Comparison of Chondroprotective Efficacy of Hyaluronic Acid and Piroxicam in Murine Model of Osteoarthritis

Noaman Ishaq,[1] Maryam Saqib,[2] Zari Salahuddin,[3] Shahzad Gul,[4] Nausheen Ata,[5] Zainab Rahman[6]

Abstract

Objective: To evaluate and compare the chondroprotective efficacy of hyaluronic acid and piroxicam in murine model of osteoarthritis.

Method: Study was carried out at Pharmacology Department, Army Medical College (AMC), Rawalpindi. Duration of this study was from May to July 2019 Osteoarthritis was induced by medial meniscus and anterior cruciate ligament resection of right knee joints in twenty-four rats. They were divided in three groups. Group A, B and C were treated with intra articular saline, hyaluronic acid and piroxicam once weekly for four weeks respectively and then gait pattern was scored. Animals were sacrificed thereafter and samples were collected for histopathology.

Results: Comparison of gait score of A, B and C groups exhibited a p value of <0.01. while, comparison of gait of group A and B, group A and C and group B and C depicted p value of <0.001, <0.001and 0.771 respectively. Likewise, collective histopathological analysis of control, piroxicam and triamcinolone groups showed p value of <0.01. While Intergroup histopathological comparison of group A and B, group A and C and group B and C showed p value of <0.001, <0.001 and 0.239 respectively.

Conclusion: Intra articular administration of hyaluronic acid and piroxicam exhibited parallel chondroprotective efficacy in murine models.

Keywords: Chondroprotective efficacy, Hyaluronic acid, Piroxicam, Osteoarthritis

How to cite: *Ishaq N, Saqib M, Salahuddin Z, Gul S, Ata N, Rahman Z. Comparison of Chondroprotective Efficacy of Hyaluronic Acid and Piroxicam in Murine Model of Osteoarthritis. Esculapio - JSIMS 2022;18(03):282-286*

Introduction

Osteoarthrosis, a less common name of Osteoarthritis (OA) is a pandemic chronic joint ailment with 30 million patients worldwide. It is a complex joint disease in which old age and female gender are major risk factors having strong association with metabolic syndrome and obesity.[1] Interleukin-1β, tumor necrosis factor α and interleukin-6 are the molecular markers that contribute to expansion of bone deformity, joint space narrowing and development of osteophytes in OA.[2,3] It is one of unfortunate diseases whom complete cure is underdevelopment. However different non pharmacological (lifestyle and dietary habits modifications), pharmacological (Viscosupplements substances, Non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids) and surgical (osteotomy, arthroscopy, and joint replacement) techniques are available to manage this disease.[4,5]

Hyaluronic acid (HA), a viscosupplement, a ubiquitinous molecule consisting of long chain of D glucuronic acid and D acetyl glucosamine is major constituent of synovial fluid. Endogenous HA through its mechanical

1. *Department of Pharmacology Nishtar Medical University Multan*
2. *Department of Pharmacology CMH Lahore Medical College and Institute Of Dentistary Lahore*
3. *Department of Pharmacology Avicenna Medical and Dental College Lahore*
4. *DHQ Hospital Haripur*
5. *Department of Pharmacology Army Medical College Rawalpindi*
6. *Army Medical College Rawalpindi*

Correspondence:
Noaman Ishaq; *Assistant Professor Pharmacology, Nishtar Medical University Multan*

Submission Date: 07-06-2022
1st Revision Date: 13 07-2022
Acceptance Date: 13-08-2022

and viscoelastic properties provide joint lubrication and allow them to move a wide range of movement.[6] HA comes in the list of prevalent investigational substance in the drug management of OA. Nowadays it is common practice to use it through Intra articular (IA) route. Viscosupplementation of diseased joints with HA produces constructive outcome to relieve pain that ultimately plays role in improvement of joint function. Numerous researches illustrated chondroprotection offered by exogenous HA alongside with its positive clinical results. Several physiological responses of HA actually lead employs its clinical regenerative effects. These mechanisms include stimulation of endogenous proteoglycan and glycosaminoglycan synthesis, anti-inflammatory effect due to reduce synthesis of mediators and viscoelasticity maintenance. It also has a distinct effect on pro-inflammatory markers comprising cytokines, leukotrienes, prostaglandins and proteases. HA also exhibits antioxidant effects promoting articular chondrocytes protection against the harm induced by oxygen-derived free radicals.[7] Piroxicam (PIRO), belongs to oxicam group of NSAIDs, is non selective cyclooxygenase (COX) inhibitor. It is one of the NSAIDs used frequently in management of OA.[8] Repeated oral administrations have serious adverse profile including gastroduodenal bleeding and renal disorder.[9] To avoid untoward effects of oral route, targeted IA therapy of PIRO is common practice when expertise of IA drug administration are available. PIRO has advantageous consequences on cartilage destruction through its hindrance of prostaglandins E2 (PGE2) synthesis which eventually results in reduced proteoglycan component in cartilage.[10]

