IJBCP International Journal of Basic & Clinical Pharmacology

Research Article

Effect of rifampicin, isoniazid on acute and subacute inflammation in male Wistar rats: an experimental study

Priya Gandigawad[1]*, Anil P. Hogade[2], P. A. Patil[2], P. R. Malur[3]

[1]Department of Pharmacology, SDM College of Medical Sciences, Dharwad, Karnataka, India, [2]Department of Pharmacology, J. N. Medical College, Belgaum, Karnataka, India, [3]Department of Pathology, J. N. Medical College, Belgaum, Karnataka, India

*Correspondence to:
Priya Gandigawad,
Email: dr.priyagandigawad@
gmail.com

ABSTRACT

Background: Tuberculosis (TB) is characterized by significant inflammation 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. To study the effect of rifampicin, isoniazid on acute and subacute models of inflammation in male Wistar rats.
Methods: The *in vivo* anti-inflammatory activity of rifampicin, isoniazid was studied using acute (carrageenan paw edema) and sub-acute (cotton pellet granuloma and histopathologic examination of grass pith) models of inflammation.
Results: Rifampicin and isoniazid used in the present study showed significant anti-inflammatory activity in acute as well as subacute models of inflammation.
Conclusion: Rifampicin and isoniazid when administered to treat TB can reduce complications of TB by virtue of its anti-inflammatory activity.

Keywords: Rifampicin, Isoniazid, Aspirin, Inflammation

INTRODUCTION

Inflammation is a complex reaction to various injurious agents such as infections, trauma, foreign bodies, tissue necrosis, physical, and chemical agents that consists of vascular responses, migration, and activation of leukocytes and systemic reactions.[1]

Inflammation is usually treated by anti-infective agents irrespective of its etiological factor. However, in case of inflammation caused by infection it needs treatment not only with anti-infective/antimicrobial but also anti-inflammatory agents. In case, antimicrobials possess anti-inflammatory activity they would be able to control not only infection, but also inflammation provided causative organisms are susceptible to them.

Tuberculosis (TB) is a chronic granulomatous infection and a major health problem in India. India is the highest TB burden country in the world and accounts for nearly 1/5th of the global burden.[2] The pathogenesis of TB involves a combination of immune and inflammatory process. This can result in pleurisy, pleural fibrosis, ureteral strictures, stricture of fallopian tubes leading to infertility, chronic pericarditis with thickening of pericardium and fibrosis, TB meningitis leading to fibrosis and its complications. Clinical trials have

demonstrated that patients given adjunctive glucocorticoid may have benefit.[3]

Current treatment for TB includes five first-line drugs isoniazid, rifampicin, pyrazinamide, ethambutol, and streptomycin. Of these, rifampicin and isoniazid are considered most effective, and pyrazinamide is mainly added to prevent development of resistance, since it acts on slowly multiplying intracellular organisms.[4]

Interestingly, certain studies have shown that rifampicin suppresses inflammatory mediators like tumor necrosis factor α (TNFα),[5] interleukin-2 (IL-2) production,[5] reactive oxygen species,[6] and prostaglandin E2 (PGE2) expression.[7] Also, it has been reported to activate human glucocorticoid receptor.[8] Similarly, isoniazid has been shown to suppress IL-1 production in *in vitro* studies[9] suggesting both of these could possess anti-inflammatory activity.

However, some studies have shown that rifampicin may have pro-inflammatory activity by increasing CD1b expression[10] and nitric oxide production.[11]

In view of controversial reports of rifampicin on inflammation and paucity of anti-inflammatory studies of isoniazid, the present study is planned to evaluate the effect of rifampicin, isoniazid on acute and subacute models of inflammation in male Wistar rats.

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 hrs 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 five groups (n=6 in each) to receive various treatments. Calculated clinical equivalent doses, 200 mg/kg of aspirin in 1% gum acacia suspension as vehicle (in aspirin group), 54 mg/kg of rifampicin (in rifampicin group), and 27 mg/kg of isoniazid (in isoniazid group), were administered orally in a single dose, while the control group received 0.5 ml of 1% gum acacia suspension orally. 1 hr after vehicle, aspirin, rifampicin, isoniazid administration, 0.05 ml of carrageenan (1% w/v) in normal saline was injected into the subplantar region of the left hind paw, as per the technique of Winter et al.[10]

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 up to the mark to ensure constant paw volume. The paw edema was measured at 0 hr (immediately after injecting carrageenan), and the procedure was repeated at 0.5, 1, 3, 4, and 5 hrs. The difference between 0 hr and subsequent reading was taken as actual edema volume.

