EFFECT OF CIPROFLOXACIN ON ACUTE AND SUBACUTE INFLAMMATION IN WISTAR RATS

Ravishankar AC[1], Priya Gandigawad N[2], Hiremath SV[2]

Medical Advisor, AstraZeneca Pharma India Ltd, Bangalore, India.[1]

[1]Dept of Pharmacology and Pharmacotherapeutics, JN Medical College, Belgaum, India.[2]

Summary

Background: Ciprofloxacin is used as a second line drug in the treatment of tuberculosis. Tuberculosis is a chronic disease characterized by significant inflammation resulting in fibrosis leading to complications like pulmonary fibrosis, constrictive pericarditis etc. Drugs possessing anti-inflammatory activity can reduce the complications of infections occurring due to inflammation and fibrosis.

Objective: To evaluate the anti-inflammatory activity of ciprofloxacin on experimentally induced acute and subacute inflammation in Wistar rats

Materials and Methods: The *in vivo* anti-inflammatory activity of ciprofloxacin was studied using acute (carrageenan paw edema) and sub-acute (cotton pellet granuloma and histopathologic examination of grass pith) models of inflammation.

Results: Ciprofloxacin exhibited significant anti-inflammatory activity in acute and sub-acute models of inflammation.

Conclusion: Ciprofloxacin when administered to treat tuberculosis can reduce complications of tuberculosis by virtue of its anti-inflammatory activity

Key Words: Ciprofloxacin, aspirin, carrageenan paw edema, cotton pellet granuloma

***Corresponding author:**
Dr. Priya Gandigawad N
Postgraduate
Department of Pharmacology,
J. N. Medical College, Belgaum, Karnataka, India, 590010
Ph: 09483936484, e-mail: dr.priyagandigawad@gmail.com

Introduction

Fluroquinolones are quinolone antimicrobials having one or more fluorine substitutions with extended activity not only to gram negative organisms but also to gram positive cocci and anaerobes. These agents inhibit DNA gyrase in gram negative and topoisomerase II in gram positive organisms (The enzyme that produces negative super coiling of DNA and thus permits transcription / replication).[1]

They are used in various infections involving urinary tract, respiratory tract, gastro intestinal tract, bone etc. Ciprofloxacin is used as a second line drug in treatment of tuberculosis.[2]

Tuberculosis, one of the oldest diseases known to affect humans, is a major cause of death worldwide. This disease, which is caused by bacteria of the Mycobacterium tuberculosis complex, usually affects the lungs, although other organs are involved in up to one-third of cases. Cell mediated immunity(CMI) plays a major role in combating this infecting. About 2–4 weeks after infection, two host responses to M. tuberculosis develop: a macrophage-activating CMI response and a tissue-damaging response. The macrophage-activating response is a T cell–mediated phenomenon resulting in the activation of macrophages that are capable of killing and digesting tubercle bacilli. The tissue-damaging response is the result of a delayed-type hypersensitivity (DTH) reaction to various bacillary antigens; it destroys unactivated macrophages that contain multiplying bacilli but also causes caseous necrosis of the involved tissues. With the development of specific immunity and the accumulation of large numbers of activated macrophages at the site of the primary lesion, granulomatous lesions (tubercles) are formed. Initially, the tissue-damaging response can limit mycobacterial growth within macrophages. As stated above, this response, mediated by various bacterial products, not only destroys macrophages but also produces early solid necrosis in the center of the tubercle. Although M. tuberculosis can survive, its growth is inhibited within this necrotic environment by low oxygen tension and low pH. At this point, some lesions may heal by fibrosis, with subsequent calcification, whereas inflammation and necrosis occur in other lesions.[3]

Inflammation, fibrosis, Calcification can lead to significant complications like pulmonary fibrosis, constrictive pericarditis, hydrocephalus, tubal stricture leading to infertility, peritoneal adhesions leading to intestinal obstruction.[4]

It could therefore be hypothesized that drugs possessing anti-inflammatory property to some extent may overcome the inflammatory reaction to infection and also its sequelae.

Present study was designed to analyze the effect of ciprofloxacin on acute and subacute inflammation in male Wistar rats.

Materials and methods

ANIMALS:

Adult male healthy Wistar rats weighing 175 ±25 g were obtained from the central animal house, J.N.Medical College Belgaum and were acclimatized to 12:12 h light - dark cycle for 10 days prior to the day of experimentation. They were maintained on standard rat chow pellet (Amrut Brand) and water ad libitum. The study was approved by the IAEC constituted as per the guidelines of CPCSEA, New Delhi.