There is presently no definitive cure for OA. HA, PIRO, and a variety of other medications are experimental and are administered to alleviate symptoms and slow the progression of illness. The goal of this experiment is to assess the chondroprotection afforded by HA and PIRO in an OA murine model and determine the medicine with the best chondroprotective effectiveness. Results of this animal study can be beneficial clinically for OA patients as we have more knowledge about efficacy of chondroprotection between these two drugs.

Material and Method

Laboratory based experimental study was the study design of this project. Center of the study was Pharmacology Department, Army Medical College (AMC), Rawalpindi. We got alliance from National Institute of Health (NIH), Islamabad for animal keeping. Ethical review committee "CREAM", AMC reviewed this project before releasing approval. Period of rat's intervention was two months from May to July 2019. Through non probability convenient sampling Twenty-four (24) adult Sprague Dawley rats, approximately of about ±500 grams were selected. Animals were separated in 03 groups with 08 in each one. Control group, HA group and PIRO group were the respective label of Group A, B and C. Standard laboratory conditions with optimum room temperature and 24 hours day/night cycle was maintained. Clean tap water for drinking and rodent chow ad libitum was provided to rats during the complete tenure of study. Right knee joint of all rats was selected for OA induction through surgical trauma. Mixture of 5% xylazine and 1% ketamine was intraperitoneally administered to anesthetize rats for surgery.[11] Joint skin was shaved and disinfected stalked by medial para patellar incision for full joint exposure. Dissections of cruciate ligament and menisci of medial side were performed following the standard protocol. Aseptic wound closure was done after the completion of procedure. Rats were conceded to move freely in their cages for a period of two weeks thereafter. Later on drugs through IA route were injected within OA induced knees of the rats. The rat model of control, HA and PIRO groups were administered with 100 microliter of 0.9% saline, 100 microliter of HA and 70 microliter PIRO once weekly for 04 weeks.[12,13] Gait score was analyzed one week after the drug administration.[14] After gait pattern scoring, rats were sacrificed by putting them in a desiccator full up of chloroform. Samples of distal femur were collected by availing angled bone cutter.[15] Histological slides was prepared by using standard technique and scored according to Modified Mankin score of histopathology.[16] By utilizing IBM SPSS version 23, statistical analysis of the results was carried out. Analysis of variance (ANOVA) and Post hoc tukey tests were used to compare the Gait score with the Modified Mankin score. Probability (p) value of ≤ 0.05 was the cut off value to determine the significance of results.

Results

One week post last dose; gait patterns of the rats were gauged by gross observations. A full length A2 (42×60cm) size blank white paper was placed on a smooth flat surface for each individual and the hind paws of each rat was heavily tainted with ink. To ensure their complete

walk progression from one border of the paper to another, a food treat was placed at the destination to allure them. This practice was aimed to compare the drug treated right leg and untreated control i.e. left leg. Amongst the eight rats of untreated control group, three rats scored 04, other four scored 03 and the last one scored 01. Their mean sums up to 3.25±0.707. On the contrary, amongst the eight rats of Hyaluronic acid (HA) group four rats notched 04, two rats scored 02, and two rats scored Zero. Their mean value came out to be 1.00± 0.756. In the interim the gait patterns of PIRO administrated group of one failed to notch up any score with a zero, four rats scored 01 and last three rats scored 02. Mean score of this group was 1.25±0.707. For evidential provision a detailed statistical scrutiny was done via ANOVA to carry out comparisons between the gait score of normal versus the drug treated groups. The p value between control and drug administered groups held a significant value as it turned out be <0.01, these results authenticates the chondroprotective efficacy of HA and PIRO. Further elaboration of inter drug group comparisons via Post Hoc Tuckey test was applied as detailed in Table 02.

X300 microscope lens were used to histopathological scoring of slides Modified Mankins scoring system was applied headed for the histopathological variations under both development and treatment of OA.

