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Combined effect of Roxithromycin and Diclofenac sodium in carrageenan induced inflammatory model in mice and rats

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ABSTRACT

Inflammation is one of the complex pathophysiological processes of the body which is both protective and destructive in nature. Many studies have been endured to study Non-steroidal anti-inflammatory drugs (NSAIDs) in combination with other drugs to obtain enhanced anti-inflammatory activity. Macrolides were proved to down regulate prolonged inflammation through various mechanisms.. Among macrolides, Roxithromycin is selected as many in- vitro studies proved that it has an anti-inflammatory effect. Apart from this, it was reported to have better plasma and higher tissue concentrations in standard animal models . Therefore, present study was aimed to evaluate the combined anti-inflammatory effect of Roxithromycin with the standard NSAID Diclofenac sodium using carrageenan paw edema model. The results demonstrated that Roxithromycin 20mg/kg in combination with Diclofenac sodium 25mg/kg has reduced the inflammation more effectively compared to Roxithomycin or Diclofenac groups both in rats and mice. However, further clinical studies are required to substantiate the results.

KEYWORDS Roxithromycin, Diclofenac sodium, Inflammation

Introduction:

Inflammation is one of the most complex pathophysiological processes of the body which is both protective and destructive in nature. It is considered as natural host defense as well as the presiding factor to many diseases i.e., from common cold to cancer1[1,2]. Anti-inflammatory drugs like NSAIDS which exert their effect through inhibition of cyclooxygenase pathway are under wider use[3]. However, in animal studies, the NSAIDS when given alone has produced limited anti-inflammatory and anti-nociceptive effects[4]. So, the research for new drugs with different anti-inflammatory mechanisms is continuously under scrutiny. Also 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. Among different antibacterials, Macrolides have been recognised to exert anti-inflammatory actions and was evidently proved through many in-vitro studies[5]. The mechanisms underlying these effects remain unclear. Macrolides are characterized by their strong accumulation within host cells, a property that sustains their activity against intracellular pathogens and is likely responsible for the modulation of cell functions[6]. Among different macrolides, Roxithromycin have been shown to affect a number of the processes involved in inflammation, including the migration of neutrophils[7], the oxidative burst in phagocytes[8] and the production of various cytokines[9]. These effects have been linked to the ability of macrolides to accumulate in mammalian cells[6]. In view of this, Roxithromycin was taken as a study drug in combination with diclofenac sodium and compared to Diclofenac sodium and roxithromycin alone.

Methodology:

In the present study, anti-inflammatory potential was assessed using carrageenan induced paw edema model. The study was conducted after Institutional Animal Ethical Committee approval(IAEC no: pharma/omc/04/2009) .The study was performed in both Albino mice and Wistar rats. 24 Albino mice and Wistar rats of either sex were taken and divided into four groups of six animals each i.e., as control, Diclofenac, Roxithromycin, combination of roxithromycin with diclofenac groups. The inflammatory response was analysed using the standard plethysmograph .An anatomical marking was made at the level of the malleolus of the right hind paw of each animal, in order to facilitate for dipping in plethysmograph. The control group received 0.9% normal saline intraperitoneally. While the diclofenac group received (diclofenac sodium 25mg/kg), the Roxithromycin group received (Roxithromycin 20mg/kg), the combination group received both diclofenac (25mg/kg) and Roxithromycin (20mg/kg) intraperitoneally respectively.

After 30 minutes rats (0.1ml) and mice (0.05ml) were injected 1 % carrageenan into the right hind paw. Immediately after the sub plantar injection of carrageenan, paw volume was measured by dipping into the mercury plethysmograph (zero hour value). Similar recordings were taken at 30, 60 and 120 minutes and results were analyzed using SPSS version 19.

Results:

The anti-inflammatory effect of Roxithromycin was seen in comparison to Diclofenac sodium and combined Roxithromycin and Diclofenac group. The study was done in both rats and mice and the results were presented.

