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Effect of anti-inflammatory activity of ranolazine in rat model of inflammation

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***Background & objectives*: Inflammatory processes are a recognized feature of atherosclerotic lesions. Ranolazine inhibits the inflammatory markers such as C-reactive protein, interleukins-1 and -6 and tumour necrosis factor-alpha. The present study was planned to evaluate the effect of anti-inflammatory activity of ranolazine in acute and sub-acute models of inflammation in rats and compare the same with that of control (gum acacia 1%) and aspirin (standard anti-inflammatory drug).**

***Methods*: Adult male Wistar rats (150-180 g) were used for the study. They were divided into three groups (n=6). One per cent gum acacia (control), aspirin (200 mg/kg body weight) and ranolazine (180 mg/kg body weight) were given orally. Acute inflammation was induced by injecting carrageenan in the left hind paw. Paw oedema volume and percentage inhibition were measured. Subacute inflammation was induced by implanting foreign bodies subcutaneously. Percentage inhibition of granuloma dry weight and haematoxylin and eosin stained sections of granulation tissue were studied.**

***Results*: In acute and subacute model study, ranolazine significantly (*P*<0.01) decreased the paw oedema volume and granuloma dry weight as compared to control and it was comparable to that of aspirin and histopathological sections showed a decrease in granulation tissue formation as compared to control.**

***Interpretation & conclusions*: Ranolazine demonstrated significant anti-inflammatory activity in acute and subacute models of inflammation and needs further evaluation for its use in reducing atherosclerosis.**

Key words Acute model - aspirin - atherosclerosis inflammation - ranolazine - subacute model

Inflammation is involved in the pathogenesis of atherosclerosis which is one of the major causes of coronary artery disease (CAD)[1]. The inflammatory response involved in acute coronary events could be related with early and late post-ACS (acute coronary syndrome) adverse cardiac events[2,3]. Inflammation is also involved in the process of post-myocardial infarction (MI) healing process. The inflammation may have deleterious effects on myocardial cells, especially in case of an exuberant inflammatory response. Ischaemic myocardium produces certain important cytokines such as interferon gamma, tumour necrosis factor-alpha (TNF-α) and interleukin-1 (IL-1). These cytokines, in turn, stimulate the production of IL-6 which triggers the inflammatory response and the platelet aggregation[4]. Inflammation directly interferes with the myocardial contractility, the vascular endothelial function and recruitment of other inflammatory mediators such as C-reactive protein (CRP)[5]. This suggests that CRP and other inflammatory markers can be considered as direct inflammatory promoters with pro-atherogenic and pro-thrombotic properties[6]. Further, it may be assumed that drugs which decrease CRP and other inflammatory mediators have additional benefits in CAD patients. It has been shown that metabolic modulator, an anti-anginal drug, ranolazine improves endothelial function by altering the inflammatory mediators[6,7]. Ranolazine has also been shown to decrease inflammatory mediators such as IL-1 and TNF-α and increase anti-inflammatory peroxisome proliferator-activated receptor gamma[6]. The present study was undertaken to evaluate the effect of oral ranolazine on acute and subacute models of inflammation in male Wistar rats and compare these effects with that of control (1% gum acacia) and standard anti inflammatory drug, aspirin.

Material & Methods

This experimental study was conducted in the department of Pharmacology, Jawaharlal Nehru Medical College (JNMC), Belagavi, Karnataka, India. Adult male Wistar rats (weighing between 150 and 180 g body weight) were obtained from the central animal house of JNMC. Animals were housed under standard laboratory conditions and acclimatized to 12 h light/dark cycle for 10 days prior to the day of experimentation. Animals had free access to food (standard rat chow pellet) and water *ad libitum.* The study was approved by the institutional animal ethics committee.

Male Wistar rats were randomly divided into control (gum acacia), standard (aspirin) and treatment (ranolazine) groups (n=6 in each). The drugs used were one per cent gum acacia orally, aspirin (200 mg/kg body weight of rat equivalent to 2222 mg of clinical dose orally) and ranolazine (180 mg/kg body weight of rat equivalent to 2000 mg of clinical dose orally)[8,9].

Acute inflammation by carrageenan-induced rat paw oedema: Acute inflammation was induced by injecting 0.05 ml of one per cent carrageenan in sub plantar region of the left hind paw. A mark was made on the leg at the malleolus to facilitate uniform dipping at subsequent readings. The paw volume was measured with the help of plethysmograph (Instrumental and Chemical Private Limited, Ambala) by mercury displacement method using plethysmograph at zero hour (immediately after injecting carrageenan). The same procedure was repeated at 0.5, 1, 3, 4 and 5 h. The difference between zero hour and subsequent reading was taken as actual oedema volume[10,11]. The percentage inhibition of oedema in the various treated groups was calculated by using the following formula[10,11]:

Percentage inhibition of oedema = 1- mean increase in paw volume in treated group/mean increase in paw volume in control group × 100

