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Comparision of Anti-Inflammatory Effect of Newer Macrolides with Etoricoxib In 0.1ml Of 1% Carrageenan Induced Rat Hind Paw Oedema By Digital Plethysmograph

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Abstract:
Introduction: *Inflammation (Latin, inflammation, to set on fire) is the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. Drugs that inhibit inflammation are called Non steroidal anti-inflammatory drugs (NSAIDS). Presently selective cox-2 inhibitors are used. But these are also having cardio-vascular complications. Macrolides are showing anti-inflammatory property by inhibiting pro inflammatory cytokines from phagocytes. The main aim of this study is to compare the anti-inflammatory effect of newer macrolides with etoricoxib in 0.1ml of 1% carrageenan induced rat hind paw oedema.*
Objectives: *1. To screen anti-inflammatory effect of roxithromycin, azithromycin, clarithromycin in experimental animal models. 2. To compare anti-inflammatory effect of roxithromycin, azithromycin, clarithromycin with etoricoxib in experimental animal models.*
Methodology: *A Randomized controlled trail was conducted in the Dept. of Pharmacology, Dr Pinnamaneni Siddhartha Institute of Medical Sciences and Research Foundation (Dr.PSIMS), Chinoutapalli, Krishna District, Andhra Pradesh with the institutional ethical committee clearance. Total rats were randomly divided into 5 groups consisting of 6 rats in each group. First group of rats were considered as controls and treated with 0.2 ml of normal saline, 1hour before injecting 0.1ml, of 1% carrageenan. Second group were considered as Standard drug and treated with etoricoxib 10mg/kg BW, 1hour before injecting 0.1ml of 1% carrageenan. Third group of rats were considered as Test-1 drug and treated with roxithromycin 20mg/kg BW, 1hour before injecting 0.1ml of 1% carrageenan to the sub-plantar region of hind paw. Fourth group of rats were considered as Test-2 group and treated with azithromycin 20mg/kg BW, 1hour before injecting 0.1ml of 1% carrageenan. Fifth group of rats were considered as Test-3 group and treated with clarithromycin 20mg/kg BW, 1hour before injecting 0.1ml of 1% carrageenan.*
Conclusion: *Roxithromycin is having same anti-inflammatory effect as that of etoricoxib. Azithromycin and clarithromycin are having less anti-inflammatory effect compared to etoricoxib. However the above preclinical experiments only give us an idea but large scale clinical trials are necessary for final assessment.*
Keywords: *Anti-Inflammatory, Azithromycin , Clarithromycin, Digital Plethysmograph, Etoricoxib, Macrolides, Roxithromycin*

I. 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 [1]. 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 non-steroidal anti-inflammatory drugs (NSAIDs). Acetyl salicylic acid is the popularly known NSAID but because of many complications at present it is withdrawn from the market. Presently selective cox-2 inhibitors are used. But these are also having cardio-vascular complications [3].

Macrolides are showing anti-inflammatory property. Macrolides the antimicrobial agents are protein synthesis inhibitors which act principally by binding to 50s ribosomal subunits and are used in the treatment of atypical pneumonia, legionnaire's pneumonia, whooping cough. It also possess anti-inflammatory effects distinct from its antimicrobial actions by decreasing proinflammatory cytokines release from the phagocytes[3], which may prove useful in the management of rheumatoid arthritis, cystic fibrosis, chronic sinusitis, asthma and diffuse bronchiolitis[4]. But the efficacy of different macrolides as an anti-inflammatory agent was not well established.

The present study was carried out to evaluate the anti-inflammatory property of newer macrolides. For this Etoricoxib was selected as standard drug where as newer macrolides Roxithromycin, Azithromycin and Clarithromycin were selected as test drugs.

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

Macrolides: The macrolides are a group of drugs (typically antibiotics) whose activity stems from the presence of a macrolide ring, a large macrocyclic lactone ring to which one or more deoxy sugars, usually cladinose and desosamine, may be attached. The lactone rings are usually 14, 15 or 16-membered. Macrolides belong to the polyketide class of natural products.

Etoricoxib: Etoricoxib is a type of medicine popularly known as a non-steroidal anti-inflammatory drug or NSAID. It is considered to belong to a new generation of NSAIDs. They effectively block the action of cyclo-oxygenase type 2 substances (COX-2) [2].

Roxithromycin: Roxithromycin is a semi-synthetic macrolide antibiotic. It is used to treat respiratory tract, urinary and soft tissue infections [5]. Roxithromycin is derived from erythromycin, containing the same 14-membered lactone ring. However, an N-oxime side chain is attached to the lactone ring. It is also currently undergoing clinical trials for the treatment of male-pattern hair loss [6]. Roxithromycin is not available in the United States. Roxithromycin has also been tested to possess antimalarial activity.

Azithromycin: Azithromycin an azalide a subclass of macrolide antibiotics for oral administration. Azithromycin is derived from erythromycin; however, it differs chemically from erythromycin in that a methyl-substituted nitrogen atom is incorporated into the lactone ring[7].

Clarithromycin: Clarithromycin is a macrolide antibiotic used to treat pharyngitis, tonsillitis, acute maxillary sinusitis, acute bacterial exacerbation of chronic bronchitis, pneumonia (especially atypical pneumonias associated with Chlamydia pneumoniae or TWAR), skin and skin structure infections[8]. In addition, it is sometimes used to treat Legionellosis, Helicobacter pylori and Lyme disease.

