

Effect of trimetazidine on acute and sub-acute models of inflammation in male wistar rats

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ABSTRACT

Background: Coronary artery disease (CAD) is the leading cause of morbidity and mortality. Pathogenesis involved in angina pectoris and acute coronary syndrome (ACS) is atherosclerosis. Inflammation drives all phases of atherosclerosis, including initiation, progression of CAD and its complications. Drugs that reduce inflammation may have added benefit in the treatment of cardiovascular disease. Studies have shown that trimetazidine inhibits the inflammatory markers like C- reactive protein, interleukins (IL-1, IL-6) and tumor necrosis factor alpha. Objective of the study was to investigate the effect of trimetazidine on acute and sub-acute models of inflammation in adult male Wistar rats.

Methods: Adult male Wistar rats (150 to 180 mg) were randomly divided into three groups (n=6 each) viz. control (vehicle), aspirin (200 mg / kg body weight of rat) and trimetazidine (5.4 mg / kg body weight of rat). Anti-inflammatory effects of these drugs were studied in acute (carrageenan induced rat paw oedema) and sub-acute (cotton pellet induced granulation tissue and histopathological examination of granulation tissue surrounding grass piths) models of inflammation. Data was expressed as mean±SEM and analysed by one way ANOVA followed by Dunnet's and Bonferroni test. p<0.05 was considered as significant.

Results: Trimetazidine group showed significant (p <0.05) anti-inflammatory effect both in acute and sub-acute models of inflammation which was comparable to that of aspirin group.

Conclusions: Results of the study indicate that trimetazidine can reduce the complications of atherosclerosis which involves inflammation as the major steps in its pathogenesis.

Keywords: Trimetazidine, Aspirin, Acute, Sub acute, Inflammation

INTRODUCTION

Coronary artery disease (CAD) is the leading cause of morbidity and mortality in the world with more than 1.4 million of new diagnosed cases per year.^{1,2} Atherosclerosis is the underlying pathogenesis of CAD leading to, angina pectoris and acute coronary syndrome (ACS).³

The inflammatory response involved in acute coronary events is related with early and late post-ACS major adverse cardiac events.⁴

Inflammation is also one of the main factors in the post-myocardial infarction healing process. The inflammation in itself may paradoxically have deleterious effects on myocardial cells, especially in case of an exuberant

inflammatory response. Ischemic myocardium produces certain important cytokines like tumour necrosis factor-alpha (TNF- α), interleukin (IL-1 and IL-6) etc. Inflammation directly interferes with the myocardial contractility, vascular endothelial function and recruitment of other inflammatory mediator like C reactive protein (CRP).⁵ Interferon gamma (IF- γ), IL-1 and TNF-alpha also stimulate the production of IL-6 which triggers the inflammatory response and platelet aggregation.⁶ Coronary angiography, percutaneous coronary intervention (PCI) and coronary artery bypass surgery (CABG) are the modalities for the detection and the treatment of CAD. Role of inflammation in the development of restenosis after PCI and CABG has also been reported.¹³

The drugs that reduce inflammation may have added benefits in the management of cardiovascular disease outcome. Interestingly certain studies have reported that metabolic modulator trimetazidine, which is an anti-anginal drug, inhibits the inflammatory markers like C reactive protein(CRP), nitric oxide products (nitrite and nitrates), interleukins (IL-1, IL-6) and TNF alpha.⁷⁻⁹ But another study suggested that trimetazidine showed no significant reduction in the levels of IL-8, TNF- α , complement 3 and 5 and CRP.¹⁰ Review of literature revealed paucity of studies regarding anti-inflammatory activity of the above mentioned drug in animals. In view of controversial reports about trimetazidine on inflammation as well as paucity of information regarding the effect of this antianginal drug on inflammation, the present study is planned to evaluate the effect of oral trimetazidine on acute and sub-acute models of inflammation in male wistar rats.

