Int J Pharm Bio Sci 2015 Oct; 6(4): (P) 277 - 282

Research Article **Pharmacology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

SCREENING OF KETOTIFEN FOR ITS ANTI-INFLAMMATORY POTENTIAL IN ALBINO RATS

M. BRAHADEESH[1*], M. MATHIVANI[2], R. N. SURESHA[3] AND RAJ KISHORE MAHATO[4]

1 Assistant Professor, Department Of Pharmacology, Velammal Medical College, Madurai.
2 Assistant Professor, Department of Pharmacology, Government Medical College, Theni.
3 Professor Department of Pharmacology, JSS Medical College, Mysore
4 Professor Department of Pharmacology, Velammal Medical College, Madurai.

ABSTRACT

The study is about the anti-inflammatory activity of ketotifen in acute and sub acute animal model. Adult albino rats of either sex weighing 150-200 grams were randomly divided into 3 groups of 6 animals in each. The control, the standard and the test group received Gum acacia (1ml of 1%), indomethacin 8 mg/kg and ketotifen 0.36 mg /kg respectively. Acute inflammatory activity was assessed by carageenan induced paw oedema, Turpentine induced arthritis and sub acute activity was assessed by cotton pellet induced granuloma model. In carageenan induced paw oedema model, percentage of inhibition of paw oedema (anti-inflammatory activity) by indomethacin and ketotifen with respect to control were 48% and 38% respectively and the test group was 77.5% of standard.The percentage of inhibition of knee arthritis by ketotifen with respect to control and standard were 60% and 40% respectively and the test group was 67.1% of standard. The percentage of inhibition of granuloma by standard and ketotifen were 49% and 34% respectively and the test group was 77.7% of standard. In this study ketotifen showed significantly good anti-inflammatory activity in carageenan induced paw model and cotton pellet induced granuloma model and good anti-inflammatory activity in turpentine induced arthritis model. It can be a promising antiinflammatory agent in inflammatory conditions apart from its conventional use in bronchial asthma.

KEY WORDS: **Ketotifen, Antinflammatory, Indomethacin, Carrageenan, Turpentine, Cotton pellet.**

M. BRAHADEESH
Assistant Professor, Department Of Pharmacology,
Velammal Medical College, Madurai. India

***Corresponding author**

INTRODUCTION

Inflammation is the basic strategy of any host defense mechanism to combat or overcome the invading pathogen or the foreign particles. The most common presentation of a patient to the doctor is a pain and inflammation[1]. Therapy of inflammation is a debate and is also incomplete since long. The introduction of sodium salicylate, acetyl salicylic acid (ASPIRIN), cortisone, gold salts and phenylbutazone for the treatment of inflammatory disorders is an important milestone in the development of clinically useful anti-inflammatory agents[2] and the newer ones like selective COX-2 inhibitors, Oxyprofen, aceclofenac etc. The currently used 3 major groups of anti-inflammatory drugs include: NSAIDs, Glucocorticoids and Disease modifying anti-rheumatic drugs (DMARDs).[3] Currently available anti-inflammatory agents are associated with unwanted side effects and have their own limitations. It has been estimated that about 34-46% of the users of NSAIDs will sustain some gastrointestinal damage due to the inhibition of the protective COX enzyme in gastric mucosa.[4] Recently developed selective COX-2 inhibitors are gastric friendly but have a potential adverse effect of prothrombotic tendency leading to MI and death. Glucocorticoids also produce an array of side-effects on chronic administration. Of late we are establishing the anti-inflammatory activity in other groups of drugs also which are not designated as conventional anti-inflammatory drugs i.e., new indications of the older drugs like chloroquine was found to be effective in the therapy of Rheumatoid arthritis, D-Penicillamine, a chealating agent used as a Disease modifying agent in rheumatoid arthritis ,Methotrexate an anticancer agent used as immunosuppressants. The mediators of inflammation are Bradykinin, C3, C5a, plasmin, thrombin, histamine, serotonin, interferon, oxygen derived free radicals, NO, PAF, Interleukins, NF-kappa B, Leukotrines, TNF-Alpha, IL-1, INF-Gamma, Prosthoglandins, lysosomal enzymes, substance P, lipoxins, prosthocyclins[5] Ketotifen is a mast cell stabilizer 2nd generation non competitive H 1 antagonist, functional leukotrine antagonist and a phosphodiesterase inhibitor[6]. It is found to inhibit neutrophil chemotaxis[7]. The drug is known to have anti-inflammatory activity due to its different action relating to mediators of inflammation; hence in this study an earnest attempt is made to explore its activity.

Objectives of the study

To evaluate the Anti-inflammatory activity of ketotifen in acute and subacute inflammatory animal models and compare it with the standard drug indomethacin.