The percentage inhibition of edema was calculated using formula:

Percentage inhibition of edema

$$= \left(1 - \frac{\text{Mean increase in paw volume in treated group}}{\text{Mean increase in paw volume in control group}}\right) \times 100$$

Subacute inflammation

Rats were divided into five 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 (25 mm × 2 mm 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, 200 mg/kg of aspirin in 1% gum acacia suspension as vehicle once daily (in aspirin group), 54 mg/kg of rifampicin once daily (in rifampicin group), 27 mg/kg of isoniazid (in isoniazid group), once daily orally, while, the control group received 0.5 ml of 1% gum acacia suspension orally. The treatment was started on the day of implantation and continued for 10 days. On 11th 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 the extraneous tissue were dried overnight at 60°C 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/100 g body weight.[12]

Percentage inhibition of granuloma dry weight was calculated using formula:

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±standard error of mean were analyzed by one-way ANOVA followed by Dunnett's *post-hoc* test and p≤0.05 was considered significant.

Table 1: Effect of various treatments on carrageenan-induced paw edema.

Time after carrageenan injection	Control	Aspirin		Rifampicin		Isoniazid	
		Paw edema in ml (mean±SEM)	Percentage inhibition	Paw edema in ml (mean±SEM)	Percentage inhibition	Paw edema in ml (mean±SEM)	Percentage inhibition
½ hr	0.38±0.01	0.20±0.01**	47.37	0.21±0.01**	44.73	0.24±0.01**	36.84
1 hr	0.54±0.01	0.24±0.01**	55.55	0.23±0.01**	57.40	0.29±0.01**	46.29
3 hrs	0.86±0.01	0.26±0.01**	69.76	0.25±0.01**	70.93	0.31±0.01**	63.95
4 hrs	0.98±0.02	0.20±0.02**	79.60	0.21±0.02**	78.57	0.24±0.01**	75.51
5 hrs	0.92±0.02	0.12±0.01**	86.95	0.13±0.02**	85.86	0.17±0.02**	81.52

Post-hoc analysis by Dunnett's test: **$p<0.01$, SEM: Standard error of mean

RESULTS

Acute studies

As expected, aspirin significantly ($p<0.01$) reduced paw edema as compared to the control throughout the observation period. Similarly, rifampicin and isoniazid also showed significant anti-inflammatory activity compared to vehicle treated groups (Table 1).

Subacute studies

The mean dry weight of 10 days old granuloma, expressed as mg percent body weights, in control group was 40.67, while aspirin (200 mg/kg) treated group it was significantly decreased ($p<0.01$) with the mean value of 25.00±0.26 and percentage inhibition of 38.53%. Similarly, rifampicin (54 mg/kg) and isoniazid (27 mg/kg) treated group exhibited decreased granuloma weight ($p<0.01$) with mean value of 25.00±0.44, 26.17±0.47 with percentage inhibition of 38.53% and 35.65% (Table 2).

Further, mean granuloma dry weight of rifampicin and isoniazid group was compared with the mean granuloma dry weight of aspirin group. It was found that, there was no significant difference ($p>0.05$) between them indicating that anti-inflammatory activity of rifampicin and isoniazid was comparable to aspirin in subacute study (Table 2).

The anti-inflammatory activity of rifampicin and isoniazid as observed in both, acute and subacute studies were further confirmed by histopathological studies. The sections of granulation tissues when stained with hematoxylin and eosin showed abundant fibrous tissue in the control group, while revealed reduced number of fibroblasts, decreased collagen content and fibrous tissue in aspirin, rifampicin, and isoniazid treated groups (Figure 1).

DISCUSSION

Results of the present study clearly indicate that rifampicin, isoniazid used in the study showed significant anti-inflammatory activity when compared with control in acute as well as subacute models of inflammation.

Table 2: Effect of various treatments on granuloma dry weight.

Drug treatment	Mean granuloma dry weight mg/100 g body weight (mean±SEM)	Percentage inhibition
Control	40.67	
Aspirin	25.00±0.26**	38.53
Rifampicin	25.00±0.44**	38.53
Isoniazid	26.17±0.47**	35.65

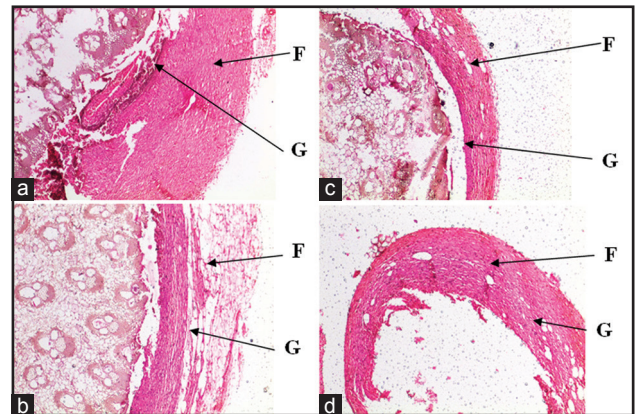*Post-hoc* analysis by Dunnett's test: **$p<0.01$, SEM: Standard error of mean

Figure 1: Photomicrographs of granulation tissue (H and E stain, ×10). As compared to control, markedly decreased granulation tissue, collagen content and fibroblast number in aspirin, rifampicin and isoniazid treated groups. F: Fibrous tissue, G: Granulation tissue, (a) Control, (b) aspirin, (c) rifampicin, (d) isoniazid.

