cruciate ligament resection in the rats. After that they were randomly divided in control group, HA group and triamcinolone group with eight rats in each group. Control group was intra articularly treated with saline water while HA and triamcinolone groups were treated with intra articularly HA and triamcinolone respectively. Later, when intervention protocol was completed, chondroprotective effects of these drugs were analyzed by radiographs. When radiographic grades of treatment group were statistically compared with control group, we found a significant *p* value of 0.001 that confirmed the chondroprotective effects of HA and triamcinolone.

Our research work is supported by 2016 research work of Zhiwei Zhang who found that HA reduces radiographic osteophytosis grading ($p<0.05$) as compared to saline treated rats' model of osteoarthritis.[21] Likewise Ai Tong worked to evaluate the chondroprotective effects of cocktail of mesenchymal cells and HA in chemical induced rat model of OA. He assessed rats through Magnetic resonance imaging and found significant (p <0.05) chondroprotective effects of this mixture.[22] Their results also favor our outcome regarding HA. Similarly, research work of Yunus Emre proved that HA exhibit chondroprotective effects at histological level (P =0.04) in rat model of OA.[23]

In 2017 Yashashri C. Shetty did research work on chemical induced models of rats. His results confirmed that triamcinolone reduces the histopathological severity of disease ($p<0.01$) as compared to disease control group.[24] Likewise research of Jeffrey S. Kroin declares that triamcinolone lessens allodynia as compared to mice of control group (p value <0.01). Their work at tissue level expressed that triamcinolone has potent anti-inflammatory effects (p value <0.01) via inhibiting expression of IL- 1β and TNFα.[25] Furthermore in vitro research of E. Frank also confirmed that triamcinolone has some chondroprotective effects on damaged and swollen cartilage (p value <0.05) via inhibiting sulfate assimilation and glycosaminoglycan loss. Results of these studies strengthening our outcome regarding chondroprotective effects of triamcinolone.[26] Consequently chondroprotective effects of HA and triamcinolone that is confirmed by current research and supported by previous research favor the use of these investigational drugs in patients of OA and

provides a template for future studies.

When Radiographs of HA group was statistically compared with triamcinolone group, it was found that HA is more efficacious than triamcinolone. These results are according to the research work of Yashashri C. Shetty who found that HA has better chondroprotective effects than triamcinolone in chemically induced murine model of OA (p <0.001).[27] Likewise, human study by Soad A Elsawy portrayed better chondroprotection offered by HA as compared to triamcinolone (p=0.01).[28] Similarly, meta-analysis by Egemen Ayhan and colleagues also verified that HA has superior chondroprotective efficacy as compared to triamcinolone.[29]

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Conclusion

Intra-articular administration of 0.2 ml (10mg/ml) hyaluronic acid and 70 µl (1.4 mg/ml) triamcinolone once weekly for 04 successive weeks reduced severity of osteoarthritis in rat model at radiographic level. Upon Comparison of hyaluronic acid and triamcinolone, it was concluded that former has better chondroprotective effects as compared to triamcinolone in rat model of osteoarthritis.

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