MATERIALS AND METHODS

Source of data

Adult wistar albino rats of either sex weighing between 150 to 250 grams were randomly selected from central animal facility, J S S Medical College, Mysore after getting prior approval from the institutional animal ethical committee.

Inclusion criteria

Rats of either size weighing 150-200 grams.

Exclusion criteria

Pregnant, diseased and debilitated rats.

Chemicals used

ketotifen 0.36 mg/kg, Indomethacin 8mg/kg, 1% Formalin, Turpentine, 1% Carrageenan, Cotton pellets(10mg each pellet), Ether.

Instruments required

Mercury Plethsmograph, Screw gauge, Tuberculin syringe, Feeding tube, mouth gag.

MODELS OF EXPERIMENT

The animals were randomly divided into 3 groups of 6 each; one group was served as control received 2% gum acacia suspension orally (without drug). Other two groups received drug Indomethacin 8 mg /kg of body weight as standard and ketotifen 0.36 mg/kg of body weight (Per-oral) as test respectively. Each rat is fed with respective drug one hour prior to the administration of Phlogestic agent.

Methodology

1. Carrageenan induced rat paw edema Animal Model[8]

0.1 ml of 1% Carrageenan is injected into the subplantar surface of right hind paw of each group. Paw volume was measured by Mercury Plethysmograph at 0 hour and at the end of 4 hours. The difference between the Zero and 4 hours gives the actual edema. From the mean difference in paw oedema volume the percentage of inhibition of oedema was calculated between the test, standard and control groups.

2. Turpentine induced Arthritis Animal Model[9]

0.1ml of turpentine oil was injected into the right knee joint of each rat .Then the lateral diameter was measured by screw gauge at 0 hour and at the end of 4 hours. Change in lateral diameter was noted. From the mean difference in lateral diameter the percentage of inhibition of oedema was calculated between the test, standard and control group.

3. Cotton pellet induced Granuloma Animal Model[10]

Four sterile cotton pellets weighing 10mg each was implanted subcutaneously in each axilla and groin of each rat in control standard and test group. Each rat was fed with respective drug for 12 days then the cotton pellets were inserted after that cotton pellets were removed along with the granulation tissue on 13th day, cleaned and dried in hot air oven for 24 hrs and dry granulation tissue weight were determined. From the mean difference in dry granulation tissue weight the percentage of inhibition of granuloma was calculated between the test, standard and control group.

Statistical methods applied

The effect of the drug under study was presented by calculating the mean and SD of the outcome parameters. One way ANOVA and post hoc test were applied to see the differences between any two groups at a time. Tests of significance were carried out at 5% level. SPSS for window (version 21) was applied in the statistical analysis.

RESULTS

CARRAGEENIN -INDUCED RAT PAW OEDEMA METHOD

Table 4

Table showing the Mean rat paw volume (cm) at 0hr and 4hr, mean difference in different groups

GROUPS	0hr (mean+/- SD)	4hr (mean+/- SD)	Mean difference in paw oedema in cms	ANOVA	Independent t- test	Mean difference in paw oedema of test group and standard with respect to control in cms	% of inhibition of oedema of test group and standard with respect to control	Mean difference in paw oedema of test group with respect to standard in cms	% of inhibition of oedema of test group with respect to standard
CONTROL (2% gum acacia)	0.69+/- 0.16	8.42+/- 0.45	7.73	F=46.95 P=0.001	t=10.847 P=0.001	-	-		-
STANDARD (Indomethacin 8 mg /kg)	1.3+/- 0.28	5.13+/- 1.03	3.83			3.9	48%	-	-
TEST (ketotifen 0.36 mg/kg)	1.7+/- 0.35	6.6+/- 0.35	4.95			2.78	38%	1.12	77.5%

The table indicates that ketotifen shows significantly good anti-inflammatory activity, and there was a statistical significance between the groups (considering the percentage of inhibition by standard as 100%)

TURPENTINE INDUCED ARTHRTIS MODEL

Table 4

Table showing the Mean lateral diameter (mm) at 0hr and 4hr, difference between the groups

GROUPS	0hr (mean+/- SD)	4hr (mean+/- SD)	Mean difference in lateral diameterin mm	ANOVA	Independent t- test	Mean difference in lateral knee diameterof test group and standard with respect to control in mms	% of inhibition of lateral knee diameter of test group and standard with respect to control	Mean difference in lateral knee diameter of test group with respect to standard	% of inhibition of lateral knee diameter of test group with respect to standard
CONTROL (2% gum acacia)	3.33+/- 0.51	8.05+/- 0.23	4.71	F=63.203 P=0.001	t=5.312 P=0.001		-		-
STANDARD (Indomethacin 8 mg /kg)	3.5+/- 0.54	5.4+/- 0.35	1.9			2.81	60%	-	-
TEST (ketotifen 0.36 mg/kg)	3.7+/- 0.12	6.53+/- 0.40	2.83			1.88	40%	0.93	67.1%

