ORIGINAL ARTICLE

RADIOGRAPHIC COMPARATIVE REGENERATIVE EFFECTS OF HYALURONATE AND PIROXICAM IN OSTEOARTHRITIS MODELS OF RATS

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Background: Osteoarthritis is the most common degenerative joint disease that affects commonly in old age. Standard treatment of this disease is still underdeveloped, however multiple drug groups delay progression or reduce the symptoms. Objective of this study was to assess and compare the regenerative effects of hyaluronate and piroxicam in a rat model of osteoarthritis at the radiographic level. **Methods:** This laboratory-based randomized trial was conducted at Department of Pharmacology Army Medical College, Rawalpindi, from May to July 2019. Resection of the medial meniscus and anterior cruciate ligament resulted in osteoarthritis in the right knee joints of 24 rats. They were separated into 3 groups of 8 rats each. For 4 weeks, groups I, II, and III received intra-articular saline, hyaluronate, and piroxicam, respectively. After one week, radiographs of the anaesthetized rats' matching knee joints were collected. **Results:** Comparison of radiograph of control group with drug treated group confirmed regenerative effects of hyaluronate and piroxicam (p=0.001). However comparison of hyaluronate and piroxicam treated groups had p=0.335 that professed both drug have equal regenerative effects in rat model of osteoarthritis. **Conclusion:** In a rat model of osteoarthritis, intra-articular injection of hyaluronate acid and piroxicam had regenerating benefits at the radiological level. Both administered medicines had similar regeneration effects.

Keywords: Regeneration, Hyaluronate, Piroxicam, Osteoarthritis, Rat model, Intra-articular

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INTRODUCTION

Osteoarthritis (OA) is one of the most common chronic joint disorders that is a prominent cause of disability globally. It is a disease of old age with in 10% men and 18% women, aged over of 60 years. Sometimes it also affects individuals in young age with a history of trauma to the affected joint. It is a multifaceted joint disease of degenerative nature, its initiation and progression mechanisms are only partly understood. Abnormal cartilage loss is a conspicuous feature of OA with perpetuated abnormal cartilage restoration and bone remodelling as contributory factors. As OA ages, joint space is also gets narrowed and compromised. Patients of OA usually present with pain (that can be severe to moderate intensity), stiffness and decreased mobility of affected joint.[1,2] Definite treatment of OA is not discovered yet. But there are large range of non-pharmacological interventions (Standard exercises, Yoga sessions, manual therapies, and joint wears) to Pharmacological interventions available to relieve symptoms and slow down the disease progression. Viscosupplement substance, Non-steroidal anti-inflammatory drugs (NSAIDs) and glucocorticoids are three big drug groups that are largely prescribed to patients of OA.[3,4]

Hyaluronate is an endogenous substance that is richly present in articular cartilage and synovial fluid. It consists of repeating β-1,4-D-glucuronic acid and β-1,3-*N*-acetylglucosamine units. Hyaluronate ensures a significant role in the biomechanics of healthy synovial fluid, where it is to some extent accountable for lubrication, viscoelasticity, shock absorbing and joint structure stabilization.[5] It is one of the favourite investigational viscosupplement used in the management of OA. Intra articular (IA) hyaluronate not only improves joint function but also alleviates pain in patients of OA. Multiple *in vitro*, animal and human researches illustrated regenerative effects of exogenous hyaluronate. Proposed mechanisms by which hyaluronate employs its chondroprotective effects and regenerative effects include increase proteoglycan and glycosaminoglycan synthesis, direct anti-inflammatory effect and viscoelasticity maintenance. Its effects on inflammatory mediators, i.e., cytokoines, prostaglandins and proteases are distinguished. Anti-oxidant property of hyaluronate provides chondrocyte protection against the harm induced by oxygen-derived free radicals.[6]

Piroxicam is an NSAID of Oxicam group. It non-selectively and reversibly inhibits

cyclooxygenase (COX), and thus exhibits anti-inflammatory and analgesic activity by decreasing the synthesis of prostaglandins, prostacyclins and thrombaxanes. Its anti-inflammatory and analgesic properties make it a suitable choice for chronic disease like OA. Besides COX inhibition it diminishes vasodilation in response to bradykinin and histamine. Piroxicam has properties to lessen the central component of nociception. Due to its gastointestinal upset prolonged use of piroxicam is not feasible. Thus intra-articular route of administration is a substitute route for OA patients.[7–9]

Presently there is no definitive cure for OA. Hyaluronate, piroxicam, and a variety of additional viscosupplement substances, and NSAIDs are under research and are used to relieve pain and delay disease progression. The goal of this study was to examine the restorative effects of hyaluronate and piroxicam in murine models of OA and determine whether this medication has regenerative benefits.

