ANTI INFLAMMATORY EFFECT OF CIPROFLOXACIN, AZITHROMYCIN AND DICLOFENAC SODIUM ON CARRAGEENAN INDUCED HIND PAW EDEMA IN MICE

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ABSTRACT

Background: Obviously, antibacterial agents are primarily directed against bacteria. However, because microorganisms can initiate an exaggerated inflammatory reaction, and as pathogens which persist in cryptic reservoirs (cells or granuloma tissue) can be the underlying cause of chronic inflammation, the hypothesis that antibacterials can down regulate inflammation. **Methodology:** Healthy adult mice weighing 20 – 30 g and aged 6-8 weeks, each group 6 mice were included. 1% carrageenan administered to produce inflammation. **Grouping:** Group 1: Normal saline 0.2 ml. i.p., Group 2: Diclofenac sodium 25mg/kg, Group 3 Ciprofloxacin 50 mg/kg, Group 4: Azithromycin 20mg/kg. Drugs were administered Intra Peritoneal. After 30 min of test drugs administration each group of mice were received subplantar administration of 0.05ml of saline (Control) or 0.05ml carrageenan (1%) for test groups 2 to 4. Paw volumes were measured by dipping in to the mercury plethysmograph at 30, 60, 120 and 180 minutes and results were tabulated. **Results**: Diclofenac, ciproflaoxin, Azithromycin inhibited paw edema in % at 30min 42.85, 28.55, 14.28, at 60min 75, 50, 25, at 120min 71.42, 42.85, 14.28, and at 180 min 50, 50, 25 respectively. **Conclusion:** Ciprofloxacin (50mg/kg) has exhibited consistent anti-inflammatory, but the anti-inflammatory activity of is less than that of Diclofenac sodium and Azithromycin also has exhibited anti-inflammatory activity, though much less when compared to Diclofenac sodium and Ciprofloxacin.

Keywords: Anti inflammatory effect, Azithromycin, Ciprofloxacin, Diclofenac Sodium, Paw edema, Mice

INTRODUCTION

Inflammation is one of the most complex pathophysiological processes involved in the host response to injury, whatever its intensity and origin [1]. Despite its initial beneficial aspect (host defense) it can become excessive, either by reacting to a non-injurious challenge (allergies or autoimmunity) or by generating excessive acute (shock or systemic inflammatory response syndrome) or long-lasting (chronic inflammatory diseases) deleterious responses [2]. This beneficial/pathological event involves a host of cellular effectors, redundant humoral mediators and enzyme cascades whose importance may vary depending on the nature of the trigger [3]. Consequently, therapeutic interventions in inflammatory diseases have a wide array of possible targets, notably phagocytes (particularly polymorphonuclear neutrophils) and their products (oxidants, enzymes and cytokines) [4].

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Any drug which can prevent or suppress any or more of the components of inflammation are termed anti-inflammatory agents, plenty of drugs have flooded the market. Anti-inflammatory drugs like NSAIDS will inhibit the cyclo-oxygenase pathway and the synthesis of prostaglandins. Another group consisting mainly of steroidal anti-inflammatory drugs exerts their action by inhibiting the enzyme phospholipase-A2. [5].

In the field of antibacterials, research is ongoing to improve antibacterial profiles and to develop new therapeutic properties, of which immunomodulatory anti-inflammatory activities are at the leading edge. Obviously, antibacterial agents are primarily directed against bacteria. However, because microorganisms can initiate an exaggerated inflammatory reaction, and as pathogens which persist in cryptic reservoirs (cells or granuloma tissue) can be the underlying cause of chronic inflammation, the hypothesis that antibacterials can down regulate inflammation by suppressing its bacterial origin has held widespread support since the beginning of antibiotic therapy and still has strong advocates [6].