ACUTE INFLAMMATION

Rats starved overnight with free access to water were divided into three groups (n=6 in each) to receive various treatments. Calculated clinical equivalent doses, 200mg/kg of aspirin in 2% gum acacia suspension as vehicle (in aspirin group) and 45mg/kg of ciprofloxacin (in ciprofloxacin group), administered orally in a single dose while, the control group received 0.5ml of 1% gum acacia suspension orally. One hour after vehicle, aspirin and ciprofloxacin administration, 0.05 ml of carrageenan (1% w/v) in normal saline was injected into the sub plantar region of the left hind paw, as per the technique of Winter et al.[16]

A mark was made on both hind paws just below the tibiotarsal junction so that the paw could be dipped in the mercury column of the plethysmometer upto the mark to ensure constant paw volume. The paw edema was measured at zero hour (immediately after injecting carrageenan) and the procedure was repeated at 0.5, 1, 2, 3, 4, 5 and 6 h. The difference between 0 hour and subsequent reading was taken as actual edema volume.

The percentage inhibition of edema was calculated using formula.[17]

$$\text{Percentage Inhibition of edema} = \left(1 - \frac{\text{Mean increase in paw volume in treated group}}{\text{Mean increase in paw volume in control group}}\right) \text{X } 100$$

SUBACUTE INFLAMMATION

Rats were divided into three groups of six in each. After clipping the hair in axillae and groin, under light halothane anesthesia, two sterile cotton pellets weighing 10 mg each and two sterile grass piths (25X2mm each) were implanted randomly, subcutaneously through a small incision. Wounds were then sutured and animals were then caged individually after recovery from anesthesia. Aseptic precautions were taken throughout the experiment.
The rats then received calculated clinical equivalent doses, 200mg/kg of aspirin in 2% gum acacia suspension as vehicle once daily (in aspirin group) and 45mg/kg of ciprofloxacin twice daily (in ciprofloxacin group) orally while, the control group received 0.5ml of 1% gum acacia suspension orally. The treatment was started on the day of implantation and continued for 10days. On eleventh day, the rats were sacrificed with an overdose of anesthesia to remove the cotton pellets and grass piths. The grass pith granulomas were preserved in 10% formalin for histopathological studies. The pellets, free from extraneous tissue were dried overnight at 60ºC to note their dry weight. Net granuloma formation was calculated by subtracting the initial weight of cotton pellet from the weights noted. Mean granuloma dry weight for various groups were calculated and expressed in mg/100g body weight.

Percentage inhibition of granuloma dry weight was calculated using formula. [17]

$$\text{Percentage Inhibition of granuloma dry weight} = \left(1 - \frac{\text{Dry weight of granuloma in treated group}}{\text{Dry weight of granuloma in control group}}\right) \times 100$$

Statistical analysis:

Data expressed as mean ± SEM were analyzed by one-way ANOVA followed by Dunnet's post hoc test and P values ≤ 0.05 was considered significant.

Results

Acute studies

As expected, aspirin significantly (P<0.01) reduced paw edema as compared to the controls throughout the observation period. Similarly ciprofloxacin also showed significant anti-inflammatory activity compared to vehicle treated groups. (Table 1)

Table 1 showing mean volume of paw edema (ml) +/- Standard Error of Mean (SEM) of control, aspirin and ciprofloxacin, and percentage inhibition of paw edema by aspirin and ciprofloxacin.

Time after carrageenan injection	Control	Aspirin		Ciprofloxacin	
	Paw edema In ml (SEM)	Paw edema In ml (SEM)	Percentage Inhibition	Paw edema In ml (SEM)	Percentage inhibition
0.5 hr	0.3500 ± 0.01291	0.1833± 0.01054	47.63 *	0.2500± 0.01291	28.57 *
1hr	0.5083 ± 0.02386	0.2667± 0.01054	47.54 *	0.3333± 0.01667	34.43 *
2hr	0.6750 ± 0.01708	0.3000± 0.01291	55.56 *	0.4083± 0.01537	39.52 *
3hr	0.8500 ± 0.01826	0.2833± 0.01054	66.67 *	0.3583± 0.02007	57.85 *
4hr	0.9250 ± 0.01118	0.2083± 0.01537	77.48 *	0.2833± 0.02108	69.38 *
5hr	0.8500 ± 0.02582	0.1333± 0.01667	84.32 *	0.2083± 0.01537	75.50 *
6hr	0.7250 ± 0.01118	0.05833± 0.01537	91.96 *	0.1000± 0.01291	86.20 *

*P < 0.01 as compared to control

Subacute studies

Mean granuloma dry weight (mg % body weight) in aspirin and ciprofloxacin were significantly (P<0.01) lower than control. (Table 2)

