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ORIGINAL ARTICLE

COMPARATIVE STUDY OF ANTI-INFLAMMATORY ACTIVITY OF NEWER MACROLIDES WITH ETORICOXIB

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ABSTRACT: The present study was designed to investigate the anti-inflammatory activity of macrolides and to compare with standard non- steroidal anti-inflammatory drug (NSAID) etoricoxib. This study was conducted in male wistar albino rats by inducing edema with 1% carrageenan. Animals were divided into 5 groups with 6 in each and paw edema volume was measured by digital plethysmograph before and 3hrs after 1% carrageenan administration. Percentage of inhibition of paw edema was calculated. Results showed macrolides having significant anti-inflammatory activity & the anti-inflammatory activity of roxithromycin was almost equally comparable with etoricoxib.
KEYWORDS: Macrolides, Anti-inflammation, Etoricoxib, Carageenan, digital plethysmograph.

INTRODUCTION: Inflammation (Latin, inflamatio, to set on fire) is the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. It is a protective attempt by the organism to remove the injurious stimuli as well as initiate the healing process for the tissue. Inflammation is not a synonym for infection. Even in cases where inflammation is caused by infection, the two are not synonymous: infection is caused by an exogenous pathogen, while inflammation is the response of the organism to the pathogen.

In the absence of inflammation, wounds and infections would never heal and progressive destruction of the tissue would compromise the survival of the organism. However, inflammation which runs unchecked can also lead to a host of diseases, such as hay fever, atherosclerosis, and rheumatoid arthritis. It is for this reason that the body normally tightly regulates inflammation.

Inflammation can be classified as either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes from the blood into the injured tissues. A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue. Prolonged inflammation, known as chronic inflammation, leads to a progressive shift in the type of cells which are present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process.

Drugs that inhibit inflammation are called anti-inflammatory agents which include glucocorticoids & non-steroidal anti-inflammatory drugs (NSAIDs). NSAIDs drugs act only peripherally by inhibiting prostaglandin synthesis & these are the commonly used anti-inflammatory drugs. NSAIDs provide effective management of pain and inflammation, but are associated with development of peptic ulcer and an increased risk of peptic ulcer bleeding[1] and perforations[2] ("Serious gastrointestinal events" in the range of 0.3% - 2.5% per year"). Chronic use of these is associated with nephrotoxicity[3 & 4] and this group of drugs are also one of the contributing factors for the development of drug induced hypertension. They can cause acute or chronic renal damage following repeated use.[5]

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Macrolides, the antimicrobial agents exert their antibiotic effect by binding irreversibly to the 50S subunit of bacterial ribosomes and inhibit translocation. The term macrolide was derived due to presence multi-membered lactone ring. Macrolide antibiotics are not only active against Gram-positive bacteria but also against some other organism like Haemophilus influenza, Mycoplasma spp., Legionella spp., and Chlamydia spp., etc. Macrolides are used as alternative to penicillin in infections caused by Streptococcal, Staphylococcal organisms. And these are drug of choice in the treatment of atypical pneumonia, legionnaire's pneumonia, whooping cough.

Non antimicrobial activity: Apart from its conventional antibacterial action macrolide antibiotics are known to have anti- inflammatory[6], immunomodulator acitivity[7 & 8] and antioxidant properties[9] and Erythromycin has been used clinically for its stimulatory effect on gastrointestinal motility.[10]

There is now abundant literature of the anti-inflammatory effect of macrolides and the underlying mechanism involved but there were no studies on the comparison of the anti-inflammatory effect of macrolides with standard anti-inflammatory drugs. Hence, the present study was carried out to evaluate the efficacy & to compare the anti-inflammatory property of the newer macrolides with etoricoxib as a standard drug.

AIMS AND OBJECTIVES:

1. To screen anti-inflammatory effect of roxithromycin, azithromycin, clarithromycin in experimental animal models.
2. To compare the anti-inflammatory effect of roxithromycin, azithromycin, clarithromycin with etoricoxib in experimental animal models.

MATERIALS AND METHODS:

MATERIALS:

Chemicals & Solutions: Carrageenan, Etoricoxib, Roxithromycin, Azithromycin, Clarithromycin.

Animals: This experiment was carried out after getting approval from Institutional animal ethics committee (IAEC). Inbreed albino male rats weighing about 200-250gm were used for the study. They were housed in clean polypropylene cages and maintained at room temperature between 27-31ºc with 12:12 hours light and dark cycle in animal house. They had free access to food and water.