Observations of the study are in agreement with the earlier reports stating that rifampicin, isoniazid may have anti-inflammatory activity,[5-8] while disagree with some earlier studies wherein, these drugs have been reported to possess pro-inflammatory activity.[10,11]

Anti-inflammatory activity of rifampicin can be attributed to its potential to block nuclear factor-κB (NF-κB) activation by TNF, which could provide a mechanism for immunosuppressive properties of these drugs. They bind

to DNA and block NF-κB activation during NF-κB gene transactivation process.[5]

Rifampicin has also been shown to inhibit activation of IL-2 promoter-reporter gene construct activated by ionomycin in Jurkat cells and may have steroid like activities.[5] Further, it binds to and activates the glucocorticoid receptor potentially leading to pharmacological glucocorticoid-like effects such as host immunosuppression.[8] It has also been described as a scavenger of reactive species[6] and inhibit PGE2 expression.[7] All of them may contribute to its anti-inflammatory activity.

Similarly, isoniazid has been shown to suppress IL-1 production in *in vitro* studies[9] suggesting both of these could possess anti-inflammatory activity.

Pathogenesis of TB involves a combination of the immune and inflammatory process. Since rifampicin and isoniazid have significant anti-inflammatory activity they can be used to reduce complications like pleurisy, pleural fibrosis, ureteral strictures, stricture of fallopian tubes leading to infertility, chronic pericarditis with thickening of pericardium and fibrosis, TB meningitis leading to fibrosis and its complications.[3] Since they have anti-inflammatory activity and reduce complications of disease daily dosing of these drugs may be more useful rather than thrice weekly dosing as in Revised National TB Control Programme, but these speculations should be confirmed clinically.

CONCLUSION

Rifampicin, isoniazid has shown significant anti-inflammatory activity in acute and subacute models of inflammation.

REFERENCES

1. Kumar V, Abbas AK, Fausto N. Robbins and Cotran Pathologic Basis of Diseases. 8th Edition. Philadelphia: Elsevier Publishers; 2010.
2. Park K. Park's Textbook of Preventive and Social Medicine. 18th Edition. Jabalpur: M/s Banarasidas Bhanot Publishers; 2005.
3. Fauci A, Braunwald E, Kasper D, Hauser S, Longo D, Jameson J, et al. Harrison's Principles of Internal Medicine. 17th Edition. New York: McGraw Hill Publishers; 2008.
4. Brunton LL, Lazo JS, Parker KL. Goodman and Gilman's the Pharmacological Basis of Therapeutics. 12th Edition. New York: McGraw Hill Publishers; 2011.
5. Pahlevan AA, Wright DJ, Bradley L, Smith C, Foxwell BM. Potential of rifamides to inhibit TNF-induced NF-kappaB activation. J Antimicrob Chemother. 2002;49(3):531-4.
6. dos Santos Fde J, Ximenes VF, da Fonseca LM, de Faria Oliveira OM, Brunetti IL. Horseradish peroxidase-catalyzed oxidation of rifampicin: reaction rate enhancement by co-oxidation with anti-inflammatory drugs. Biol Pharm Bull. 2005;28(10):1822-6.
7. Yuhas Y, Azoulay-Alfaguter I, Berent E, Ashkenazi S. Rifampin inhibits prostaglandin E2 production and arachidonic acid release in human alveolar epithelial cells. Antimicrob Agents Chemother. 2007;51(12):4225-30.
8. Mlambo G, Sigola LB. Rifampicin and dexamethasone have similar effects on macrophage phagocytosis of zymosan, but differ in their effects on nitrate and TNF-α production. Int Immunopharmacol. 2003;3(4):513-22.
9. Kucharz EJ, Sierakowski SJ. Studies on immunomodulatory properties of isoniazid: v. Influence of isoniazid on secretion of interleukin-1. J Hyg Epidemiol Microbiol Immunol. 1992;36(1):119-22.
10. Tentori L, Graziani G, Porcelli SA, Sugita M, Brenner MB, Madaio R, et al. Rifampin increases cytokine-induced expression of the CD1b molecule in human peripheral blood monocytes. Antimicrob Agents Chemother. 1998;42(3):550-4.
11. Yuhas Y, Berent E, Ovadiah H, Azoulay I, Ashkenazi S. Rifampin augments cytokine-induced nitric oxide production in human alveolar epithelial cells. Antimicrob Agents Chemother. 2006;50(1):396-8.
12. Turner RA. Screening Methods in Pharmacology. New York and London: Academic Press Inc.; 1965.