II. Materials And Methods

A Randomized controlled trail was conducted in the Dept. of Pharmacology, Dr Pinnamaneni Siddhartha Institute of Medical Sciences and Research Foundation (Dr.PSIMS), Chinoutapalli, Krishna District, Andhra Pradesh with the institutional ethical committee clearance. Total Male albino rats were divided into 5 groups consisting of 6 rats in each group. A mark is made at the ankle joint (tibio-tarsal joint) of each rat. Initial paw volume of each rat is measured before giving drug by using digital plethysmograph.

Control group rats 0.2 ml of normal saline is administered orally 1hour before injecting 0.1ml of 1% carrageenan to the sub-plantar region of the hind paw. Standard group rats etoricoxib 10mg/kg BW is administered orally 1hour before injecting 0.1ml of 1% carrageenan to the sub-plantar region of hind paw. Test-1 group rats roxithromycin 20mg/kg BW is administered as single oral dose 1hour before injecting 0.1ml of 1% carrageenan to the sub-plantar region of hind paw. Test-2 group rats azithromycin 20mg/kg BW is administered as single oral dose 1hour before injecting 0.1ml of 1% carrageenan to the sub-plantar region of hind paw. Test-3 group rats clarithromycin 20mg/kg BW is administered as single oral dose 1hour before injecting 0.1ml of 1% carrageenan to the sub-plantar region of hind paw and the paw volume of each rat is measured after 3hours. The anti-inflammatory property was assessed by using Digital Plethysmograph.

Carrageenan Induced Paw Oedema 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 sulphated polysaccharide obtained from sea weed (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.

A 1% w/v suspension of carrageenan is 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 is injected. Test drug is usually administered intraperitoneally, according to body weight, half an hour before the carrageenan challenge. A mark is made at the ankle joint (tibio-sacral joint) of each rat. Paw volume up to the ankle joint is measured in drug treated and untreated groups before and 3hours after carrageenan challenge using a plethsmograph filled with mercury. Oedema is found out and % of reduction in edema is calculated using the following formula.
%reduction in oedema= Mean oedema in control group-mean oedema in drug treated group is divided by Mean oedema in control and multiplied by hundred

The method is simple, easy and short lasting as well as reproducible. However, it is non-specific and difficult to quantify. It is also difficult to examine cells and their modification by anti-inflammatory drugs. One must avoid injecting the irritant in both the hind paws of the animal on account of severe pain. The carrageenan causes unalleviated pain and deformity. Other irritants such as formalin, mustard oil, snake venom, dextran and polyvinylpyrollidone, histamine, bradykinin, 5-hydroxytryptamine or egg white can replace carrageenan in this model.

II. Materials

Chemicals & Solutions: Carrageenan,Etoricoxib**,** Roxithromycin, Azithromycin, Clarithromycin, Double distilled water, Normal saline

Animals: Albino male rats weighing about 200-250gm.

Equipment: Digital plethysmograph, Insulin syringes, Tuberculin syringes, Infant feeding tube, Hypodermic syringe, Measuring jar, Glass beakers, Animal weighing balance, Animal cages, Cotton, Spirit, Stopwatch, Glass rod, Motor and pestle.

Statistical Analysis: Descriptive statistics, Kruskal wallis, Mann-whitney U test were applied.

III. Results And Discussion

Table:1 Descriptive statistics, Comparision of mean paw oedema among five groups

Group	N	Min	Max	Mean	Median	SD	%
Normal saline (Control)	6	.63	.97	.83	.87	.11	-
Etoricoxib (Standard)	6	.13	.40	.25	.22	.11	70%
Roxithromycin (Test-1)	6	.13	.43	.29	.32	.14	64%
Azithromycin (Test-2)	6	.47	.90	.71	.75	.15	15%
Clarithromycin (Test-3)	6	.27	.83	.53	.53	.19	36%
Test applied: Kruskal wallis; p<0.01; HS							

The standard group with etoricoxib 10 mg/kg body weight showed inhibition of rat paw oedema by 70%.
The test-1 group with roxithromycin 20 mg/kg body weight showed inhibition of rat paw Oedema by 64%
The test-2 group with azithromycin 20 mg/kg body weight showed inhibition of rat paw oedema by 15 %
The test-3 group with clarithromycin 20 mg/kg body weight showed inhibition of rat paw oedema by 36%
In comparison between standard and test drugs observed % of inhibition of rat hind paw oedema, better results were observed with etoricoxib compared to test drugs. Among the test drugs, roxithromycin showed similar % of inhibition as that of etoricoxib. But azithromycin and clarithromycin showed less % of inhibition.

Table-2: Comparison of mean paw oedema between the groups

Comparison		p-value	Inference
Control	Standard	<0.01	HS
	Test-1	<0.01	HS
	Test-2	0.24	NS
	Test-3	<0.01	HS
Standard	Test-1	0.49	NS
	Test-2	<0.01	HS
	Test-3	<0.01	HS
Test-1	Test-2	<0.01	HS
	Test-3	0.03	S
Test-2	Test-3	0.13	NS
Test applied: Mann-whitney U			

IV. Summary& Conclusion

Newer macrolides compared with etoricoxib are having anti-inflammatory property. In newer macrolides roxithromycin is having same anti-inflammatory effect as that of etoricoxib. Azithromycin and clarithromycin are having less anti-inflammatory effect. So newer macrolides are more suitable for patients who

are having infection associated with inflammation. However the above preclinical experiments only give us an idea but large scale clinical trials are necessary for final assessment.

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