Rats :

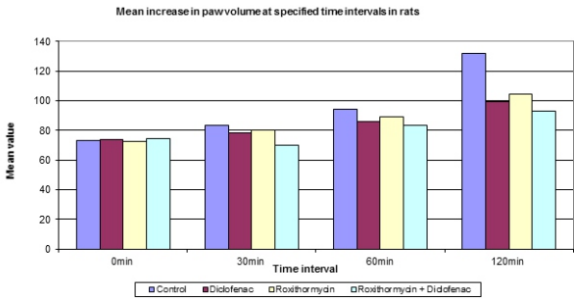
The mean percentage increase in paw volume of Roxithromycin was more compared to Diclofenac sodium, P value is 0.1 and is statistically not significant. The mean percentage increase in paw volume of combined Roxithromycin and Diclofenac sodium was much less compared to Diclofenac sodium, P value is 0.01 and is statistically significant. The mean percentage increase in paw volume of combined Roxithromycin and Diclofenac group was less compared to control, P value is 0.001 and is highly statistically significant. (Refer Table 1; Fig 1)

Table 1 : mean increase in paw volume at specified time intervals in rats

	0min	30min	60min	120min
Control	73.3 + 1.04	83.3 + 1.38	94.16 + 1.67	131.66 + 4.4
Diclofenac Na (25mg/kg)	73.8 + 0.73	78.6 +1.19	86.16 + 1.53	99.66 + 7.76
Roxithromycin 20mg/kg	72.6 +1.33	80.5 +2.2	89.33 +1.62	104.66 + 2.39
Diclofenac Na (25mg/kg) + Roxithromycin (20mg/kg).	74.3 + 1.79	70.16 + 2.76	83.66 + 1.76	93.16 + 1.64

Number of animals (n=6) ; Mean + standard error

Figure 1: Mean increase in paw volume at specified time intervals in rats



Mice :

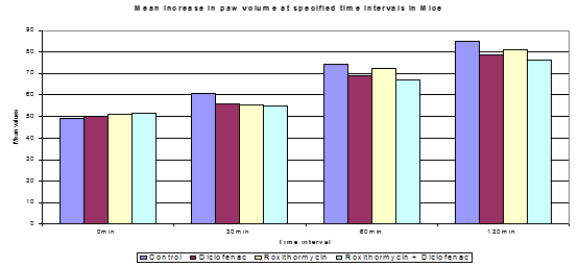
The mean percentage increase in paw volume of Roxithromycin was more compared to diclofenac, P value is 0.1 and is statistically not significant. The mean percentage increase in paw volume of combined Roxithromycin and Diclofenac was less compared to control P value is 0.02 and is statistically significant. The mean percentage increase in paw volume of Roxithromycin was significant compared with control with P-value 0.01. (Refer Table 2; Figure 2)

TABLE –2 : Mean increase in paw volume at specified time intervals in mice

	0min	30min	60min	120min
Control	49.16 + 0.98	60.66+ 1.12	74.5 + 4.28	85 + 4.4
Diclofenac	50.15 +0.72	56 + 1.26	69+ 1.74	78.5 +1.83
Roxithormycin	51 + 0.52	55.5 +0.44	72.33 + 4.34	81 + 1.76
Roxithormycin + Diclofenac	51.33 + 0.43	54.83 +1.13	66.83 + 1.78	76 +1.38

Number of animals (n=6) ; Mean + standard error

Figure 2: Mean increase in paw volume at specified time intervals in rats



When compared between rats and mice, Roxithromycin group was less effective compared to Diclofenac group (20mg/kg) in reducing edema both in rats and mice. In rats and mice combined group (Roxithromycin 20mg/kg + Diclofenac 25mg/kg) is more effective compared to Diclofenac group and Roxithromycin group in reducing the paw edema.

Discussion

Inflammation is one of the most complex pathophysiological processes involved in the host response to injury, whatever its intensity and origin system, and various cells within the injured tissue[1]. Plenty of drugs which can prevent or suppress any or more of the components of inflammation have flooded the market and there is continuing flow of preparation[2,3]. 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. As the 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.

Macrolide antibiotics are characterized by their strong accumulation within host cells, a property that sustains their activity against intracellular pathogens and is likely responsible for the modulation of cell functions[6]. Besides their antibacterial activity, some macrolides, particularly those derived from erythromycin A, are beneficial in various clinical inflammatory settings, including diffuse pan bronchiolitis , asthma, atherosclerosis and lung cancer. 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*[10].