METHODS

Present experimental study involved the use of adult male Wistar rats weighing between 150-200 g body weight. Animals were obtained from central animal house of J. N. Medical College, Belagavi, India. Animals were housed under standard laboratory conditions and acclimatized to 12-hrs light/dark cycle for 10 days prior to the day of experimentation. They had free access to food (standard rat chow pellet- amrut brand) and water *ad libitum*. The drugs required for this experiment were obtained from the hospital pharmacy.

All the procedures were performed in accordance with the guidelines for institutional animal ethics committee constituted as per the recommendations of committee for the purpose of control and supervision of experiments on animals (CPCSEA).

The adult human clinical doses of the drugs were converted into rat equivalent doses with the help of converting table.¹⁴ Male wistar rats were randomly divided into control, standard and treatment groups (n=6 in each group).The drugs (with their adult therapeutic dose in parenthesis) used were 1% gum acacia orally

(control), aspirin (200 mg/kg Body weight of rat equivalent to 2222 mg of clinical dose orally) and trimetazidine (5.4 mg/Kg Body weight of rat equivalent to 60mg of clinical dose orally).^{11,12,14,15}

Acute inflammation was induced by injecting 0.05 ml of 1% carrageenan in sub plantar region of 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 by mercury displacement method at zero hour (immediately after injecting carrageenan). The same procedure was repeated at 0.5,1,3,4 and 5 hour. The difference between 0 hour and subsequent reading was taken as actual oedema volume.¹⁶ The percentage inhibition of oedema in the treated groups were then calculated by using the formula:¹⁶

$$\text{Percentage Inhibition of oedema} = 1 - \frac{\text{Mean increase in paw volume in treated group}}{\text{Mean increase in paw volume in control group}} \times 100$$

Sub-acute inflammation was induced in the same above groups after a wash out period of 48 hours. In overnight starved (with water *ad lib*) rats, after clipping the hair in axilla and groin, two sterile cotton pellets weighing 10 mg and two sterile grass piths (25x 3 mm) were implanted subcutaneously, through a small incision, under light thiopentone anaesthesia. Wounds were then 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 twenty four hours, regularly for ten days. On eleventh day the rats were sacrificed with an overdose of thiopentone 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 cotton pellet (10 mg) from the weights noted. Mean granuloma dry weight and percentage inhibition for aspirin and trimetazidine treatment groups was calculated and expressed as mg/100 gm of body weight. The percentage inhibition of granuloma dry weight was calculated using the formula:¹⁶

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

The grass piths were preserved in 10% formalin for haematoxylin and eosin staining.¹⁶ The results were expressed as mean±SEM and analyzed using ANOVA followed by post hoc test of Dunnett's Bonferonni test. p<0.05 was considered as statistically significant.

RESULTS

The mean paw oedema volumes in ml in trimetazidine (5.4 mg/kg) treated group at ½ hour, 1 hour, 3 hours, 4 hours and 5 hours were 1.15±0.05, 1.20±0.04, 1.07±0.02, 1.01±0.02 and 0.94±0.01 respectively (Table 1, Figure 1),

with the calculated percentage inhibitions of 12.5%, 43.75%, 70.76%, 83.72% and 95.60% respectively (Table 1, Figure 3). Inhibition of paw oedema volume in trimetazidine treatment group showed no significant difference at ½ hour, but there was statistically significant (p <0.05) at 1h and (p <0.01) at 3 hours, 4 hours, 5 hours, when compared to that control (Table 1, Figure 1). There was significant difference (p <0.05) when the trimetazidine treated group was compared to that of aspirin at ½ and 1 hour, however, there was no significant difference at 3 hours, 4 hours and 5 hours. (Table 2, Figure 2).

Table 1: Effect of aspirin and trimetazidine treatment on carrageenan induced paw oedema in comparison with control.