Equipment: Digital plethysmograph.

Digital plethysmograph is used for screening the anti-inflammatory drugs. It is an instrument for determining and registry the variations in the size or volume of a limb, as the arm or leg.

Carrageenan Induced Paw Edema Model: To study the acute and sub-acute phases of inflammation in rats. Carrageenan is a widely used irritant or inflammogen. Chemically, it is a sulfated polysaccharide obtained from seaweed (rhodophyceae). The experimental tissue injury caused by this irritant initiates a cascade of inflammatory events leading to formation of exudates. The inflammation induced by it is biphasic in nature. The first phase is attributed to the release of histamine, 5-hydroxy tryptamine (serotonin) and kinin while the second phase is related to the release of prostaglandins.

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PROCEDURE: Weighed the animals before the start of experiment. Animals were divided into 5 groups. A mark was made at the ankle joint (tibio-tarsal joint) of each rat and 1% w/v suspension of carrageenan was prepared freshly in normal saline and injected into sub planter region of left hind paw (usually 0.1ml in rats).In control group animals only vehicle was injected. Test drug was administered intraperitoneally, according to body weight, half an hour before the carrageenan challenge**.**

Paw volume up to the ankle joint was measured in drug treated and untreated groups before and 3hours after carrageenan challenge using a plethysmograph filled with mercury. Edema was found out and % of reduction in edema was calculated.

Experimental design: total 5 groups with 6 animals in each

Group I: control normal saline 0.2 ml per oral

Group II: standard drug etoricoxib 10mg/kg per oral, 1/2 hour before carrageenan inj.

Group III: test 1 - roxithromycin 20mg/kg per oral, 1/2 hour before carrageenan inj.

Group IV: test 2 - azithromycin 20mg/kg per oral, 1/2 hour before carrageenan inj.

Group V: test 3 – clarithromycin20mg/kg per oral, 1/2 hour before carrageenan inj.

Statistical analysis:

% of reduction in edema was calculated by following formula:

$$\frac{\text{Mean edema in control - mean edema in drug treated group X 10}}{\text{Mean edema in control}}$$

Results were analyzed by using an unpaired T test.

RESULTS: The anti-inflammatory activity of Macrolides & etoricoxib was measured by using digital plethysmograph. Oedema was induced by intra plantar injection of carrageenan. Hind paw volume (ml) was determined as a measure of oedema formation, before, and at timed intervals (30 min ± 6 h), after intraplantar carrageenan injection. The mean paw volume and % of inhibition of edema was calculated. The mean paw volume of all groups was shown in table 1.

The mean paw volume of etoricoxib was 0.25 whereas for roxithromycin, azithromycin and clarithromycin were 0.294, 0.705 & 0.527 respectively. These results showed all drugs were reducing the paw volume after carrageenan induced inflammation. These results were compared with control group. After noting mean paw volume we calculated the percentage of inhibition of paw edema by using above mentioned calculation.

It was found that all drugs (both standard and test drugs) showed significant inhibition of % of rat hind paw edema. (Table 2) & better results are shown by etoricoxib with % inhibition of paw edema was 70% compared to test drugs (Table 3). But roxithromycin showed almost equal (64.7%) % of inhibition as that of etoricoxib. Azithromycin and Clarithromycin showed less % of inhibition of rat hind paw edema (Table 4).

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S.NO	ACUAL PAW VOLUME				
	Normal saline (Control)	Etoricoxib (Standard)	Roxithromycin (TEST-1)	Azithromycin (TEST-2)	Clarithromycin (TEST-3)
1.	0.866	0.167	0.433	0.900	0.833
2.	0.967	0.167	0.134	0.466	0.533
3.	0.634	0.266	0.233	0.733	0.267
4.	0.766	0.367	0.134	0.600	0.567
5.	0.867	0.400	0.434	0.767	0.433
6.	0.866	0.133	0.400	0.767	0.533
MEAN	0.827	0.250	0.294	0.705	0.527
S.D	0.114	0.113	0.144	0.151	0.185
S.E	0.046	0.046	0.058	0.061	0.075
%	0%	70%	64.7%	15%	34.7%

Table 1: COMPARISION OF MEAN ACTUAL PAW VOLUME

Note: % - % of inhibition of paw edema volume

Analysis	Normal saline Vs. Etoricoxib	Normal saline Vs. Roxithromycin	Normal saline Vs. Azithromycin	Normal saline Vs. Clarithromycin
t-value	4.92	5.04	1.33	3.58
p-value	<0.05	<0.05	>0.05	<0.05

