

ORIGINAL ARTICLE

INTRA-ARTICULAR PIROXICAM AND TRIAMCINOLONE IN A RAT MODEL OF OSTEOARTHRITIS; ELEVATION AND COMPARISON OF CHONDROPROTECTIVE EFFICACY

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Background: Osteoarthritis is the most common chronic degenerative joint disease. Definite treatment of osteoarthritis is still undiscovered. This study was designed to evaluate and compare the chondroprotective efficacy of piroxicam and triamcinolone in rat model of osteoarthritis. **Methods:** This laboratory based experimental study was conducted in Pharmacology Department, Army Medical College, Rawalpindi, from April-June 2019. Osteoarthritis was induced by medial meniscectomy and anterior cruciate ligament resection in knee joints of twenty-four rats. They were divided in three groups with eight rats in each. Group I, II and III were control, piroxicam and triamcinolone groups that were treated by intra articular saline, piroxicam and triamcinolone once weekly for four weeks respectively and then gait pattern was scored. Animals were euthanized thereafter and samples were taken for histopathological analysis. **Results:** Comparison of gait score of control, piroxicam and triamcinolone groups exhibited a *p*-value of <0.01. Intergroup comparison of gait of group I and II, group I and III and group II and IV depicted *p*-value of <0.001, 0.013 and 0.013 respectively. Likewise histopathological comparison of control, piroxicam and triamcinolone groups showed *p*-value of <0.01. While Intergroup histopathological comparison of group I and II, group I and III and group II and IV showed *p*-value of <0.001, <0.001 and 0.008 respectively. **Conclusion:** Comparison of control group with treatment group proved chondroprotective efficacy of piroxicam and triamcinolone. On comparison of treatment groups, it was concluded that piroxicam has better chondroprotective efficacy as compared to triamcinolone.

Keywords: Chondroprotective efficacy; Osteoarthritis; Piroxicam; Triamcinolone

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INTRODUCTION

Osteoarthritis (OA) is one of the most common joint diseases that is affecting over 250 million persons around the world. It can affect any joint of body but most frequently affects knee, hip, spine or small joints of fingers. It is a degenerative joint disease that is characterized by cartilage degradation, bone remodelling, osteophyte formation and synovium inflammation. Pain and joint dysfunction are characteristic feature of OA that worsen over time, resulting in disability and distinctly reduced quality of life.¹ Definite treatment of OA is still not discovered. Non pharmacological interventions, i.e., assistive devices (foot wear and joint braces), encouraging individuals to adapt healthy life style and to commence moderate intensity exercises are the first preferences to manage a patient of OA. Pharmacological therapy is added when non pharmacological interventions become ineffective. Different drug groups are used to alleviate the severity of symptoms and to delay the progression of disease. Nonsteroidal anti-inflammatory drugs

(NSAIDs), corticosteroids and visco-supplement substances are widely used drug groups in the treatment of OA. Surgical interventions including arthroscopy and joint replacement can be an option for selected patients after the failure of pharmacological management.²⁻⁴

Piroxicam is an NSAID, an enolic acid oxamic derivative, that non selectively inhibit cyclooxygenase (COX) which leads to inhibition of prostaglandin production. Besides pain alleviation, inhibition of prostaglandin synthesis also suppresses lesions of OA. Long half-life ($t_{1/2}$) of approximately 50 hours is one of the reasons that make it suitable for use in chronic ailment like OA.⁵ Triamcinolone is an intermediate acting synthetic glucocorticoid nature of corticosteroid with half-life of 18–36 hours and a molecular weight of 434 Da. It was introduced in the market about 50 years ago and is still used in variety of diseases. It is also being used in the management of OA for decades. It reduces the inflammatory component of OA. Triamcinolone binds to intracellular glucocorticoid receptors and inhibits

transcription and translation of genes involved in prostaglandin synthesis and leukotrienes release that are culprit of inflammation in OA. Moreover, it also augments lipocortin expression that modulates anti-inflammatory effects. These factors ultimately lessen the inflammation of synovium and other articular structures.^{6,7}

Definite cure of OA is not currently available. Piroxicam, triamcinolone and many other NSAIDs and corticosteroids are investigational and are used to reduce symptoms and delay the progression of disease. Aim of this study is to compare the chondroprotective efficacy of piroxicam and triamcinolone and to find the drug with maximum chondroprotective efficacy among these two drugs in OA induced rats.

