RESEARCH ARTICLE

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Effect of Rifampicin on Acute and Subacute Inflammation in Male Wistar Rats - An Experimental Study

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ABSTRACT
Tuberculosis is associated with significant inflammation and fibrosis resulting in complications like pleural fibrosis, ureteral strictures, stricture of fallopian tubes leading to infertility, chronic pericarditis with thickening of pericardium and fibrosis, tubercular meningitis leading to fibrosis etc. Rifampicin when given in these conditions, by virtue of their anti-inflammatory activity may reduce complications of the disease. Based on the controversial reports on influence of rifampicin on inflammation, rifampicn was investigated for its anti-inflammatory activity in both acute (carrageenan induced rat paw edema) and subacute (foreign body induced granuloma) models of inflammation using male Wistar rats. Rifampicin when compared with control showed significant inhibition ($p<0.01$) of rat paw edema in acute model and granuloma dry weight in subacute model of inflammation, indicating significant anti-inflammatory activity. Histopathological examination of grass pith revealed markedly reduced fibroblasts and collagen in rifampicin which was similar to aspirin when compared to control. These results clearly indicate that rifampicin has anti-inflammatory property.

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]

Tuberculosis 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 global burden.[2] The Pathogenesis of tuberculosis 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, tubercular meningitis leading to fibrosis and its complications. Clinical trials have demonstrated that patients given adjunctive glucocorticoid may have benefit. But glucocorticoids are invariably associated with various adverse effects on chronic use, [3] so search for newer anti-inflammatory drugs continues.

In case of inflammation caused by infection it needs treatment not only with anti-infective / antimicrobial but also anti-inflammatory agents. In case, antimicrobials posses anti-inflammatory activity they would be able to control not only infection but also inflammation and its sequelae.

Interestingly, certain studies have shown that rifampicin suppresses inflammatory mediators like tumor necrosis factor α (TNFα) ,[4] interleukin -2 (IL-2) production,[4] reactive oxygen species,[5] PGE2 expression.[6] Also it has been reported to activate human glucocorticoid receptor,[7] suggesting that it could possess anti-inflammatory activity.

However some studies have shown that rifampicin may have pro inflammatory activity by increasing CD1b expression [8] and nitric oxide (NO)production.[9]

Current treatment for tuberculosis include 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. [3]

In view of controversial reports of rifampicin on inflammation and paucity of anti-inflammatory studies the present study was planned to evaluate the effect of rifampicin on acute and subacute models of 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 1% gum acacia suspension as vehicle (in aspirin group) and 54 mg/kg of rifampicin (in rifampicin 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 rifampicin 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.[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 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, 3, 4 and 5 h. The difference between 0 hour and subsequent reading was taken as actual edema volume.

The percentage inhibition of edema was calculated using formula.

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

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 1% gum acacia suspension as vehicle once daily (in aspirin group) and 54 mg/kg of rifampicin once daily (in rifampicin 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. [10]

Percentage inhibition of granuloma dry weight was calculated using formula.

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

STATISTICAL ANALYSIS:

Data expressed as mean ± SEM were analyze 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 rifampicin also showed significant anti-inflammatory activity compared to vehicle treated groups. (Table 1)

Time after carrageenan injection	Control	Aspirin		Rifampicin	
	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 *
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 *

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

*P < 0.01 as compared to control

SUBACUTE STUDIES

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

Control	Aspirin		Rifampicin	
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*

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

*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 rifampicin treated groups indicating their anti-inflammatory action.

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

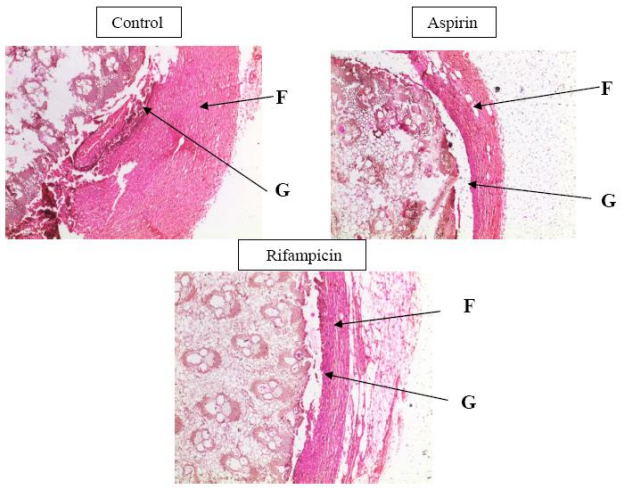

Figure 1 showing photomicrographs of granulation tissue (H & E stain 10x)
F denotes fibroblasts and G denotes granulation tissue.

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

DISCUSSION

As mentioned in the introduction, the present study was planned to investigate the influence of rifampicin on acute as well as subacute inflammation in male Wistar rats.

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

These observations of the study are in agreement with the earlier reports stating that rifampicin may have anti-inflammatory activity[4,5,6,7], while, disagree with some earlier studies wherein, rifampicin have been reported to possess pro-inflammatory activity.[8,9]

Anti inflammatory activity of rifampicin can be attributed to its potential to block 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.[4]

Rifampicin has also been shown to inhibit activation of IL-2 promotor-reportor gene construct activated by ionomycin in Jurkat cells and may have steroid like activities[4]. Further it binds to and activates the glucocorticoid receptor potentially leading to pharmacological glucocorticoid like effects such as host immunosuppression.[7] It has also been described as a scavenger of reactive species [5] and inhibit PGE2 Expression.[6] All of them may contribute to its anti-inflammatory activity.

Pathogenesis of tuberculosis involves a combination of immune and inflammatory process. Since rifampicin, has significant anti-inflammatory activity it 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, tubercular meningitis leading to fibrosis and its complications.[3]

CONCLUSION

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

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