Table 2: COMPARISION OF MEAN ACTUAL PAW VOLUME BETWEEN CONTROL, STANDARD AND TEST DRUGS BY 'T' test

Note: p<0.05 - Statistically significant, p>0.05 - Statistically not significant

Analysis	Etoricoxib Vs. Roxithromycin	Etoricoxib Vs. Azithromycin	Etoricoxib Vs. Clarithromycin
t-value	0.818	4.14	3.71
p-value	>0.05	<0.05	<0.05

Table 3: COMPARISION OF MEAN ACTUAL PAW VOLUME BETWEEN STANDARD AND TEST DRUGS BY 'T' test

Note: p<0.05 - Statistically significant, p>0.05 - Statistically not significant

Analysis	Roxithromycin Vs. Azithromycin	Roxithromycin Vs. Clarithromycin	Azithromycin Vs. Clarithromycin
t-value	4.82	2.437	1.92
p-value	<0.05	<0.05	>0.05

Table 4: COMPARISION OF MEAN ACTUAL PAW VOLUME BETWEEN VARIOUS TEST DRUGS

Note: p<0.05 - Statistically significant, p>0.05 - Statistically not significant

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DISCUSSION: The present study was carried out to evaluate the anti-inflammatory property of newer macrolides such as roxithromycin, azithromycin and clarithromycin and comparing with standard drug etoricoxib.

Percentage of inhibition of paw edema can be considered for the anti-inflammatory activity of drug which was measured by digital plethysmograph. All the animals except group 1 showed anti-inflammatory action. Etoricoxib, the standard drug showed highest anti-inflammatory activity with70% of inhibition of paw volume. Etoricoxib is a selective COX-2 inhibitor[11]. The anti-inflammatory activity is due to inhibition of prostaglandin synthesis by inhibiting cyclooxygenase enzyme. Prostaglandins are powerful mediators of inflammation. Etoricoxib was being used in rheumatoid arthritis, osteoarthritis, acute gouty arthritis, ankylosing spondylitis, low back pain, acute postoperative pain and primary dysmenorrhea.

From the results it showed that newer macrolide antibiotics also found to have anti-inflammatory action which are in supportive with study conducted by Angela Ianaro et al in 2000.[9] The anti-inflammatory activity was more with roxithromycin and almost equal to standard drug etoricoxib. The signs of the inflammatory response (rubor, tumor, calor, dolar, and functio laesa) are induced by vascular changes & cellular changes. Macrolides have been shown to affect several steps of the inflammatory process.

The main targets for macrolides appear to be the phagocytes and particularly, the neutrophils. These drugs accumulate in neutrophils and delivered to the site of action. They affects the neutrophils migration[12], proliferation of lymphocytes[13], and differentiation of monocytes[14] in inflammatory process Macrolides also inhibit the production and secretion of Interleukins and TNF, GM -CSF in monocytes[15] mast cells[16], macrophage inflammatory protein (MIP-1) in macrophages and leukocytes[17]. Clarithromycin was shown to suppresses the production of interleukins by fibroblast-like cells of the synovial membrane.[18]

Phagocytes and inflammatory mediators like cytokines, oxidants and enzymes playing an important role in inflammatory diseases including bronchiectasis, Diffuse Pan Bronchiolitis and chronic bronchitis. All above mentioned disease respond to macrolide therapy.[19&20]

Roxithromycin as antimicrobial & anti-inflammatory agent effective not only in reducing the persistent inflammation in atherosclerotic plaque but also against microorganism like Chlamydia which is linked with coronary heart disease.[21]

Macrolides also have immunomodulator effects which are produced by interfering the production of the components of both innate & adaptive immunity.[22] These effects distinct from its antimicrobial actions. So macrolides with its anti-inflammatory, antimicrobial and immunomodulator property are better useful in the management of rheumatoid arthritis, cystic fibrosis, chronic sinusitis, asthma and diffuse bronchiolitis, COPD, bronchiectasis,[8&23] as in some of these conditions infection may act as triggering factor for establishing the disease condition.

CONCLUSION: Newer macrolides are having a significant anti-inflammatory activity and roxithromycin almost has equal anti-inflammatory activity as that of standard drug etoricoxib**.** So newer macrolides are more suitable for patients in disease conditions where there is infection associated with inflammation like COPD, Chronic sinusitis, bronchiolitis, and cystic fibrosis.

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