MATERIAL AND METHODS

It was a laboratory based experimental study that was conducted in department of Pharmacology and Therapeutics, Army Medical College (AMC), Rawalpindi in collaboration with National Institute of Health (NIH), Islamabad. Ethical approval was taken from ethical review committee of "Centre for Research in Experimental and Applied Medicine (CREAM)", AMC. Period of animal's intervention was two months from April to June 2019. Animals was procured and cherished in animal house of NIH during the complete tenure of the study. Preliminary twenty-four (24) adult male or non-pregnant female rats of Sprague Dawley breed, 08–10 weeks old and weighing about 500 grams were selected through non probability 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 group, piroxicam group and triamcinolone respectively. Animals were kept in standard environment with temperature ranged $25\pm 5^{\circ}\text{C}$, adequate humidity and 12 hours day night cycle to maintain circadian rhythm. Free access to clean drinking water and standard rodent diet *ad libitum* was provided 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.⁸ Skin of the joint was shaved and disinfected 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 removed and transacted respectively. Aseptic closure of wound was done after the completion of the surgery. Animals were allowed to move freely in the cage for two weeks thereafter.⁹ Then intra articular drugs were administered in the corresponding joint of the rats. Rats of control group,

piroxicam group and triamcinolone groups were injected with 0.2 ml of normal saline, 50 μl (2mg/ml) and 70 μl (1.4 mg/ml) triamcinolone once weekly for 04 weeks.^{10–12} We waited for one week more before analyzing the gait score.¹³ After gait pattern was scored, 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 Slide preparation.¹⁵ Obtained data was analyzed using SPSS version 23. Gait score and Modified Mankin score both were Quantitative parameters and compared through ANOVA followed by *Post hoc tukey test*. The differences between two observations were considered statistically significant if the *p*-value was equal or less than 0.05 ($p\leq 0.05$).

RESULTS

None of the rat's surgical wound got infected nor any animal died till the end of study. One week after the last IA injection gait pattern of the rats was scored by allowing them to walk full length of A2 (42×60 cm) size paper. Walk pattern of the rats was observed visually with naked eye and footprint made by the right injected leg was compared with the left non injected one to analyse weight bearing throughout the movement. Gait score of three rats of control group was 04 while four and one rats of this group scored 03 and 01 respectively. Meanwhile gait patterns of one, four and three rats of piroxicam group were scored 0, 01 and 02 respectively. Likewise, gait pattern of two rats of triamcinolone group scored three while six rats of this group scored 02. The mean gait score of control group, piroxicam group and triamcinolone group were 3.25 ± 0.707 , 1.25 ± 0.707 2.25 ± 0.463 respectively with a significant *p* value of <0.01 when compared via ANOVA. Intergroup comparison of gait score via *Post hoc tukey test* exhibited *p* value as described in table-2

Histopathological analysis of all slides was done under X400 lens. Histological changes of OA were scored according to Modified Mankin scoring system. Score of slides of control group was ranging from 13–10. Six out of eight slides showed marked while rest of two showed mild irregularity in perichondrium. One slide had marked, six had moderate and one had mild fibrosis of perichondrium. Six slides had moderate to mark while two slides had mild to moderate irregularity of organization. All slides showed moderate to marked increase in cellularity of chondrocytes. Five slides showed 10–20% while three slides showed 20–80% necrosis of chondrocytes. Fibrinoid degeneration was feature of all the slides of control group. Mean score of control group was 11.50 ± 1.195

Slides of group piroxicam group scored around 5–8. Mild irregularity of perichondrium was the feature of all

slides. One had none, six had mild, while one had moderate fibrosis of perichondrium. Seven had mild to moderate while one had moderate to marked irregularity in organization. One slide showed no, four slides showed mild and three slides showed moderate to marked increase in cellularity of chondrocytes. 10-20% necrosis of chondrocyte was the feature of all slides. None of the slides exhibited fibrinoid degeneration. It showed features of mild perichondrium fibrosis, moderate irregularity in organization, hypercellularity and 10–20% of chondrocytes necrosis. While mean \pm SD of piroxicam group was 6.50 ± 1.195 .

Slides of triamcinolone group scored from 7–10. Seven slides of triamcinolone showed mild to moderate while one slide showed moderate to marked irregularity of perichondrium. Five slides had mild while three slides had moderate fibrosis of perichondrium. Six slides had mild to moderate while two slides had moderate to marked irregularity of organization. Seven had 10–20% while one had 20-80% chondrocytes necrosis. Fibrinoid degeneration was the feature of three slides of this group. Mean score of this group was 8.83 ± 2.390 . Comparison of control group with drug group via ANOVA exhibited *p* value of <0.01 . Intergroup comparison of groups via *Post hoc tukey test* depicted *P* values as showed in table-2.

In short, we observed a visible decrease of mean gait score and mean modified mankin score of piroxicam group and triamcinolone group as compared to control group. Inter group gait and histological score comparison of control group with drug groups the *p*-value of <0.001 each time that confirmed chondroprotective efficacy of piroxicam and triamcinolone. However, comparison of these two drugs depicted that IA piroxicam has better chondroprotective efficacy as compared to IA triamcinolone.

Table-1: Mean \pm SD of gait score and modified Mankin score of all groups.