Under the aforementioned conditions, the control group's histology slide scores ranged from 10 to 13. Six of the eight slides had substantial irregularities in the perichondrium, whereas the other two had moderate irregularities. Similarly, six slides showed substantial cellular organisation irregularity, while two slides showed minor irregularity. On the other hand, perichondrium fibrosis was seen in one of the control slides, moderate in six, and mild in one. All slides in this group showed a significant rise in chondrocyte cellularity index, with five films showing a considerable increase in chondrocyte clusters with 10-20% chondrocyte necrosis, and the other three slides showing a modest increase in chondrocyte clusters. All slides include fibrous degeneration as a characteristic. The control group's mean score was 11.50 ±1.195.

In comparison to the other groups, the HA's score ranged between 04-07, the lowest possible. All slides showed modest to moderate perichondrium irregularity in comparison to the control group. Four slides showed perichondrium fibrosis, whereas the other half did not. All except one slide in the study exhibited mild to moderate perichondrium irregularity, while the other slides had moderate to noticeable irregularity. The cellularity of chondrocytes was somewhat increased on five slides, but not on the other nine. One picture indicated a significant increase in chondrocyte clusters in seven of the images. The chondrocyte necrosis on all of the slides was between 10 and 20 percent. All of the slides showed no signs of fibrinoid degeneration. The HA group had a mean score of 5.50±1.195.

By conducting procedure in similar manner the slides of group PIRO group scored an estimated range of 5-8. Slight perichondrial irregularity was homologous characteristic in all slides. Whereas, regarding perichondrial fibrosis in the eight slides one was devoid of any relevant findings, six had slight, whereas one had modest fibrosis of perichondrium. In addition mild and moderate to marked irregularity in cellular organization was the feature of one and seven slides respectively. Particularly regarding chondrocytes one slide exhibits none, four slides exhibit slight and three slides exhibit modest to mark increased in chondrocytes cellularity regardless of that all slide showed the feature of 10-20% chondrocyte necrosis. None of the slides exhibited fibrinoid degeneration. Summing up the findings the PIRO group displayed wide array of features like, modest unevenness in organization, hypercellularity and minor cellular necrosis. PIRO group's mean score was calculated to be 6.50±1.195.

Hence, a noticeable decrease of the variables including means of gait and Modified Mankins scores were seen in drug treated groups in competition to the disease

Table 1: *Mean± SD of Gait score and Modified Mankin scoring system*

Groups	Mean ± SD of histopathology	Mean ± SD of histopathology	P value
Group A (control group)	3.25± 0.707	11.50± 1.195	
Group B (HA group)	1.00± 0.756	5.50± 1.195	<0.001*
Group C (piroxicam group)	1.25± 0.707	6.50± 1.195	

P value was < 0.01 when ANOVA was applied

Table 2: *Tuckey test is applied when the results are significant*

Groups	Gait analysis score	Histopathology score
A and B	<0.001	<0.001
A and C	<0.001	<0.001
B and C	0.771	0.239

group with an all-rounder significant p value of <0.01, validating the regenerative properties of HA and PIRO. Amongst inter drug group comparison both drugs depicted their chondroprotective effects on parallel level.