Time after carrageenan injection	Control	Aspirin	Trimetazidine	Time after carrageenan injection	Control
	Paw oedema; ml (Mean ±SEM)	Paw oedema; ml (Mean ±SEM)	Percentage inhibition	Paw oedema; ml (Mean ±SEM)	Percentage inhibition
½ hour	1.15±0.02	0.94±0.02**	50%	1.15±0.05	12.5%
1 hour	1.35±0.03	1.04±0.02**	60.3%	1.20±0.04 *	43.75%
3 hours	1.29±0.03	0.97±0.01**	73.18 %	1.07±0.02**	70.76%
4 hours	1.20±0.03	0.89±0.01**	96.14%	1.01±0.02**	83.72%
5 hours	1.14±0.01	0.89±0.02**	95.62%	0.94±0.01**	95.60%

Post hoc analysis by Dunnett's test: * p<0.05, ** p<0.01 comparison with Control.

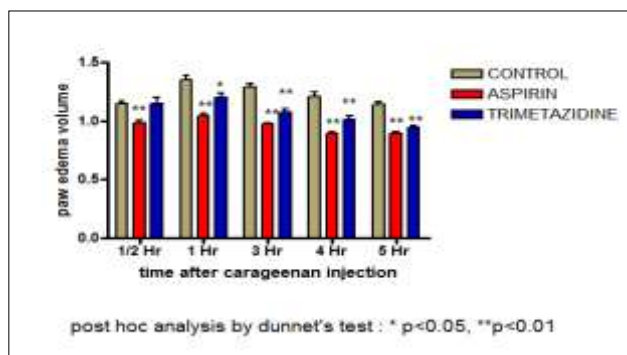


Figure 1: Paw oedema in millilitre (ml) after carrageenan injection in comparison with control.

The mean dry weight of ten day old granuloma, expressed as mg percent (mg/100 g) body weight of rat in control group was 18.15±0.15. 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 12.93±0.33 and percentage inhibition of 28.76%. Similarly, trimetazidine (5.4 mg/kg), exhibited statistically significant (p <0.01) decrease in granuloma dry weight with the mean values of 12.83±0.21 and percentage inhibition of 29.31% respectively when compared to control. (Table 3, Figure 4, 5) There was no significant (p >0.05) difference in mean granuloma dry weights of trimetazidine group when compared to that of aspirin (Table 3).

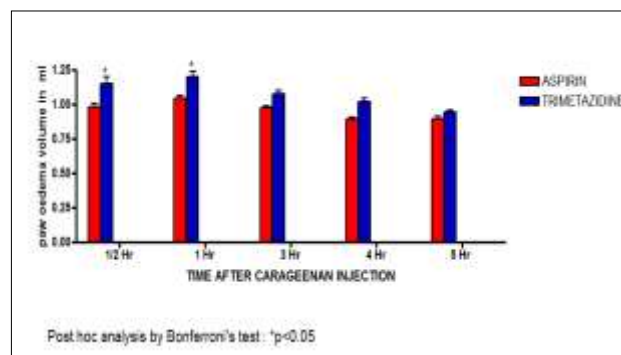


Figure 2: Paw oedema in millilitre (ml) after carrageenan injection in comparison with aspirin.

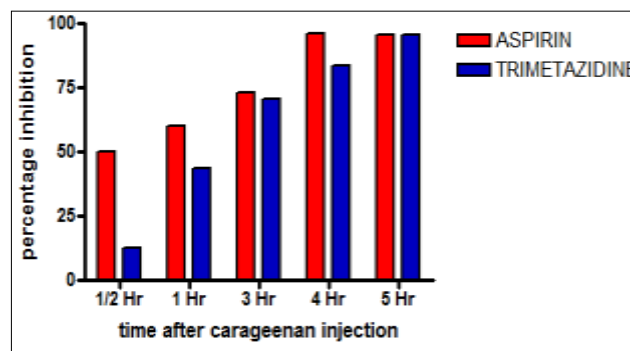


Figure 3: Percentage inhibition of paw oedema.

