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Research Article

Effect of ticlopidine on acute and subacute models of inflammation in male wistar rats: an experimental study

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ABSTRACT

Background: Atherosclerosis and its complications remains the major cause of death and premature disability. Atherogenesis involves elements of inflammation, a process that now provides a unifying theme in the pathogenesis of the disease. Anti-platelet drugs are currently used in the treatment of atherosclerosis and its complications. Our study evaluated the influence of ticlopidine on acute and sub-acute models of inflammation in male Wistar rats.
Methods: Male wistar rats (150-200g) were divided into three groups i.e. control, Aspirin and ticlopidine (n=6 animals in each group). The effect of ticlopidine, administered orally, on inflammation was studied using acute (carrageenan induced rat paw edema) and sub-acute (cotton pellet granuloma and histopathological examination of grass piths) models. Experiment was conducted according to the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) guidelines. Analysis was done using one way ANOVA followed by Post Hoc Test of Dunnets. $P<0.05$ was considered as statistically significant.
Results: Ticlopidine showed significant inhibition of rat paw oedema in acute model ($P<0.01$) and granuloma dry weight, in subacute model of inflammation when compared to control ($P<0.01$). Histopathological examination of grass pith revealed markedly reduced fibroblasts, granulation tissue, fibrous tissue and collagen in ticlopidine group when compared to control.
Conclusions: Ticlopidine exhibited a significant anti-inflammatory activity in acute and sub-acute models of inflammation.

Keywords: Ticlopidine, Aspirin, Carrageenan, Inflammation

INTRODUCTION

Cardiovascular diseases remain the major cause of death and premature disability in developed societies. Current predictions estimate that by the year 2020 cardiovascular diseases, notably atherosclerosis and hypertension will become leading global causes of total disease burden.[1]

Atherogenesis involves elements of inflammation, a process that now provides a unifying theme in the pathogenesis of the disease.[2-4] Key inflammatory factors in atherothrombosis include activated endothelial cells, like inflammatory leucocytes, smooth muscle cells and platelets.[4]

Platelet activation leads to surface expression of P-selectin, which promotes the formation of platelet-leukocyte complexes, surface expression of CD-40 Ligand and also platelet itself releases various inflammatory mediators such as Platelet activating factor

(PAF), Platelet factor-4, RANTES (regulated upon activation normal T-cell expressed and secreted) and Tissue factor. Thus, drugs that simultaneously block thrombotic occlusion and reduce inflammation may have added benefits in the treatment of cardiovascular disease.[4]

Ticlopidine, an antiplatelet drug, is clinically used for the secondary prevention of transient ischemic attacks (TIA), ischemic stroke, and coronary heart diseases, which are closely associated with atherosclerosis. Several clinical studies of ticlopidine in the prevention of stroke have shown it to be an effective prophylactic agent not only in patients with previous transient cerebral ischemia but also in patients with a previous complete stroke.[5] Recently, studies revealed ticlopidine attenuates progression of atherosclerosis in apolipoprotein E and low density lipoprotein receptor double knockout (apoE/LDLR-/-) mice and improve the endothelial function in those mice.[6] The size of the atherosclerotic plaques and the number of macrophages were significantly reduced by ticlopidine treated mice as compared to their nontreated counterparts.[6]

Studies have shown that, inflammation drives all phases of atherosclerosis, including initiation, progression and thrombotic complications of the lesion.[3] In view of paucity of anti-inflammatory studies of ticlopidine, the present study was planned to evaluate the effect of ticlopidine on acute and sub-acute models of inflammation in male Wistar rats.

METHODS

Animals used: 18 male wistar rats (150-200 g) were used for present study. They were fed with standard pellet diet and water ad libitum. All animals were acclimatized 12:12 h light - dark cycle for one week before the experiment session. All experiments were done following the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals). The study was approved by Institutional Animal Ethical Committee of J. N. Medical College, Belagavi.

Adult male healthy Wistar rats weighing 150-200 g were obtained from the central animal house, J. N. Medical College, Belgavi 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. Aspirin was administered in the dose of 200 mg/kg body weight of rat, equivalent to 2222 mg of clinical dose orally.[7,8] Ticlopidine was administered in the dose of 45 mg/kg body weight equivalent to 500 mg of clinical dose orally.[7,8] Carrageenan (Sigma Co. St. Louis) was administered as a suspension in 1% warm normal saline given in the volume of 0.05 ml per rat paw.

Acute inflammation was produced by injecting carrageenan into one of the hind paws and sub-acute inflammation by randomly implanting a foreign body subcutaneously in axilla and groin as described below.

Carrageenan induced rat paw oedema

Rats were divided into three groups of six each. They were starved overnight with water ad libitum prior to the day of experiment. Group I (control) received 0.5ml of 1% gum acacia suspension, orally; group II (standard) received aspirin 200mg/kg orally in 1% gum acacia suspension and group III received ticlopidine 45 mg/kg orally in 1% gum acacia suspension.[7,8] Aspirin was taken as the standard anti-inflammatory drug.