Subacute inflammation by foreign body-induced granuloma method: Subacute inflammation was induced in all the groups after a washout period of 48 h. In overnight starved (with water *ad libitum*) rats, after clipping the hair in axilla and groin, two sterile cotton pellets weighing 10 mg and two sterile grass piths (25 mm×2 mm) were implanted subcutaneously, through a small incision, under light thiopentone anaesthesia. Wounds were sutured, and animals were caged individually after recovery from anaesthesia. Aseptic precautions were taken throughout the procedure. The treatment was started after implantation and was repeated every 24 h, regularly for 10 days. On the 11th day, the rats were sacrificed with an overdose of anaesthesia to remove cotton pellets and grass piths. The pellets, free from extraneous tissue were dried overnight at 60°C to note their dry weight. Net granuloma weight was calculated by subtracting initial weights of the cotton pellet (10 mg) from the weights noted. Mean granuloma dry weight for various groups was calculated and expressed as mg/100 g of body weight. The percentage inhibition of granuloma dry weight was calculated using the formula[12]:

Percentage inhibition granuloma dry weight = 1- dry weight of granuloma in treated group/dry weight of granuloma in control group × 100

The grass piths were preserved in 10 per cent formalin for haematoxylin and eosin (H&E) staining.

Statistical analysis: The results were analyzed using ANOVA followed by *post hoc* test of the Dunnet's and Bonferonni test.

Results

In acute model of carrageenan-induced inflammation, the mean paw oedema volumes for control group at ½, 1, 3, 4 and 5 h intervals, were 1.15±0.03, 1.31±0.01, 1.24±0.02, 1.26±0.02 and 1.16±0.02 ml, respectively (Table). The mean paw

Table. Effect of ranolazine treatment on carrageenan-induced paw oedema when compared with control and aspirin

Time after carrageenan injection (h)	Control	Aspirin		Ranolazine	
	Paw oedema volume (ml)	Paw oedema volume (ml)	Per cent inhibition	Paw oedema volume (ml)	Per cent inhibition
½	1.15±0.03	0.98±0.01**	54	1.10±0.01	29.15
1	1.31±0.01	1.08±0.02**	63.3	1.09±0.03**	39.07
3	1.24±0.02	0.98±0.01**	74.25	1.05±0.01**	49.32
4	1.26±0.02	0.85±0.03**	97.14	1.04±0.02**	71.07
5	1.16±0.02	0.87±0.02**	96.2	0.97±0.02**	88.25

Values shown as mean±SEM. ***P*<0.01 compared with control.

oedema volumes in aspirin (200 mg/kg) treated group were 0.98±0.01, 1.08±0.02, 0.98±0.01, 0.85±0.03 and 0.87±0.02 at ½, 1, 3, 4 and 5 h, respectively, with the calculated percentage inhibitions of 54, 63.3, 74.25, 97.14 and 96.2 per cent, respectively. Aspirin-treated group showed significant (*P*<0.01) inhibition of paw oedema volume when compared to control (Table). Ranolazine (180 mg/kg) showed no significant difference at ½ h but showed a significant inhibition (*P*<0.01) of paw oedema at 1, 3, 4 and 5 h, when compared to that of control. There was no significant difference when compared to aspirin treated group at ½ and 1, 3, 4 and 5 h (Table).

In subacute study, foreign-body-induced granuloma method, the mean dry weight of ten day old granuloma, expressed as mg percent (mg/100 g) body weight of rat in control group was 18.25±0.29. In aspirin (200 mg/kg) treated group, the mean dry weight of ten day old granuloma was significantly (*P*<0.01) decreased as compared to that of control with values 12.83±0.35 and percentage inhibition of 28.94 per cent. Similarly, ranolazine (180 mg/kg) treatment group exhibited significant (*P*<0.01) decrease in granuloma dry weight (*P*<0.01) with the mean values of 12.62±0.55 and percentage inhibition of 29.15 per cent, respectively, when compared to control. There was no significant difference in mean granuloma dry weights of ranolazine treatment groups when compared to that of aspirin treated group.

In the H and E staining (10× & 40×), sections of granulation tissue showed abundant fibrous, tissue with dense inflammatory infiltrates in the control group, while it showed sparse inflammatory infiltrate with reduced number of fibroblasts, fibrous tissue and decreased collagen content in aspirin and ranolazine treated groups when compared to that of control (Figure).

Discussion

In this study acute inflammation was induced by injecting carrageenan in rat hind paw and anti-inflammatory action was assessed by measuring the paw oedema volume. Ranolazine, treatment showed significant inhibition of paw oedema as compared to control. When these drugs were compared to aspirin, the effect of ranolazine was comparable to aspirin. The anti-inflammatory effect of ranolazine on acute inflammation in the present study can be explained on the basis of its effect on one of the inflammatory mediators. Ranolazine is reported to decrease CRP in patients with stable CAD[13,14]. Subacute inflammation was induced by implanting cotton pellet and grass pith subcutaneously. Ranolazine showed a significant decrease in the granuloma dry weight when compared to control. There was no significant difference in granulation tissue dry weight between aspirin and ranolazine treatment group.