Table 2: Effect of trimetazidine treatment on carrageenan induced paw oedema in comparison with Aspirin

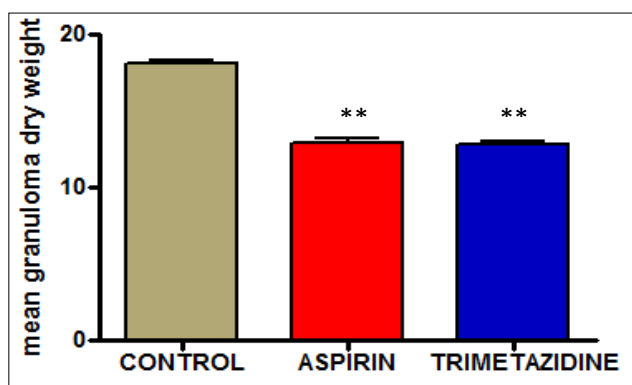
Time after carrageenan injection	Aspirin Paw oedema in ml (mean ± SEM)	Trimetazidine Paw oedema in ml (mean ± SEM)	ANOVA result		Post-hoc analysis by bonferroni's test
			F 2,10	p- value	
½ hour	0.94±0.02	1.15±0.05*	8.259	<0.0001	p <0.05,
1hour	1.04±0.02	1.20±0.04*	9.140	<0.0001	P <0.05
3hour	0.97±0.01	1.07±0.02	16.81	<0.0001	p >0.05
4hour	0.89±0.01	1.01±0.02	15.17	<0.0001	p >0.05,
5hour	0.89±0.02	0.94±0.01	22.28	<0.0001	P >0.05

Post hoc analysis by Bonferroni's Test: * p< 0.05 comparison with aspirin

Table 3: Effect of trimetazidine treatment on granuloma dry weight when compared with control.

Drug treatment (Oral Doses in mg/Kg)	Mean granuloma dry weight mg/100 gm body weight (mean±SEM)	Percentage inhibition
Control	18.15±0.1529	-
Aspirin (200)	12.93±0.3365 **	28.76%
Trimetazidine (5.4)	12.83±0.2148 **	29.31%

Post hoc analysis by Dunnet's test: ** p<0.01 when compared with control.



Post hoc analysis of dunnet's ** p<0.01 when compared with control

Figure 4: Mean granuloma dry weight in comparison with control.

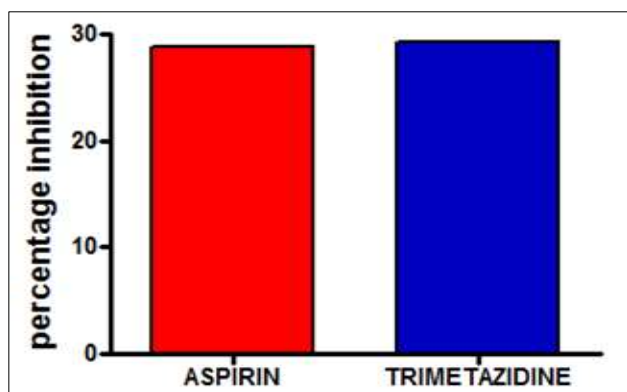
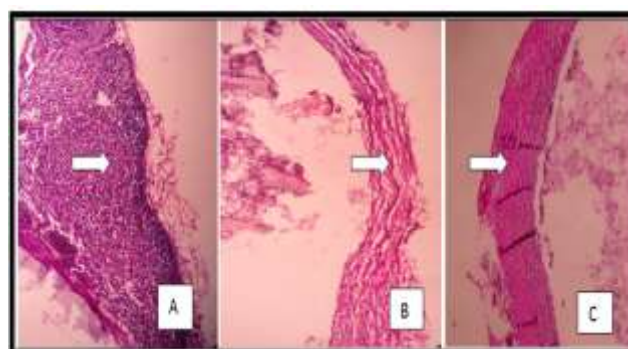


Figure 5: Percentage inhibition of granuloma dry weight.