The table indicates that ketotifen shows good antiinflammatory activity between the groups and there was a statistical significance between the groups (considering the percentage of inhibition of standard as 100%)

COTTON WOOL PELLET INDUCED -GRANULOMA MODEL

Table showing the mean dry granulation tissue weight in different drug groups

GROUPS	Mean dry granulation tissue in mgs	ANOVA	Independent t-test	Mean dry granulation tissue weight of test group and standard with respect to control in mgs	% of inhibition of dry granulation of test group and standardwith respect to control	Mean dry granulation tissue weight of test group with respect to standard	% of inhibition of dry granulation tissue of test group with respect to standard
CONTROL (2% gum acacia)	90.33	F=743.46 P=0.001	t=11.178 P=0.001		-		-
STANDARD (Indomethacin 8 mg /kg)	46.33			44	49%	-	-
TEST (ketotifen 0.36 mg/kg)	60			30.3	34%	13.7	77.7%

The table indicates that Ketotifen shows significantly good anti-inflammatory activity, and there was a statistical significance between the groups (considering the percentage of inhibition of standard as 100%)

DISCUSSION

The drug ketotifen have been investigated in this study for their anti-inflammatory potential and compared with the standard reference drug Indomethacin. In the present study the acute experimental inflammatory models studied includes, Carrageenin rat paw oedema, Turpentine induced arthritis. The sub acute inflammatory model includes Cotton pellet induced granuloma model. In all the experimental inflammatory models, Indomethacin was used as Standard drug and ketotifen was used as test drug.The percentage inhibition of carageenan induced rat paw oedema by Indomethacin compared with control was 48% while that of test drug was 38%. Hence the anti-edema activity of the test group is comparable with that of the standard. The percentage inhibition of paw oedema by the test group considering the inhibition of paw oedema by standard as 100% is 77.5%. Thus ketotifen shows significant anti-inflammatory activity comparable with standard drug Indomethacin in Carrageenin induced rat paw oedema model.The percentage inhibition of Turpentine induced knee arthritis by standard compared with control was 60% and that of test group was 40% respectively. Therefore cetrizine shows a good antiarthritic activity as compared to the standard drug. The percentage inhibition of knee arthritis by the test group considering the percentage inhibition of standard as 100% was 67.1%.Thus ketotifen shows good anti-inflammatory activity comparable with standard drug Indomethacin in Turpentine induced arthritis model. The percent inhibition of cotton pellet induced dry granulation tissue weight by Indomethacin compared with control was 49% and that of the test group was 34%. Ketotifen showed a significantly good antigranuloma effect as compared to the standard drug. The percentage inhibition of dry granuloma weight by the test group considering the percentage inhibition of standard as 100% was 77.7%.Thus ketotifen shows significantly good anti-inflammatory activity comparable with standard drug Indomethacin in Cotton pellet induced granuloma model. The anti-inflammatory activity of the Standard drug was high in Turpentine induced arthritis model but little less in cotton pellet induced granuloma model and carageenan induced paw oedema model. The anti-inflammatory activity of ketotifen was significantly good and almost similar in cotton pellet induced granuloma model and carageenan induced paw oedema model and was good in turpentine induced arthritis model. This study is in accordance with the study- 'Effect of Ketotifen on chemiluminescence of human neutrophils', wherein ketotifen was found to inhibit the chemotaxis of neutrophils[7].The other possible mechanism of its anti-inflammatory action could be possibly due to decrease expression of CD28 and Eotaxin[11].In the present study ketotifen in its therapeutically permissible dose showed promising results in acute models and also in subacute models of experimental inflammation when compared with the standard potent drug in domethacin. Different assays based on other inflammation parameters like erythema, pain, etc needs to be done. Further studies are required to support these findings in humans as the animal data cannot be directly extrapolated on humans. Hence ketotifen can be used to combat inflammation alone or with other conventional anti-inflammatory agents to combat inflammation as like NSAIDs, steroids, LT receptor antagonists, Mast cell stabilizers, cytotoxic drugs, cytokine modulator etc. during the period of administration.

CONCLUSION

In the present study, ketotifen has showed significantly good anti-inflammatory activity in acute models and good activity in a sub acute model of inflammation, in comparison to the standard Indomethacin. This type of study is valuable for identifying lead compounds for anti-inflammatory drugs, keeping in mind the side effects of NSAIDs and corticosteroids. Further studies need to be done in various other acute and chronic inflammatory models along with the human studies to strengthen the results and prove the safety and efficacy of long term administration ketotifen as potential anti-inflammatory agent in routine clinical practice.

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