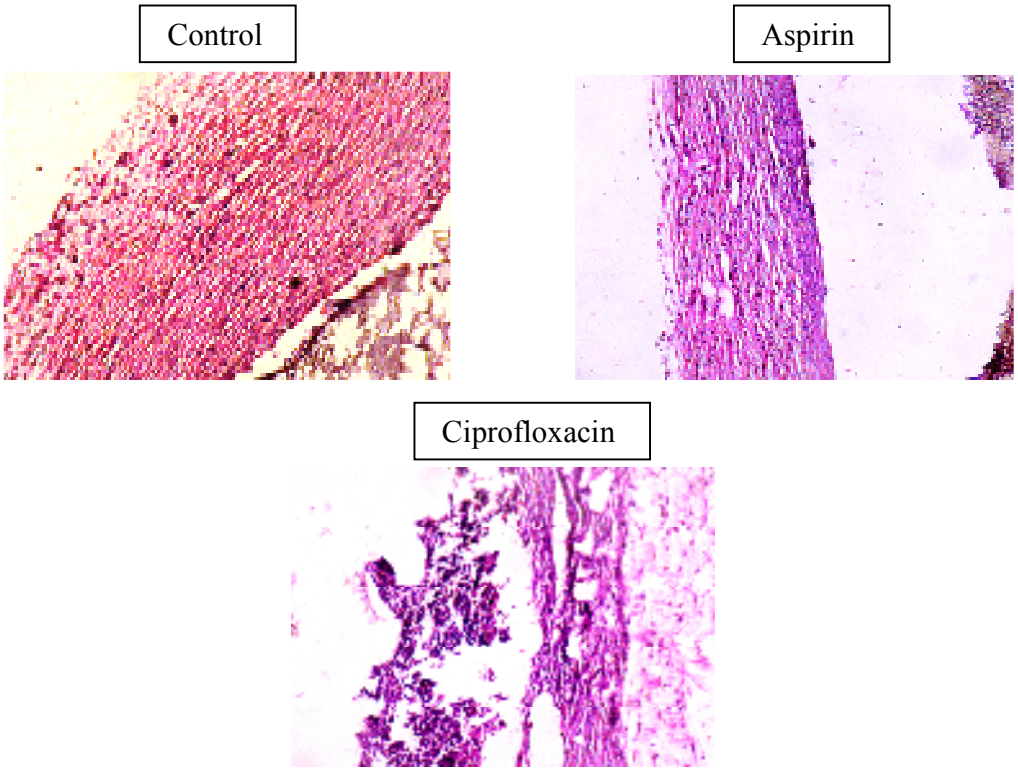
Table 2 showing grnuloma dry weight (mg % body weight) of control, aspirin and ciprofloxacin, and percentage inhibition of granuloma dry weight by aspirin and ciprofloxacin.

Control	Aspirin		Ciprofloxacin	
Granuloma dry weight	Granuloma dry weight	Percentage Inhibition	Granuloma dry weight	Percentage inhibition
40.83 ± 0.9098	24.67 ± 1.116	39.58*	27.33 ± 0.7149	33.07*

*P < 0.01 as compared to control

The granulation tissue sections stained with haematoxylin and eosin revealed a marked reduction in thickness of collagen content and fibroblast number as compared to control in both aspirin and ciprofloxacin treated groups indicating their anti-inflammatory action (Fig1).

Figure 1 showing photomicrographs of granulation tissue (H & E stain 10x)



As compared to control, markedly decreased collagen content and fibroblast number in drug treated groups

Discussion

Earlier studies have analyzed the influence of ciprofloxacin on various inflammatory mediators , it has been reported that ciprofloxacin may have anti inflammatory action mainly through PGE_2 which reduces Interleukin [IL]-6 IL-10,[5] IL-12 & IL-18,[6] Intercellular Adhesion Molecule 1 [ICAM 1], Tumor necrosis factor [TNF] α , Interferon [INF] γ[7] and inhibit P65, NFkappaB activation.[7] Also ciprofloxacin on its own is known to increase cAMP,[8] reduce CD 14, Toll like receptors,[9] pro inflammatory cytokines (TNF α, INF γ, Nitric oxide[NO],[10] IL-1, IL-8,[11] IL- 12 & IL-18 [6]). Inhibit expression of ICAM 1, B7.1, B7.2 and CD 40.[6]

However various invivo models have shown ciprofloxacin to induce inflammation in tendon leading to its rupture in rats, [12] induce skin inflammation by increasing PGE_2 & 6 keto PGF1 alpha from dermal fibroblasts.[13] In addition, PGE_2 which is induced by ciprofloxacin has also been reported to posses pro inflammatory activity like induction of COX 2,[4] pro inflammatory cytokines,[14] P19, P40[14] and produce clinical signs of inflammation like fever, hyperalgesia, edema etc.[15]

Results of the present study clearly indicate that ciprofloxacin when administered in clinically equivalent dose showed significant anti-inflammatory activity in acute and subacute models of inflammation.

