ORIGINAL ARTICLE

CHONDROPROTECTIVE EFFECTS OF INTRA-ARTICULAR HYALURONIC ACID AND TRIAMCINOLONE IN MURINE MODEL OF OSTEOARTHRITIS

Noaman Ishaq[1], Shahzad Gul[2], Mahwish Gul[3], Nausheen Ata[4], Saba Batool[1], Quratulain Mehdi[5]

[1]Department of Pharmacology, Nishtar Medical University, Multan, [2]Rheumatology Department, Pakistan Institute of Medical Sciences (PIMS), Islamabad, [3]Department of Pharmacology, Ayub Medical College, Abbottabad, [4]Department of Pharmacology, Army Medical College, Rawalpindi, [5]Department of Pharmacology, Quetta Institute of Medical Sciences, Quetta-Pakistan

Osteoarthritis is a heterogeneous disease of joints that affects mainly older population. Definitive cure of osteoarthritis is still undiscovered. This study was designed to evaluate and compare the chondroprotective effects of hyaluronic acid and triamcinolone in murine model of osteoarthritis. **Methods:** This Laboratory based experimental study was carried out in Pharmacology Department, Army Medical College Rawalpindi, from April–June 2019. Osteoarthritis was induced by medial menisectomy and anterior cruciate ligament resectioning of knee joints of twenty-four rats which were then divided into three groups with eight rats in each. Group I, II and III were named control, hyaluronic acid and triamcinolone groups respectively, which were given intra-articular injections of these drugs once weekly for four consecutive weeks and then gait pattern was scored. Animals were sacrificed thereafter and samples were collected for histopathological analysis. **Results:** Comparison of gait score of control, hyaluronic acid and triamcinolone groups exhibited a *p* value of <0.01 while intergroup comparison between group I and II, group I and III and group II and III depicted *p*-value of <0.001, 0.016 and 0.003 respectively. Likewise, collective histopathological analysis of the three groups showed *p*-value of <0.01 while intergroup comparison of group I and II, group I and III and group II and III showed *p*-value of <0.001 for all. **Conclusion:** Comparison of control group with treatment groups proved chondroprotective effects of hyaluronic acid and triamcinolone. Additionally, hyaluronic acid proved to provide better chondroprotection as compared to triamcinolone.

Keywords: Chondroprotective; Hyaluronic acid; Osteoarthritis; Triamcinolone

Citation: Ishaq N, Gul S, Gul M, Ata N, Batool S, Mehdi Q. Chondroprotective effects of intra-articular hyaluronic acid and triamcinolone in murine model of osteoarthritis. J Ayub Med Coll Abbottabad 2022;34(1):82–6.

INTRODUCTION

Osteoarthritis (OA) is a chronic debilitating disease of joint that mostly affects old age population. Almost 240 million people are suffering from OA globally.[1] Pathogenesis of OA is complex that includes mechanical wear and tear in addition to inflammatory component caused by interleukin-1 (IL-1) and tissue necrosis factor α (TNF-α).[2] non-pharmacological approaches, i.e., education and exercise are the first treatment options to combat OA. If non-pharmacological options are not enough to relieve symptoms, then different drugs might become useful such as non-steroidal anti-inflammatory drugs (NSAIDs), glucocorticoids and visco-supplement substances. Joint replacement surgeries are also considered in few patients if disease involves knee or hip joint.[3]

Hyaluronic acid (HA) is a natural component of synovial fluid, joint capsule and articular cartilage. It is used in the management of OA for many years. Definite mechanism of action of HA is still a matter of debate. A proposed mechanism is that HA restores joint lubrication, improves shock absorption and other joint mechanics. It also contributes in the synthesis of endogenous HA and other extra cellular matrix components by synovial fibroblasts in addition to lowering the proteoglycan loss and apoptosis of chondrocytes. To some extent, it also reduces endogenous HA degradation and production of inflammatory cytokines and pain mediators.[4]

Triamcinolone (TRI), an intermediate acting synthetic glucocorticoid, has been used in a variety of diseases including OA for decades. Triamcinolone exhibits its anti-inflammatory activity via binding to intracellular glucocorticoid receptors and down regulating the expression of genes in prostaglandin synthesis and leukotriene release. It not only decreases size of osteophytes but also reduces the severity of structural changes of cartilage in OA.[5,6]

Induction of OA via surgical transection of ligaments and menisci is one of the most popular techniques to develop OA models. Medial meniscus and anterior cruciate ligament (ACL) are most commonly transacted ligaments to induce OA in knee joint of murine models. Resection of menisci and ligaments leads to post traumatic changes that has close

similarities with OA changes.[7] Definitive cure of OA is not currently available as yet. HA, TRI and many other visco-supplement substances and glucocorticoids are investigational agents and are used to reduce symptoms and delay the progression of disease. Aim of this study is to compare the chondroprotective effects of intra-articular (IA) HA and TRI and to find the drug offering maximum chondroprotection among these two in OA induced murine model.