MATERIAL AND METHODS

This randomized control study was carried out in a laboratory setting. It was conducted in partnership with the National Institute of Health (NIH) in Islamabad at the Department of Pharmacology and Therapeutics, Army Medical College Rawalpindi. This study was approved by the Ethical Review Committee of the Centre for Research in Experimental and Applied Medicine.

The animal intervention lasted two months, i.e., May to July 2019. The animals were kept in the experimental animal facility of National Institutes of Health. Initially, 24 mature male or non-pregnant female Sprague Dawley rats aged 8–10 weeks and weighing 400–500 g were chosen using a non-probability handy sampling technique. They were randomly assigned to 3 groups, of 8 rats each, the disease control, hyaluronate, and piroxicam groups (Group I, II, and III, respectively). Free access to fresh drinking water and standard rodent feed was provided throughout the study.

Osteoarthritis was induced in the right knee joint of the rats by standard surgical procedure. The rats were anaesthetized with 5% xylazine and 1% ketamine prior to surgery.[10] The skin around the joint was shaved aseptically, and the joint was totally exposed by a para patellar incision on the medial side. Anterior cruciate ligament was explored and transacted followed by identification and resection of medial meniscus. Thereafter, the wound was aseptically closed using a surgical stapler.

After surgery, the animals were allowed 3 weeks to roam freely inside the cage.[11] The rats' disease-induced joint was treated with intra-articular medicines. For four weeks, rats were given 100 µL of saline water, 30 µL of Hyaluronate (HA), and 70 µL of Piroxicam (PIRO) respectively to group I, II, and III respectively.[12–14]

The rats were then given intraperitoneal injections of 10% xylazine and 1% ketamine before being transported to the Radiology Department of a private hospital for radiographs of their knee joints. With the help of radiologist, Kellgren and Lawrence grading system was used to assess the severity of OA. The grading system used by Kellgren and Lawrence is as follows:[15,16]

Grade 0: No OA radiographic characteristics seen
Grade 1: Possible osteophyte lipping and dubious joint space narrowing (JSN) on anteroposterior weight-bearing radiograph
Grade 2: Obvious osteophytes and potential JSN
Grade 3: Multiple osteophytes, confirmed JSN, sclerosis, and potential bone deformity
Grade 4: Large osteophytes, JSN, severe sclerosis, and evident bone deformity

Animals were killed with a lethal dose of chloroform after radiographic grading.

Data was analysed on SPSS-23. ANOVA followed by the Post Hoc Tukey test was used to compare differences in groups considering $p \leq 0.05$ as statistically significant.

RESULTS

Radiographs from illness group I were rated as grade 4, grade 3, and grade 2 on 2 (25%), 4 (50%), and 2 (25%) radiographs respectively. Figure-1 shows an X-Ray image of a control group rat with joint deformity and sclerosis and a grade 3 JSN. The radiograph of group I is characterised by osteophytes and bone deformities.

Following injection of HA, radiographic alterations in group II revealed no OA in 2 (25%), dubious changes in 4 (50%), and moderate changes in 2 (25%) radiographs with grades 0, 1, and 2, respectively. The X-Ray in Figure-2 shows a rat from the HA group with very minor changes, displaying the OA phenotype. This radiograph has a grade of 2. Radiographs of this group indicated no or ambiguous OA changes.

Group 3 received IA piroxicam once weekly for 4 weeks and had small alterations of grade 2 and 1 in half (50%) of the rats and doubtful changes in another half (50%) of the animals respectively. Figure-3 depicts an X-ray of a Group 3 rat with minimal OA changes of grade 2. In this group, majority of the radiographs revealed probable JSN and osteophyte lipping.

ANOVA was used to compare the three groups. HA and PIRO had regeneration effects at the radiographic level ($p<0.001$ between groups 1 and 2, <0.001 between groups 1 and 3, and 0.335 between groups 2 and 3). This indicated regenerative effects of both drugs when compared with non-treated group and equal effects when compared with each other.

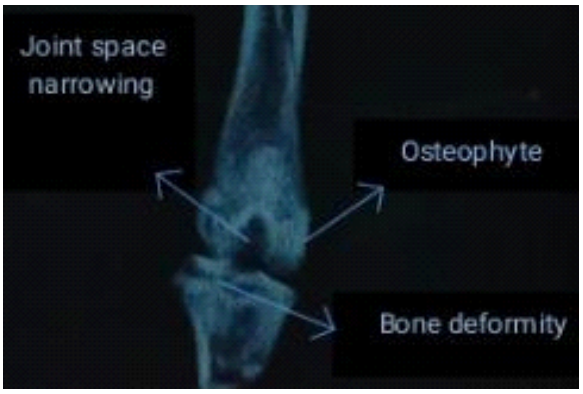

Figure-1: Radiograph of a rat of group 1 (Control)