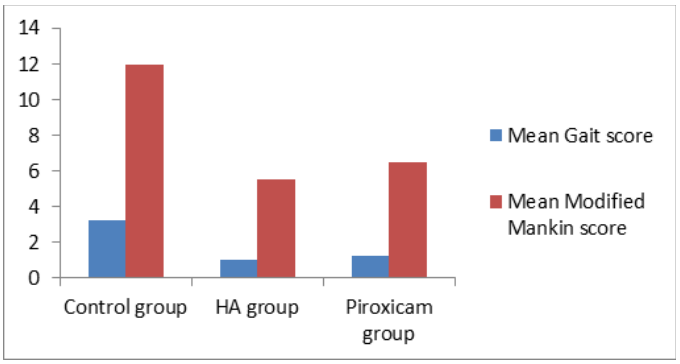

Fig 1: *Bar Chart of Mean Gait and mean Histopathology score*

Discussion

Osteoarthritis (OA) is the most communal type of arthritis in the world, characterised by persistent joint pain and dysfunction. Increased average life expectancy and the obesity epidemic explain the majority of the global rise in OA. OA has significant macroeconomic consequences in addition to its personal costs; as a result, OA will be a growing public health challenge in the future decades.[17] The definitive therapy for OA has yet to be established. Many medication categories are still under study and are availed to treat traits and lessens the course of illness These are viscosupplement compounds, nonsteroidal anti-inflammatory drugs (NSAIDs), and glucocorticoids.[18] Many investigations on the chondroprotective effectiveness of different viscosupplement substances, NSAIDs, and glucocorticoids have been conducted all over the globe. In a murine model of OA, the goal of this project was to relate the chondroprotective effects of hyaluronic acid (HA), a viscosupplement material, and piroxicam (PIRO), an NSAID. When the scores of gait and histology of the control and HA groups were evaluated after the interventional procedure was completed, we identified significant changes that confirm the chondroprotective benefits of HA. Our results matched those of Zhenqinq's study from 2018. He compared rabbit HA-interrupted OA models to a disease group and concluded with a substantial p value of 0.05.[19] Yunus Emre used a 27 mm drill bit to cause chondrocyte abnormalities in rats' knees. In contrast to control group mouse mice, he discovered that HA had regenerating effects on cartilage (p-value 0.001). 20 Similarly, our findings are corroborated by a 2020 study by Li Jung Kang and colleagues, who developed a surgically induced OA mouse model and discovered that HA had chondroprotective effects (p value 0.01) when compared to a vehicle-treated group.[21] When the Gait and histology scores of the PIRO and control groups were matched, we discovered statistically considerable results which proved PIRO's chondroprotective effectiveness. Similarly, Park and his colleagues found the same outcomes in their investigation. In comparison to saline-treated rats, their research found statistically significant changes (p value 0.05) in joint swelling ratings in IA PIRO intervention rats.[22] Meanwhile, Ijaz ul Haq and colleagues observed a regenerative effect of PIRO (p value 0.05) in rodent OA models, which supports our findings.[23] Similarly, Aziza's study has revealed that PIRO intramuscular treatment lowers articular swelling and arthritis score statistically substantially (p 0.05) in vehicle-induced arthritis in mice.[24] Her results corroborated previous research on PIRO's chondroprotective properties. After comparing the chondroprotective efficacy of the HA and PIRO groups, we discovered that both drugs have equal chondroprotective efficacy. Our work is unusual in that both medicines are routinely recommended by doctors for the treatment of OA, and no in vitro, animal, or human investigation has examined the chondroprotective effectiveness of these two treatments to our knowledge.

Conclusion

In rat models, intra-articular injection of hyaluronic acid and piroxicam decreased the severity of OA, as shown by better mean gait and histopathological scores. Both medications have identical chondroprotective effectiveness, according to the comparison.

Conflict of Interest *None*

Funding Source *None*

References

1. Abramoff B, Caldera FE. Osteoarthritis: pathology, diagnosis, and treatment options. Med Clin. 2020; 104 (2):293-311.
2. Norman B, Pedoia V, Noworolski A, Link TM, Majumdar S. Applying densely connected convolutional neural networks for staging osteoarthritis severity from plain radiographs. J dig imag. 2019;32(3):471-7.