MATERIAL AND METHODS

This laboratory based experimental study was conducted in Department of Pharmacology and Therapeutics at Army Medical College (AMC) Rawalpindi and National Institute of Health (NIH) Islamabad. Ethical approval was endeavoured from ethical review committee of "Centre for Research in Experimental and Applied Medicine (CREAM)", AMC. Interventional period was two months, from May to July 2019, during which a total of twenty-four (24) adult male and non-pregnant female Sprague Dawley rats (weighing about 500 gram) were procured and nurtured in animal house of NIH. They were randomly divided into three (03) groups, with eight (08) rats in each group. Group I, II and III were labelled as Control, hyaluronic acid (HA) and triamcinolone (TRI) group respectively. Animals were kept in an optimal environment with temperature maintained at 25±5 ˚C. Adequate humidity and 12 hours' day-night cycle along with standard diet and water were ensured. Osteoarthritis (OA) was induced in right knee joint of all rats with surgery. Before surgery rats were anesthetized with intra-peritoneal injection of 5% xylazine and 1% ketamine.[8] Right knee joint of rats was shaved and disinfected in a sterilized environment. After that, a para-patellar incision was made on medial side for complete joint exposure. Then anterior cruciate ligament was identified and transacted. Wound was closed with surgical stapler after the completion of the surgery. Animals were allowed to move freely in the cage for two weeks thereafter.[9] Intra-articular (IA) injections were administered in the corresponding joint of the rats. Rats of control group, HA group and TRI groups were injected with 0.2 ml of normal saline, 0.2 ml (10mg/ml) HA and 70 µl (1.4 mg/ml) TRI once weekly for four successive weeks respectively.[10–12] Gait pattern was analysed one week after the last dosage of drugs.[13] Subsequently, animals were euthanized with toxic dosage of chloroform and sample of distal femur was obtained by using angled bone cutter.[14] After tissue collection and histological slide preparation, these slides were scored using Modified Mankin score.[15] Results were analysed using SPSS version 23. Gait score and Modified Mankin score both are Quantitative parameters and were compared through ANOVA followed by Post Hoc Tukey 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

Gait pattern of the rats was noted with naked eye one week after the last dose. It was done by heavily staining the hind paws of the rats and they were allured by food to walk full length of A2 (42×60 cm) size paper. The footprint made by the drug treated right leg was compared with the non-treated left leg. Control group's gait score of three rats was 04, four rats was 03, whereas one rat had 01 score. The mean gait score of this group was 3.25±0.707. On the other hand, two rats of Hyaluronic acid (HA) group scored 0, four rats scored 04 and two rats scored 02. Its mean score was 1.00±0.707. In the triamcinolone (TRI) group, the gait score of two rats was 03 while six rats of this group scored 02 and its mean gait score was 2.25±0.463. Comparison of control group with the drug treated groups via ANOVA showed p-value of <0.01 which validates the chondroprotective effects of HA and TRI. Intergroup comparison via Post Hoc Tukey test depicted p-values as showed in Table-1 and the graphical representation of the results is presented in figure-1.

The slides were observed and analyzed under x100 and x400 lens after histological slides preparation. Modified Mankins scoring system was used to score the histopathological changes of osteoarthritis (OA). The score of slides of control group ranged from 10–13. Marked irregularity in perichondrium was seen in six out of eight slides while rest showed mild irregularity. Marked fibrosis of perichondrium was seen in one slide, moderate in six and mild in one slide. Moderate irregularity of cellular organization was seen in six slides whereas it was mild in two slides. Marked increase in cellularity of chondrocytes was seen in all slides of this group. Five slides displayed moderate increase in chondrocytes cluster and 10–20% of chondrocyte necrosis whereas mild increase in chondrocytes clusters and 20-80% chondrocyte necrosis was seen in three slides. All slides showed fibrinoid degeneration. Mean score of control group was 11.50±1.195.