Among the antibacterial agents used in various inflammatory skin diseases, sulfonamides in the treatment of Wegener's granulomatosis, and tetracyclines for inflammatory acne; the latter were first assessed in 1968

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to 1970 in rheumatoid arthritis (RA) [7]. Rifampicin were less effective in this disease, although Rifamycin was beneficial in ankylosing spondylitis and juvenile RA. It must be noted that all these agents impair phagocyte functions, and particularly their potent oxidant-generating system

Besides their antibacterial activity, some macrolides, particularly those derived from erythromycin are beneficial in various clinical inflammatory settings, including diffuse panbronchiolitis (DPB) in Japanese patients and cystic fibrosis (CF) its genetic counterpart in Caucasians, asthma, atherosclerosis and lung cancer [8]. Although the existence of an underlying intracellular pathogen, generating chronic inflammation has not been ruled out in some diseases (eg,asthma and atherosclerosis), there is some evidence that macrolides may modulate inflammatory responses both in vitro and in vivo.

Aim: The present study was carried out to look for anti-inflammatory activity of ciprofloxacin and azithromycin and comparison with standard anti-inflammatory drug Diclofenac sodium.

MATERIALS AND METHODS

Study design: An experimental animal based study

Ethics approval: The study was approved institutional ethics committee

Study location: Osmania Medical College, Hyderabad

Study duration: 6 months

Inclusion criteria: Healthy adult mice weighing 20 – 30 g and aged 6-8 weeks, were included in the present study.

Raring of mice: Animal: The animals were kept in wire bottomed cages in a room under standard condition of illumination with a 12 - h light-dark cycle at 25 ± 1° C.

Sample size: In each group n=6

Grouping:

Group 1 (Control Group): administered normal saline 0.2 ml. i.p.
Group 2 (Standard Group): administered Diclofenac sodium 25mg/kg i.p [9]
Group 3 (Test Group 1): administered Ciprofloxacin 50 mg/kg i.p [10]
Group 4 (Test Group 2): administered Azithromycin 20mg/kg i.p. [11]
Drugs preparation:

1.**Diclofenac Sodium (Tab. Voveran):** Intraperitoneal preparation of Diclofenac sodium tablet 50mg was diluted with 20 ml of double distilled water at room temperature. The solution was freshly prepared each time, before/during experimental procedure. This solution has a concentration of 2.5mg/ml.

2. **Ciprofloxacin (Tab. Cifran):** Intraperitoneal preparation of Ciprofloxacin tablet 250mg was diluted with 30 ml of double distilled water at room temperature. The solution was freshly prepared each time, before/during experimental procedure. This solution has a concentration of 8.3mg/ml.

3. **Azithromycin (Tab. Azax):** Intraperitoneal preparation of Azithromycin tablet 250mg was diluted with 50 ml of double distilled water at room temperature. The solution was freshly prepared each time, before/during experimental procedure. This solution has a concentration of 5mg/ml.

4. **Carrageenan:** 1% carrageenan administered to produce inflammation

Study variables:

Independent variables: Diclofenac sodium, Ciprofloxacin, Azithromycin

Dependent variables: Paw edema (Volume)

Procedure: After 30 min of test drugs administration each group of mice were received subplantar administration of 0.05ml of saline (Control) or 0.05ml carrageenan (1%) for test groups 2 to 4. An anatomical marking is made at the level of malleolus of the right hand paw of each animal in order to facilitate for dipping in plethysmograph. Paw volumes were measured by dipping in to the mercury plethysmograph at 30, 60, 120 and 180 minutes and results were tabulated. The difference between the two readings at different intervals indicates the actual edema. In this manner the edema volumes was designated as Vc and Vt in the control and drug treated group respectively.

Percentage of inhibition of edema [12] =Vc-Vt/Vc X 100

Statistical analysis: To analysis within the group at different time interval t test was used and to compare between the groups ANOVA was applied.