Roxithromycin have been shown to affect a number of the processes involved in inflammation, including the migration of neutrophils, the oxidative burst in phagocytes and the production of various cytokines[11,12]. These effects have been linked to the ability of macrolides to accumulate in mammalian cells[6] and play an important role in their anti inflammatory activity as polymorphonuclear lymphocytes also contribute to inflammation and tissue damage[13],.

Therefore, we have taken roxithromycin combined with Diclofenac sodium and studied the

anti –inflammatory effect in comparison to individual treatment of roxithromycin and diclofenac sodium using the Carageenan Paw edema model. The results shown that Combined anti-inflammatory effect of Roxithromycin+Diclofenac sodium is more compared to individual groups. This may be because the roxithromycin itself has anti-inflammatory properties which were proved earlier. Roxithromycin in combination with Diclofenac may prove to provide better therapeutic implications.

Conclusion:

All the three groups tested were able to reduce the inflammation provoked by carrageenan in the paw edema model, in both rats and mice. Combined group (Roxithromycin 20mg/kg +Diclofenac 25mg/kg) reduced the inflammation more effectively. Therefore, Roxithromycin can be considered for their anti-inflammatory activity. However, further studies on other anti-inflammatory models have to be done to substantiate the above results.

References:

1. Robbins and Cotran Pathologic Basis of Disease. Page no 48-85. -7th edition. Kumar. Abbas and Fausto.
2. Pharmacology and Pharmacotherapeutics. R.S.Satoskar. 20th edition. Classification of anti inflammatory drugs: 161.
3. Basic and Clinical pharmacology by Bertram G Katzung. steroidal anti-inflammatory drugs. 640 George P. Chrousos. MD
4. Mario I. Ortiz Synergistic interaction between diclofenac and pyrilamine on nociception, inflammation, and gastric damage in rats Canadian Journal of Physiology and Pharmacology, 2017, Vol. 95, No. 1 : pp. 51-58
5. Essentials of Medical Pharmacology. 6th edition. K.D.Tripathi newer macrolides, 729-730.
6. Nilsen.O.G (1995). Pharmacokinetics of macrolides. Comparison of plasma. Tissue and free concentration with specialy reference to Roxithromycin. Infection23. Supp1.1,S5-9.
7. Aanderson. R. (1989). Erythromycin and Roxithromycin potentiate human neutrophil locomotion in vitro by inhibition of leukoattractant-6
8. activated superoxide generation and autooxidation. Journal of Infectious Diseases 159. 966-73.
8. Hand. g. K.. Van Rensburg. C.E. And King-Thompson, N. L. (1990). Antibiotic inhibition of the respiratory burst response in human polymorphonuclear leukocytes. Antimicrobial Agents and chemotherapy 34, 863-70.
9. Bailly, S.. Pocidalo, J. J., Fay. M. and Gougerot-Pocidalo. M. A. (1991). Differential modulation of cytokine production by macrolides: interleukin-production is increasedby spiramycinand erythromycin. Antimicrobial Agents and Chemotherapy 35, 2016-9.
10. Kadota.J. Salito.o.kohno,s,Abek.shirai and kawakami Ketal(1994) Roxithromycin treatment in patients with chronic lower respiratortract disease-its clinical efficacy and effect on cytokine(journal of the Japanese Association for infectious diseases)68.27-33
11. Pechere JC. Auckenthaler R. Invitro activity of Roxithromycin against respiratory and skin pathogens. J. Antimicrobial chemothraphy. 1987 Nov. 20 Suppl B: 1-5.
12. Labro,M. T., el Benna. J. and Babin-Chevaye. C. (1989). Comparison of the in-vitro

effect of several macrolides on the oxidative burst of human neutrophil. Journal of Antimicrobial Chemotherapy24. 561-72.
13. Mitsuyama, T., Tanaka. T., Hidaka, k., Abe. M. and Hara, N. (1995). Inhibition by erythromycin of superoxide anion production by humanpolymorphonuclear leukocytes through the action of cyclic AMP-dependent protein kinase. Respiration 62, 269-73.