Minimum score of 04–07 was seen in HA group. All slides showed mild to moderate irregularity of perichondrium. Four slides showed mild fibrosis of perichondrium while it was absent in the rest. Moderate to marked irregularity of perichondrium was seen in one slide while the remaining slides had mild to moderate changes. Five slides showed mild increase in cellularity of chondrocytes while it was absent in the rest. Mild increase in chondrocytes clusters was seen in seven slides whereas it was moderate in one slide. All slides

showed 10–20% chondrocyte necrosis. Fibrinoid degeneration was not seen in any of the slides. Mean score of this group was 5.50±1.195. The score of the slides of TRI group ranged from 07–10. Mild to moderate irregularity of perichondrium was seen in seven slides with moderate to marked irregularity in one slide. Mild fibrosis of perichondrium was a feature of five slides while it was moderate in three. Mild to moderate irregularity of organization was seen in six slides whereas it was moderate to moderate in the remaining slides. Seven slides displayed 10–20% whereas one slide had 20–80% chondrocytes necrosis. Three slides exhibited fibrinoid degeneration which was absent in the rest. Mean score of this group was 8.83±2.390.

Comparison of the control group with the drug treated group via ANOVA exhibited *p* value <0.01, thus confirming the chondroprotective effects of HA and TRI. Intergroup comparison of groups via Post Hoc Tukey test depicted *p* values as showed in table-1 along with the graphical interpretation shown in Figure 01. A noticeable decrease of mean gait score and mean Modified Mankin score was seen in the drug treated groups as compared to the control group. Gait and histological scores comparison of control group with drug treated groups exhibited *p*-value of <0.01 each time that confirmed chondroprotection awarded by HA and TRI. However, comparison of these two drugs depicted that HA has superior chondroprotective effects as compared to TRI.

Table-1: Intergroup comparison of Gait score and modified Mankin score when Post Hoc Tukey test is applied

Groups	Gait score	Modified Mankin score
Group I and II	<0.001	<0.001
Group I and III	0.016	<0.001
Group II and III	0.003	<0.001

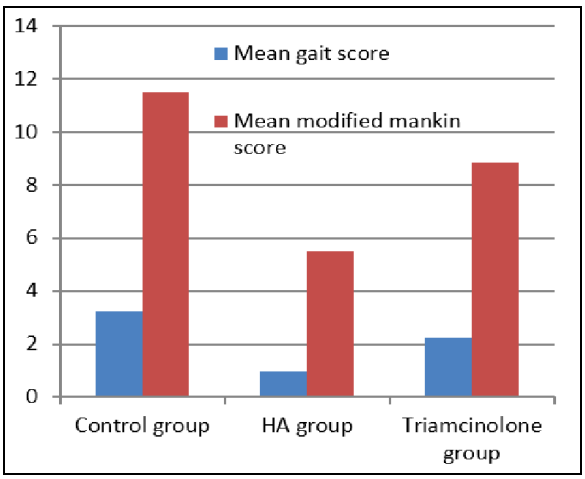

Figure-1: Graphical representation of mean gait and mean modified Mankin score

DISCUSSION

Osteoarthritis (OA) is a chronic degenerative joint disease that is characterized by cartilage degeneration and synovial hypertrophy. As of 2019, osteoarthritis is estimated to affect millions of people all around the globe and is the 15th leading cause of years lived with disability.[16] Definite treatment of the disease is still not discovered. Many drug groups are investigational and are used to slow down the disease progression and to relieve symptoms. These include NSAIDs, glucocorticoids and viscosupplements.[17] Many studies were conducted all over the world to evaluate and compare the chondroprotection offered by different NSAIDs, glucocorticoids and visco-supplements. This study was designed to evaluate and compare chondroprotective effects of hyaluronic acid (HA), a visco-supplement substance, and triamcinolone (TRI), a glucocorticoid, in murine model of OA. After the completion of interventional protocol, when gait score and Modified Mankin score of control and HA groups were compared, we found statistically significant differences that confirmed the chondroprotective effects of HA. Our results were in accordance to a Zhenqinq's research conducted in 2018. He compared HA treated rabbit model of OA with control group and found a significant *p* value of <0.05.[18] Likewise in 2017, Yunus Emre created chondrocyte defect in knees of rats by 27 mm drill bit. He also declared that HA exhibits chondroprotective efficacy when compared with the rats of control group (*p*-value <0.001).[19] In 2016, Tatsuya Tamura worked on antigen induced rat model of OA and found a *p* value of <0.01 when HA treated group was compared with vehicle treated group.[20]