Groups	Mean \pm SD of Gait score	Mean \pm of Modified Mankin score	<i>p</i> -value
Group I (Control group)	3.25 ± 0.707	11.50 ± 1.195	<0.01
Group III (Piroxicam group)	1.25 ± 0.707	6.50 ± 1.195	
Group IV (Triamcinolone group)	2.25 ± 0.463	8.83 ± 2.390	

Table-2: 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	0.013	<0.001
Group II and III	0.013	0.008

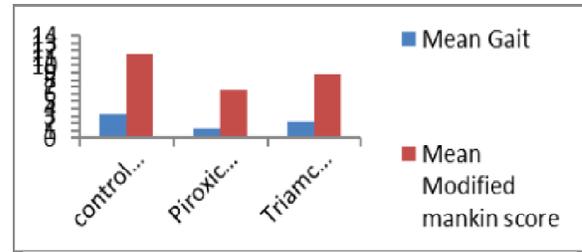


Figure-1: Graphical representation of mean Gait score and mean modified Mankin score

DISCUSSION

Osteoarthritis (OA) is one of the common diseases of joints that can involve any joint of the body. It is the disease of old age but can also arise in young age or childhood.¹⁶ Definite treatment of the disease is still not discovered. Many drug groups are investigational and used to slow down the disease progression and to relieve symptoms. These include NSAIDs, corticosteroids and visco-supplements. Many studies were carried out to confirm and compare chondroprotective efficacy of different NSAIDs, corticosteroids and visco-supplements. We chose piroxicam, an NSAID of oxamicam derivative and triamcinolone, a synthetic corticosteroid for comparison in OA induced rat model. Comparison of gait and histopathology score of piroxicam group with control group confirmed chondroprotective efficacy of piroxicam. A similar result found in studies of Park and his colleagues. It has proven in their study that there was a statistically significant difference ($p < 0.05$) of joint swelling and PGE2 level in IA piroxicam treated rats as compared to IA saline treated rat models of OA.¹⁷ Meanwhile research work of Ijaz ul Haq and his colleagues also proved chondroprotective efficacy of piroxicam ($p < 0.05$) in rabbit models of OA that also reinforce our results.¹⁸ Likewise research work of Aziza and Hana revealed that Intra muscular therapy of piroxicam reduces joint oedema and arthritic index statistically significantly ($p < 0.05$) in Freud Adjuvant induced arthritis models of rat.¹⁹ Their findings also strengthen our results regarding chondroprotective efficacy of piroxicam. Likewise, meloxicam, an oxamicam derivative fellow drug of piroxicam, is used in chemically induced rat model of OA. It significantly decreased ($p = 0.02$) heat stimulation of affected joint as well as TNF- α level as compared to control group that also strengthened our results.²⁰

Likewise, when gait score and modified mankin score of triamcinolone group and control group were compared, the chondroprotective efficacy of triamcinolone was confirmed. These results were

in accordance to 2016 research work of Jeffrey S. Kroin and fellows who claimed that triamcinolone reduces allodynia as compared to mice of control group (p value <0.01). Their work at tissue culture level expressed that triamcinolone has potent anti-inflammatory effects with p value <0.01 via inhibiting expression of IL- 1β and TNF α . Thus, it acclaimed triamcinolone chondroprotective efficacy.²¹ In 2017 Yashashri C. Shetty and his colleagues did research work on chemical induced models of rats. Their results confirmed that triamcinolone reduces the histopathological severity of disease ($p < 0.01$) as compared to disease control group. In vitro study of E. Frank and fellows affirmed that triamcinolone has some chondroprotective effects on injured and inflamed cartilage with a p value <0.05 via inhibiting sulphate incorporation and glycosaminoglycan loss.²¹ Similarly Heard BJ performed research work on dexamethasone, a sister drug of triamcinolone. They concluded that dexamethasone reduces severity of OA (p value of <0.05). His research work also strengthens our results.

After comparison of piroxicam group and triamcinolone group, we found that piroxicam has better chondroprotective efficacy as compared to triamcinolone, our study is unique and distinct in a way that these drugs are commonly prescribed by the physicians for the treatment of OA and to best of our knowledge there is no in vitro, animal and human study conducted till date that compared the chondroprotective effects of these two drugs. However, Mehmet Mufit and his colleagues compared chronic effects of IA tenoxicam, a sister drug of piroxicam, and IA methylprednisolone, A sister synthetic corticosteroid of triamcinolone in healthy rats. Their results expressed that there is statistically significant ($p < 0.05$) reduction of neutrophils in tenoxicam treated rat group that strengthens our finding.²² Likewise Ouedrago and his fellows conducted a human study and compared the effects of oral NSAIDs with IA steroid in patients of knee OA. They concluded that NSAIDs significantly decrease ($P < 0.01$) visual analogue pain scale as well as severity of X-ray grading of osteoarthritis. Their results are also in favour of our outcome.²³

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CONCLUSION

IA administration of Piroxicam and triamcinolone reduced severity of OA in rat models as shown by improved mean gait score and mean Modified Mankin score of histopathology. Upon comparison it was concluded that Piroxicam has better chondroprotective efficacy as compared to triamcinolone in rats' model of OA.

DISCLOSURE

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

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

AUTHORS' CONTRIBUTION

NI: Conceptualization, animal intervention, data collection, data analysis, write-up. NA: Data collection, data analysis, wrote-up. MS: Data analysis, critical review, proof reading. BS: Critical review, accountability for all aspects of work. AZ: Data collection, write-up. AM: Data collection, proof reading.

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