Histopathological examination of granulation tissue (Figure 6)

The anti-inflammatory effect of trimetazidine as observed in both acute and sub-acute studies were further confirmed by histopathological studies. The sections of granulation tissues when stained with haematoxylin and eosin showed increased fibroblasts, thick fibrous tissue and dense inflammatory infiltrate in the control group (A) whereas aspirin (B) and trimetazidine (C) treated groups revealed reduced number of fibroblasts, scanty collagen tissue and decreased thickness of fibrous tissue and sparse inflammatory infiltrate.



Sections of granulation tissues stained with haematoxylin and eosin. Arrow indicates increased fibroblasts, thick fibrous tissue and dense inflammatory infiltrate in the control group; (A) and in Aspirin; (B) and Trimetazidine; (C) treated groups arrow indicates reduced number of fibroblasts, scanty collagen tissue, decreased thickness of fibrous tissue and sparse inflammatory infiltrate.

Figure 6: Photomicrographs of granulation tissue. (H and E-stain), 10X.

DISCUSSION

The present study was planned to investigate the effects of trimetazidine on acute and sub-acute inflammation in male wistar rats. The results of the present study reveal anti-inflammatory effect of trimetazidine on acute and sub-acute inflammatory models.

In acute study, inflammation was induced by injecting carrageenan in rat hind paw and anti-inflammatory action was assessed by measuring the paw oedema volume. Trimetazidine showed significant inhibition of paw oedema volume as compared to that of control. When compared to aspirin, effect of trimetazidine was comparable to aspirin ($p > 0.05$).

Sub-acute inflammation was induced by implanting subcutaneously cotton pellet and grass pith. Granuloma dry weight and the histopathology of granulation tissue were studied. In cotton pellet induced granuloma method, trimetazidine, exhibited significant decrease in the granuloma dry weight when compared to that control. There was no statistical significant difference in granulation tissue dry weight between aspirin and trimetazidine. The H and E stained granulation tissue, showed dense inflammation with abundant fibrous tissue in the control group, while it revealed reduced number of fibroblasts, fibrous tissue and decreased granulation tissue, collagen content with sparse inflammation in aspirin and trimetazidine.

The metabolic modulator, trimetazidine also known as partial fatty acid oxidation (pFOX) inhibitor is used in the management of angina by virtue of their mechanism affecting the various events of angina. Because metabolism shifts the oxidation of fatty acids in ischemic myocardium, the oxygen requirement per unit of ATP produced increases. One of the major events involved in angina, myocardial infarction and atherosclerosis (CAD) is inflammation.^{11,12} In addition, inflammation is also involved in the restenosis processes in the patients who have undergone PCI.¹³ Therefore, it can be speculated that drugs affecting the processes of inflammation could be of beneficial effect in the management of cardiovascular events.

The anti-inflammatory effect of trimetazidine on acute inflammation in the present study can be explained on the basis of their effect on one of the inflammatory mediators. The anti-inflammatory effect of trimetazidine can be attributed to decrease the levels of TNF α , NO products and CRP.⁷⁻¹⁰ Though the results of the present study do not point at actual mechanism of trimetazidine on inflammation, this effect can be attributed to the effect of trimetazidine to decrease TNF α as reported in previous studies.⁷⁻¹⁰

This study shows that in addition to anti anginal activity, trimetazidine may also possess anti-inflammatory properties that could be of additional benefit in the

treatment of atherosclerosis and their complications like reinfarction and infarct extension, and also in preventing cardiovascular events like restenosis after PCI. However these speculations need to be confirmed clinically. Clearly, the role of trimetazidine as anti-inflammatory agents is an area that warrants further investigation and has to be confirmed by clinical trials.

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Conflict of interest: None declared

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