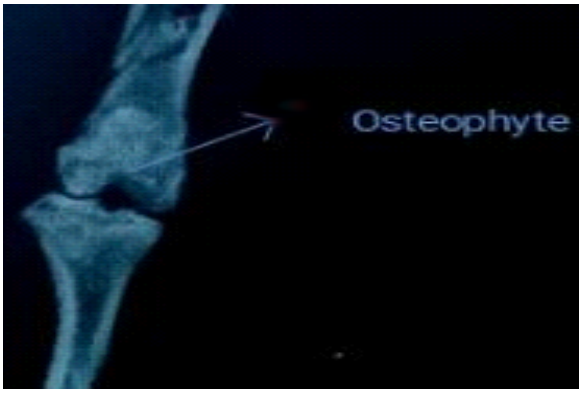

Figure-2: Radiograph of a rat of group 2 (HA group)

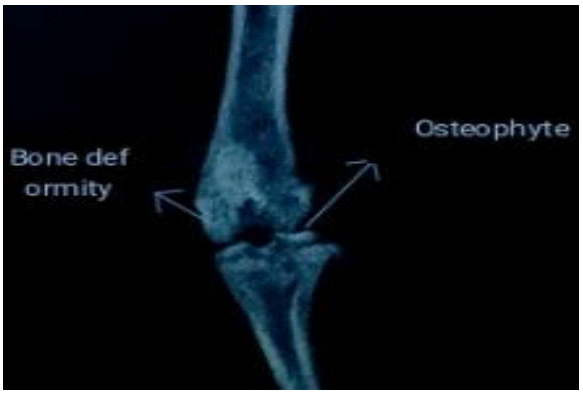

Figure-3: Radiograph of a rat of group 3 (piroxicam group)

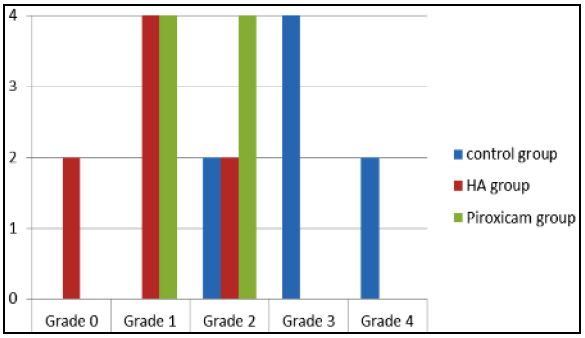

Figure-4: Kellegren and Lawrence grading of OA knee joints of rats of all groups

DISCUSSION

Osteoarthritis (OA) is a disease of old age that can involve any joint of the body but commonly involves hand, knee, hip and spinal facets. Irregular pain is the main problem with which patients usually present. Cartilage degeneration is the pathological factor of OA that is followed by joint inflammation. Different drug groups are used to alleviate symptoms and delay the cartilage degeneration. Viscosuplement substances, NSAIDs and corticosteroids are three major drug groups that are used to manage patients of OA.[17,18] When the treatment groups' radiographic grades were compared to the disease group, significant regeneration benefits of hyaluronate and piroxicam were confirmed (p=0.001). In the case of hyaluronate, Arafat and Kamel's investigation yielded identical findings. In albino rats models, they discovered a substantial (p<0.02) regeneration impact of Hyaluronate.[19] Our findings are supported by Zhiwei Zhang's work who found that hyaluronate decreases radiographic osteophytosis grade (p<0.05) when compared to a saline-treated rat model of osteoarthritis.[20] Our results are corroborated by Li Jung Kang *et al*[21] who established a surgically induced OA mice model and found that HA had regenerative effects when compared to a vehicle-treated group.

Regarding regenerative effects of piroxicam, similar results were found in a study by Park *et al* who reported that there was a statistically significant difference of joint swelling and PGE2 level in IA piroxicam treated rats as compared to IA saline treated rat models of OA.[22] Research work of Aziza[23] revealed that therapy of piroxicam significantly reduces joint oedema and arthritic index in Freud adjuvant induced arthritis models of rat that also supports our findings.

Although both drugs are often prescribed by rheumatologists for the treatment of OA, no *in vitro*, animal, or human studies have yet been undertaken to compare the regeneration benefits of these two drugs. Both hyaluronate and piroxicam had equivalent regeneration effects in rat models of OA after comparing the two groups in our study.

CONCLUSION

As compared to saline treated group, both hyaluronic acid and piroxicam intra-articular exhibit equal regenerative effects in a rat model of OA.

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Contribution of Authors

NI: Study design, Animal intervention
SB: Study design, Critical review
SMQ: Study design, Proof reading
HMIA: Biostatistics, Writing
MWAK: Animal intervention
SJ: Biostatistics

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