Correspondingly, differences of gait score and Modified Mankin score between rats of TRI group and disease control group were statistically significant that confirmed its beneficial effects. These results were supported by 2016 research work of Jeffrey S. Kroin who claimed that TRI reduces allodynia as compared to mice of control group (*p* value <0.01).[21] In 2017, Yashashri C. Shetty and his colleagues did research work on chemically induced models of rats and concluded that TRI lessens the histopathological severity of disease (*p*<0.01) as compared to disease control group.[22] In vitro study of E. Frank also affirmed that TRI has some chondroprotective effects (*p* value <0.05) on injured and inflamed cartilage inhibiting sulphate incorporation and glycosaminoglycan loss.[23]

When gait and Modified Mankin score of HA group was statistically compared with TRI group, it was analysed that HA is more efficacious than TRI. These results are according to the research work of

Yashashri C. Shetty who found that HA has better chondroprotective effects than TRI in chemically induced murine model of OA (p<0.001).[22] Likewise, human study by Soad A Elsawy and his colleagues depicted superior chondroprotection offered by HA as compared to TRI (p=0.01).[24] Similarly, meta-analysis by Egemen Ayhan and colleagues also verified that HA has better chondroprotective effects as compared to TRI.[25]

CONCLUSION

Intra-articular administration of 0.2 ml (10 mg/ml) hyaluronic acid and 70 µl (1.4 mg/ml) triamcinolone once weekly for 04 successive weeks reduced mean gait score and mean Modified Mankin score for histopathological changes in rat model of osteoarthritis. Additionally, while comparing hyaluronic acid and triamcinolone treated groups, it was concluded that the former has better chondroprotective effects in murine model of osteoarthritis.

ACKNOWLEDGEMENT

Authors are indebted to Dr. Hussain Ali, Head of Laboratory Animal Facility, National Institute of Health, Islamabad for his supervision regarding induction of osteoarthritis and intra-articular drug administration in rats. We are also thankful to National University of Medical Sciences and Army Medical College, Rawalpindi, for providing us opportunity and facilities to be carry out this research project.

Disclosure: We are highly obligated to National University of Medical Sciences, Rawalpindi for funding our project.

Conflict of interest: There is no conflict of interest declared by any author.

AUTHORS' CONTRIBUTION

NI: Animal investigation, data collection, data analysis, write-up. SG: Data collection, data analysis, write-up. MG: Data analysis, result interpretation, critical review, proof reading. NA: Critical review, accountability for all aspects of work. SB: Literature review, write-up. QM: Data collection, proof reading.