The histopathology of the granulation tissue revealed a decrease in cellular infiltration in ranolazine treated group as compared to that of control. This can be attributed to a decrease in levels of inflammatory mediators such as TNFα, CRP and IL6 which, in turn, could have decreased the cellular infiltration to the site of inflammation[15]. This decrease in cytokines level (like TNFα) could be because of decrease in fibroblasts which is known to increase the level of this cytokine[16]. This was associated with a decrease in number of fibroblasts in the subacute study.

The anti-anginal drug, ranolazine has a cardioprotective action by blocking the sodium current that facilitates calcium entry through the sodium-calcium exchanger[7,17]. One of the major events involved in angina, myocardial infarction (MI) and atherosclerosis is inflammation[13,18]. Inflammation is also involved in the restenosis processes in those patients who have

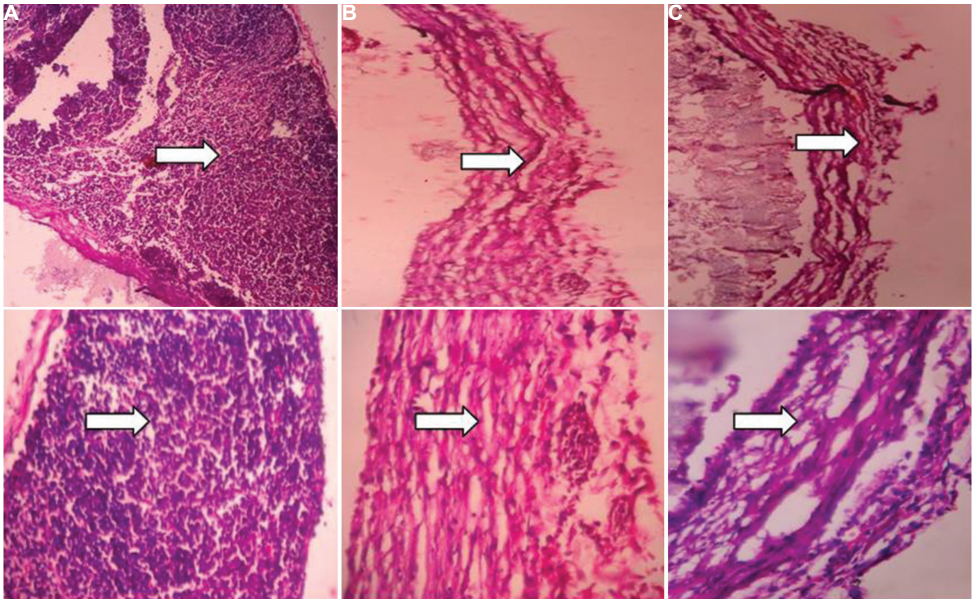

Figure. Photomicrographs of granulation tissue of aspirin and ranolazine when compared with control (H&E staining; upper panels, 10×; lower panels, 40×). Markedly reduced inflammatory mediators, fibroblasts, fibrous tissue and collagen were seen in ranolazine treatment group when compared to that of control. Arrow indicates increased fibroblasts, thick fibrous tissue and dense inflammatory infiltrate in the control group (**A**) and reduced number of fibroblasts, scanty collagen tissue, decreased thickness of fibrous tissue and sparse inflammatory infiltrate in aspirin (**B**) and ranolazine group (**C**).

undergone percutaneous coronary intervention (PCI)[19]. The results of the present study revealed that ranolazine could be exhibiting multiple mechanisms in CAD, *i.e.* anti-inflammatory in addition to their pharmacological actions. This drug may be useful in reducing the inflammation involved in atherosclerosis and also post-ischaemic complications such as reinfarction and infarct extension, and also in preventing cardiovascular events like restenosis after PCI.

The study had limitations. Chronic anti-inflammatory effect of ranolazine was not studied. This would have given some information on long term therapeutic benefit in CVD patients. The effect of the drug on the inflammatory mediators such as IL, TNF-alpha and CRP has also not been investigated.

In conclusion, this experimental study was conducted to know the effect of anti-anginal drugs ranolazine on inflammation in both acute (carrageenan-induced rat paw oedema) and subacute (foreign-body-induced granuloma) models of inflammation in male Wistar rats. Ranolazine showed significant inhibition of rat paw oedema in acute model and granuloma dry weight, in subacute models of inflammation when compared to control. The anti-inflammatory effect of ranolazine was comparable to aspirin in acute and subacute models of inflammation. Drugs that reduce inflammation may have added benefits in the treatment of cardiovascular disease and also will be useful in reducing the post ischaemic complications. The role of ranolazine as anti-inflammatory agent warrants further investigation and has to be confirmed by clinical trials.

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***Conflicts of Interest*:** None.

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