Conclusion

Ciprofloxacin has significant anti-inflammatory activity and when given to a patient suffering from tuberclulosis, may reduce complications of inflammation and fibrosis.

Acknowledgement

The authors are grateful to the Principal, J. N. Medical College, Belgaum for providing facilities and Dr. P. R. Malur, Professor and Head, Dept of Pathology for his guidance in microscopic studies.

References

1. Rang HP, Dale MM, Ritter JM, Moore PK. Text book of pharmacology.5th edition. Edinburgh: Churchill Livingstone Publishers; 2003.

2. Laurence L Bruton, John S Lazo, Keith L Parker. Goodman & Gilman's The Pharmacologic Basis of Therapeutics. 11th edition. New York: Mc Graw Hill Publishers; 2006.

3. Fauci, Braunwald, Kasper, Hauser, Longo, Jameson. Harrison's Principles of Internal Medicine. 17th edition. New York: Mc Graw Hill Publishers; 2008.

4. Christopher Haslett, Edwin R. Chilvers, Nicholas A. Boon, Nicki R. Colledge. Davidson's Principles and Practice of Medicine. 19th edition. London: Churchill Livingstone Publishers; 2002

5. Chae BS, Shin TY, Kim DK, Eun JS, Leem JY, Yang JH. Prostaglandin PGE_2 mediated dysregulation of Proinflammatory cytokine production in Pristane induced lupus mice. Archives of pharmacal research 2008; 31(4):503-510.

6. Hideo Kohka Takahashi, Hiromi Iwagaki, Dong Xue, Goutarou Katsuno, Sachi Sugita, Kenji Mizuno et al. Effect of ciprofloxacin –induced Prostaglandin PGE_2 on Interleukin 18 treated monocytes. Anti microbial agents and Chemotherapy 2005; 49(8):3228-3233.

7. Gomez PF, Pillinger MH, Attur M, Marjanovic N, Dave M, Park J et al. Resolution of inflammation : Prostaglandin E_2 dissociates nuclear trafficking of individual NfkappaB sub units (P65, P50) in stimulated rheumatoid synovial fibroblasts. Journal of Immunology 2005; 175(10): 6924-30

8. More AR, Willough DA.The role of cAMP regulation in controlling inflammation. Clin Exp Immunol 1995; 101:387-389

9. Katsuno G, Takahashi HK, Iwagaki H, Sugita S, Mori S, Saito S et al. The effect of ciprofloxacin on CD14 and toll – like receptor-4 expression on human monocytes. Shock 2006; 25(3): 247-53.

10. Kolios G, Manousou P, Bourikas L, Notas G, Tsagarakis N, Mouzas I et al. Ciprofloxacin inhibits cytokine –induced nitric oxide production in human colonic epithelium. European Journal of Clinical Investigation 2006; 36(10): 720-729.

11. Guy Lahat, Drora Halperin, Eli Barazovsky, Itamar Shalit, Micha Rabau, Josef Klausner et al. Immunomodulatory effects of ciprofloxacin in TNBS-induced colitis in mice. Inflammatory bowel disease 2007; 13 (5): 557-565.

12. Michael Casparian J, Michael Lundi, Daniel Hinthorn et al . Quinolones and Tendon rupture. Southern Medical Journal 2000; 93(5): 488-491.

13. kohji Shimoda, Nobuhiko Wagai & Michiyuki Kato. Stimulation of Prostaglandin production by Quinolone phototxicity in Balb/c 3T3 mouse fibroblast cells in vitro. Fundamental & Applied Toxicology 1997; 36: 157- 162

14. Amir F, Sheibanie, Iman Tadmori, Huie Jing, Evros Vassiliou & Doina Ganea. Prostaglandin E2 induces IL-23 production in bone marrow derived dendritic cells. FASEB Journal 2004; 18: 1318-1320

15. Kumar, Abbas, Fausto. Robbins and Cotran Pathologic Basis of Diseases. 7th edition. Philadelphia: Elsevier Publishers; 2005.

16. Turner R A. Screening Methods in pharmacology. New York and London: Academic Press Inc: 1965.

17. Chakradhar V Lagishetty, Suresh Ramnath Naik. Polyamines: Potential anti-inflammatory agents and their possible mechanism of action. Indian journal of Pharmacology. 2008; 40 (3): 121-125