REFERENCES

1. Ghouri A, Conaghan PG. Prospects for therapies in osteoarthritis. Calcif Tissue Int 2020;13(1):1–12.
2. Woodell-May JE, Sommerfeld SD. Role of inflammation and the immune system in the progression of osteoarthritis. J Orthop Res 2020;38(2):253–7.
3. Reginster JY, Veronese N. Highly purified chondroitin sulfate: A literature review on clinical efficacy and pharmacoeconomic aspects in osteoarthritis treatment. Aging Clin Exp Res 2020;7(1):1–11.
4. Salwowska NM, Bebenek KA, Żądło DA, Wcisło-Dziadecka DL. Physiochemical properties and application of hyaluronic acid: a systematic review. J Cosm Dermatol 2016;15(4):520–6.
5. Alves JC, Santos A, Jorge P, Lavrador C, Carreira LM. The intra-articular administration of triamcinolone hexacetonide in the treatment of osteoarthritis. Its effects in a naturally occurring canine osteoarthritis model. PLoS One 2021;16(1):1–20.
6. Van Heugten AJ, de Boer W, de Vries WS, Markesteijn CM, Vromans H. Development and validation of a stability-indicating HPLC-UV method for the determination of triamcinolone acetonide and its degradation products in an ointment formulation. J Pharm Biomed Analysis 2018;149(5):265–70.
7. Bapat S, Hubbard D, Munjal A, Hunter M, Fulzele S. Pros and cons of mouse models for studying osteoarthritis. Clin Trans Med 2018;7(1):1–3.
8. Paglia DN, Kanjilal D, Kadkoy Y, Moskonas S, Wetterstrand C, Lin A, *et al*. Naproxen treatment inhibits articular cartilage loss in a rat model of osteoarthritis. J Orthop Res 2020;12(1):1–8.
9. Ma TW, Wen YJ, Song XP, Hu HL, Li Y, Bai H, *et al*. Puerarin inhibits the development of osteoarthritis through antiinflammatory and antimatrix-degrading pathways in osteoarthritis-induced rat model. Phytotherapy Res 2020;12(22):1–15.
10. Kim SH, Mi-Won SO, Jang SW, Kang MJ, Ma KW. Stable liquid pharmaceutical composition containing HA or its pharmaceutically acceptable salt and hyaluronic acid or its pharmaceutically acceptable salt and the manufacturing method thereof. 2016; p.1–18.
11. 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 Pharmacol 2019;102(1):79–89.
12. Siebelt M, Korthagen N, Wei W, Groen H, Bastiaansen-Jenniskens Y, Müller C, *et al*. Triamcinolone acetonide activates an anti-inflammatory and folate receptor–positive macrophage that prevents osteophytosis in vivo. Arthritis Res Ther 2015;17(1):352–64.
13. 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. Osteoarthritis Cartilage 2015;23(1):151–60.
14. Aguwa US, Nnamdi OS, Nnabuihe ED, Elizabeth EC, Ogechi A, Nzube OB, *et al*. Evaluating the Effect of Chloroform Inhalation as a Method of Euthanasia on the Cerebellum and Hippocampus of Adult Wistar Rats. J Adv Med Pharm Sci 2020;27(1):14–25.
15. 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. Osteoarthritis Cartilage 2019;27(7):1084–93.
16. Steinberg J, Southam L, Roumeliotis TI, Clark MJ, Jayasuriya RL, Swift D, *et al*. A molecular quantitative trait locus map for osteoarthritis. Nat Commun 2021;12(1):1309.
17. Jain KB, Ravikumar P. Recent advances in treatments of cartilage regeneration for knee osteoarthritis. J Drug Del Sci Technol 2020;23(8):1–12.
18. 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):1590–7.
19. 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. Acta Orthop Traumatol Turc 2017;51(4):331–6.
20. Tamura T, Higuchi Y, Kitamura H, Murao N, Saitoh R, Morikawa T, *et al*. Novel hyaluronic acid–methotrexate

conjugate suppresses joint inflammation in the rat knee: efficacy and safety evaluation in two rat arthritis models. Arthritis Res Ther 2016;18(1):79–89.
21. Kroin JS, Kc R, Li X, Hamilton JL, Das V, van Wijnen AJ, *et al*. Intraarticular slow-release triamcinolone acetate reduces allodynia in an experimental mouse knee osteoarthritis model. Gene 2016;591(1):1–5.
22. Shetty YC, Patil AE, Jalgaonkar SV, Rege NN, Salgaonkar S, Teltumbde PA, *et al*. Intra-articular injections of ketamine and 25% dextrose improve clinical and pathological outcomes in the monosodium iodoacetate model of osteoarthritis. J Basic Clin Physiol Pharmacol 2017;28(6):543–53.
23. Frank E, Hung HH, Krishnan Y, Senter B, Bodick N, Grodzinsky A. Dose-dependent chondroprotective effects of triamcinolone acetonide on inflamed and injured cartilage using an in vitro model. Osteoarthritis Cartilage 2019;27(1):176–84.
24. Elsawy SA, Hamdy M, Ahmed MS. Intra-articular injection of hyaluronic acid for treatment of osteoarthritis knee: Comparative study to intra-articular corticosteroids. Egy Rheumatol Rehab 2017;44(4):143–6.
25. Ayhan E, Kesmezacar H, Akgun I. Intraarticular injections (corticosteroid, hyaluronic acid, platelet rich plasma) for the knee osteoarthritis. World J Orthop 2014;5(3):351–61.

Submitted: May 5, 2021	*Revised: June 20, 2021*	*Accepted: June 21, 2021*

Address for Correspondence:
Mahwish Gul, Department of Pharmacology, Ayub Medical College, Abbottabad-Pakistan
Email: dr.mahwishayub@gmail.com