RESULTS

Table 1. Paw edema (ml) at different time intervals
Table 2. Percentage of inhibition of edema in Mice

Group and Drugs	Paw edema (ml) at different time intervals			
	30 min	60 min	120 min	180 min
1: Normal saline	0.14±0.02	0.08±0.02	0.07±0.01	0.04±0.02
2: Diclofenac sodium	0.08±0.02 **	0.02±0.01 **	0.02±0.01 **	0.02±0.01
3: Ciprofloxacin	0.10±0.01 *	0.04±0.02 *	0.04±0.01 *	0.02±0.01
4: Azithromycin	0.12±0.01 $$	0.06±0.02 $$	0.06±0.02 $$	0.03±0.01

DISCUSSION

Time	Standard	Ciprofloxacin	Azithromycin
30 min	42.85%	28.57%	14.28%
60 min	75%	50%	25%
120 min	71.42%	42.85%	14.28%
180 min	50%	50%	25%

In the present the mean percentage of mice paw edema inhibition is more with Ciprofloxacin and are statistically significant at all-time intervals except at 180 minutes (P-value >0.05). The mean percentage of mice paw edema inhibition by Ciprofloxacin compared with Diclofenac sodium. Here P-value not significant at all-time intervals. The mean percentage of mice paw edema inhibition by Azithromycin not statistically significant at all-time intervals but less than Ciprofloxacin and Diclofenac sodium.

Carrageenan works via the Bcl10, NF-κB, IκBα pathway to activate inflammation mediators. This pathway initially involves phosphorylation steps followed by nuclear translocation of phospho-NF-κB.

Ciprofloxacin is also well known to have a positive immune modulating effect [13]. By positive immunomodulating e□ect it has anti-inflammatory activity. Ciprofloxacin augments the humoral and cellular immunity by attenuating the proinflammatory cytokines such as TNF-alpha, IL-1 beta, IL-4, IL-6, IL-8 and IL-12 and stimulating the anti-inflammatory cytokines such as IL-2, IL-10, soluble TNF receptor 1&2, IL-1 receptor antagonist and colony stimulating factors [14].

Ciprofloxacin displays excellent activities against numerous respiratory tract pathogens. It is known to strongly accumulate in human neutrophils and to easily penetrate epithelial cells.

human nasal epithelial cells (HNECs). Inhibition of the IL-8 response by ciprofloxacin could be attributed to immunomodulatory e□ects [15].

fluorquinolones with a cyclopropyl-moiety at position N1 like ciprofloxacin and moxifloxacin expression of pro-inflammatory cytokines in human monocytes is suppressed by moxifloxacin in vitro and in vivo [16]. The macrolides have long been associated with anti-inflammatory benefits in patients with chronic pulmonary inflammatory disorders. Multiple in vitro and in vivo anti-inflammatory e□ects respiratory tract of bronchiolitis patients [8]. Macrolides like Azithromycin have been shown to a□ect a number of the processes involved in inflammation, including the migration of neutrophils, the oxidative burst in phagocytes and the production of various cytokines. According to Amsden etal These e□ects have been linked to the ability of macrolides to accumulate in mammalian cells (play an important role in their anti-inflammatory activity as polymorphonuclear lymphocytes also contribute to inflammation and tissue damage).The macrolides have long been associated with anti-inflammatory benefits in patients with chronic pulmonary inflammatory disorders [17].

Macrolids like zithromycin inhibited arachidonic acid release in the same way as a $cPLA_2$ inhibitor, while indomethacin had no e□ect. Further comparison revealed that in LPS-stimulated J774A.1 cells, the $cPLA_2$ inhibitor showed the same profile of inhibition as azithromycin in inhibiting PGE_2, IL-6, IL-12p40 and arachidonic acid release [18].

CONCLUSION

In conclusion it may be said that Ciprofloxacin (50mg/kg) has exhibited consistent anti-inflammatory, but the anti-inflammatory activity of is less than that of Diclofenac sodium (25mg/kg) and Azithromycin (20mg/kg) also has exhibited anti-inflammatory activity, though much less when compared to Diclofenac sodium (25mg/kg) and Ciprofloxacin (50mg/kg).

Limitations: The Ciprofloxacin and Azithromycin are well-tolerated antimicrobial agents. Although their anti-inflammatory activity is secondary to anti-infective capacity, these results suggest that further work should be conducted to investigate the mechanism of these e□ects.

Conflict of interest: Nil

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