3. Wang Z, Jones G, Winzenberg T, Cai G, Laslett LL, Aitken D, et al. Effectiveness of Curcuma longa Extract for the Treatment of Symptoms and Effusion-Synovitis of Knee Osteoarthritis: A Randomized Trial. Annals Intern Med 2020; 173(11):861-9.
4. Hussain SM, Cicuttini FM, Alyousef B, Wang Y. Female hormonal factors and osteoarthritis of the knee, hip and hand: a narrative review. Climacteric. 2018; 21(2): 132-9.
5. Varela-Eirin M, Loureiro J, Fonseca E, Corrochano S, Caeiro JR, Collado M, Mayan MD. Cartilage regeneration and ageing: targeting cellular plasticity in osteoarthritis. Age res rev. 2018; 42(1):56-71.
6. Sudirman S, Ong AD, Chang HW, Kong ZL. Effect of fucoidan on anterior cruciate ligament transection and medial meniscectomy induced osteoarthritis in high-fat diet-induced obese rats. Nut. 2018;10(6): 686-99.
7. Holyoak DT, Tian YF, van der Meulen MC, Singh A. Osteoarthritis: pathology, mouse models, and nanoparticle injectable systems for targeted treatment. An biomed eng. 2016;44(6):2062-75.
8. Leighton R, Fitzpatrick J, Smith H, Crandall D, Flannery CR, Conrozier T. Systematic clinical evidence review of NASHA (Durolane hyaluronic acid) for the treatment of knee osteoarthritis. Rheumatol. 2018;10(1):43-54.
9. Kim SH, Park KW, Kim JM, Ho MJ, Kim HT, Song SH, et al. Pharmacokinetics and four-week repeated-dose toxicity of hyaluronic acid and ketorolac combination following intra-articular administration in normal rats. Regul Toxicol and Pharmacol. 2019; 102(1):79-89.
10. Li X, Gao Y, Liu J, Zhang G, Zhang T. A rapid analysis of piroxicam in beagle plasma applying evaporation-free liquid-liquid extraction by supercritical fluid chromatography-tandem mass spectrometry. J Chromat B. 2018; 1100(1):93-9.
11. Mohammadi-Samani S, Zojaji S, Entezar-Almahdi E. Piroxicam loaded solid lipid nanoparticles for topical delivery: preparation, characterization and in vitro permeation assessment. J Drug Del Sci Technol. 2018; 47(1):427-33.
12. Kim SR, Ho MJ, Kim SH, Cho HR, Kim HS, Choi YS, et al. Increased localized delivery of piroxicam by cationic nanoparticles after intra-articular injection. Drug des develop therap. 2016;10(1):3779-87.
13. Mechelinck M, Kupp C, Krüger JC, Habigt MA, Helmedag MJ, Tolba RH, et al. Oxygen inhalation improves postoperative survival in ketamine-xylazine anaesthetised rats: An observational study. PlOS One 2019; 14(12): 1-14.
14. Xing D, Wu J, Wang B, Liu W, Liu W, Zhao Y, et al. Intra-articular delivery of umbilical cord-derived mesenchymal stem cells temporarily retard the progression of osteoarthritis in a rat model. Intern J Rheum Dis. 2020; 23(6):778-87.
15. Kim SH, Mi-Won SO, Jang SW, Kang MJ, Ma KW, inventors; Dong-A ST Co Ltd, assignee. Stable liquid pharmaceutical composition containing piroxicam or its pharmaceutically acceptable salt and hyaluronic acid or its pharmaceutically acceptable salt and the manufacturing method thereof. Uni Stat Pat US 2016; 12(14);1-18.
16. Kumar A, Bendele AM, Blanks RC, Bodick N. Sustained efficacy of a single intra-articular dose of FX006 in a rat model of repeated localized knee arthritis. Osteo Car 2015;23(1):151-60.
17. Takahashi I, Matsuzaki T, Kuroki H, Hoso M. Joint unloading inhibits articular cartilage degeneration in knee joints of a monosodium iodoacetate-induced rat model of osteoarthritis. Ost Car 2019; 27(7):1084-93.
18. Yang Y, Li P, Zhu S, Bi R. Comparison of early-stage changes of osteoarthritis in cartilage and subchondral bone between two different rat models. Peer J. 2020; 20(8):8934-52.
19. Latourte A, Kloppenburg M, Richette P.. Emerging pharmaceutical therapies for osteoarthritis. Nat Rev Rheumatol. 2020; 16(12): 673-88.
20. Jain KB, Ravikumar P. Recent advances in treatments of cartilage regeneration for knee osteoarthritis. J Drug Del Sci Technol 2020; 23(8):1-12.
21. Hong Z, Gao H, Su Y, Xu B, Wu Z. Effect and mechanism of total alkaloids of strychnine on papain induced rabbit knee osteoarthritis. Biomed Res.2018; 29(8):1590-97.
22. Akman YE, Sukur E, Senel A, Sukur NE, Talu CK, Ozturkmen Y. The comparison of the effects of a novel hydrogel compound and traditional hyaluronate following micro-fracture procedure in a rat full-thickness chondral defect model. Actaorthop Traumatol Tur. 2017; 51(4):331-6.
23. Kang LJ, Yoon J, Rho JG, Han HS, Lee S, Oh YS, et al.. Self-Assembled Hyaluronic Acid Nanoparticles for Osteoarthritis Treatment. Biomat. 2021; 10(1): 120967-76.
24. Park CW, Ma KW, Jang SW, Son M, Kang MJ. Comparison of piroxicam pharmacokinetics and anti-inflammatory effect in rats after intra-articular and intramuscular administration. Biomol Ther. 2014; 22(3): 260-66.

Authors Contribution

NI: Conceptualization of Project
MS : Data Collection
ZS: Literature Search
SG: Statistical Analysis
NA: Drafting, Revision
ZR: Writing of Manuscript