Thirty minutes after aspirin and ticlopidine administration, 0.05ml of 1% w/v carrageenan suspension was injected into the sub-plantar region of left hind paw. A mark was put on the hind limb at the malleolus to facilitate uniform dipping at subsequent readings. The paw oedema volume in millilitres was measured with the help of a plethysmograph by mercury displacement method at zero hour i.e. immediately after injecting carrageenan. The same procedure was repeated at 0.5, 1, 3, 4 and 5 hours.[9] The percentage inhibition of oedema in the various treated groups was then calculated by using the formula.[9]

Percentage inhibition of edema = 1- Mean increase in paw volume in treated group/Mean increase in paw volume in control group X 100

Foreign body induced granuloma method

Rats were divided into three groups of six each. Under thiopentone anesthesia, each rat was implanted subcutaneously with two sterile cotton pellets weighing l0mg each and two sterile grass piths (25x2mm) through a small incision in all rats. Wounds were then sutured and animals were caged individually after recovery from anaesthesia. Aseptic precautions were taken throughout the experiment. The treatment was started on the day of implantation and was repeated every twenty-four hours, regularly, for ten days.[10]

On the eleventh day, the rats were sacrificed to remove the cotton pellets and grass piths. The grass piths were preserved in 10% formalin for histopathological studies. Sections were stained with haematoxylin and eosin, and the granulation tissue in each group was studied microscopically. The pellets, free from extraneous tissue, were dried overnight at 60°C to note their dry weight. Net granuloma formation was calculated by subtracting initial weight of cotton pellet (10mg) from the weights recorded. Mean granuloma dry weight for various groups was calculated and expressed as mg/100 gm body weight.[11,12] The percentage inhibition of granuloma dry weight was calculated using the formula.

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

Statistical analysis

The data for all the groups was expressed as Mean ±SEM and were analysed by one way ANOVA (Analysis of variance) followed by Dunnet's test using Graph pad prism software and P < 0.05 was considered statistically significant.

RESULTS

In the present study, ticlopidine in therapeutic equivalent dose was investigated for its possible anti-inflammatory effect, in acute and sub-acute models of inflammation.

Table 1: Effect of aspirin and ticlopidine treatment on carrageenan induced paw edema when compared with control group.

Time after carrageenan injection	Control Paw edema in ml (Mean ± SEM)	Aspirin		Ticlopidine		ANOVA Result
		Paw edema in ml (Mean ± SEM)	Percentage inhibition %	Paw edema in ml (Mean ± SEM)	Percentage inhibition %	P value
½ hr	1.167±0.04	1.033± 0.06	12	1.113± 0.03	5	>0.05
1 hr	0.85 ± 0.01	0.77± 0.02*	10	0.75± 0.01**	11.76	<0.006
3 hr	0.82± 0.02	0.34± 0.01**	58.53	0.35± 0.01**	57.31	<0.0001
4 hr	0.89 ± 0.01	0.30± 0.01**	66.29	0.29± 0.01**	67.41	<0.0001
5 hr	0.89 ± 0.01	0.25 ± 0.01**	71.9	0.26± 0.01**	70.78	<0.0001

Post hoc analysis by Dunnet's Test: *P<0.05; **P<0.01.

Table 2: Effect of aspirin and ticlopidine treatments on granuloma dry weight when compared with control group.

No.	Drug Treatment	Mean granuloma dry weight mg/100gm body weight (Mean ± SEM)	Percentage inhibition
1.	Control	22.83 ± 1.138	---
2.	Aspirin	15 ± 0.81**	34.29%
3.	Ticlopidine	11.83 ± 0.65**	48.18%

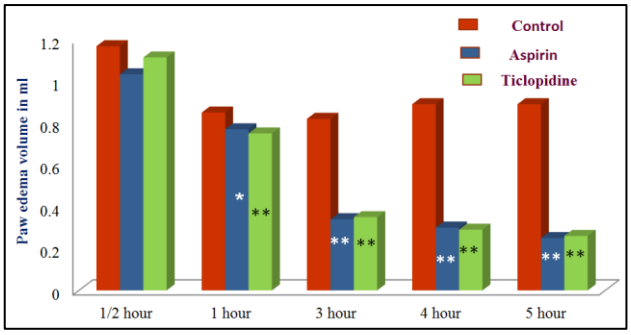
ANOVA: P < 0.0001; Post hoc analysis by Dunnet's Test: **P < 0.01.

Carrageenan induced acute inflammation

The edema volume in millilitres (ml), as measured by mercury displacement using a plethysmograph, for control group at ½ h, 1h, 3h, 4h, and 5h, was 1.167± 0.04, 0.85 ± 0.01 , 0.82 ± 0.02, 0.89 ± 0.01 and 0.89 ± 0.01 (Table- 1) respectively, while the corresponding mean volumes in aspirin (200 mg/kg) treated group was 1.033 ± 0.06, 0.77 ± 0.02, 0.34 ± 0.01, 0.30 ± 0.01 and 0.25 ± 0.01 respectively (Table- 1, Graph-1), with percentage inhibition 12%, 10%, 58.53%, 66.29% and 71.9% respectively indicating significant (P < 0.01) anti-inflammatory activity of aspirin (Table - 1, Graph - 2).

Ticlopidine in the dose of 45 mg/kg showed significant inhibition (P < 0.01) of paw edema at ½ h, 1h, 3h, 4h, and 5h, with mean edema volume of 1.11± 0.03, 0.75 ± 0.01, 0.35 ± 0.01, 0.29 ± 0.01 and 0.26 ± 0.01 (Table- 1, Graph- 1) and percentage inhibition of 5%, 11.76%, 57.31%, 67.41%, 70.78% respectively indicating its anti-inflammatory activity (Table- 1, Graph- 2) .

The above results clearly show the anti-inflammatory effect of ticlopidine in acute model of inflammation when compared to control.



Post hoc analysis by Dunnet's Test: * P < 0.05; **P<0.01

Figure 1: Carrageenan induced paw edema when compared with control group.

Sub-acute inflammation (foreign body induced granuloma method)

The mean granuloma dry weight of cotton pellet in control group was 22.83± 1.138, while in aspirin treated group, it was significantly decreased (P<0.01) with the

mean value of 15±0.81 and percentage inhibition of 34.29%. Similarly, ticlopidine treated group exhibited statistically significant decrease in granuloma weight (P<0.01) with mean value of 11.83 ± 0.65 (Table - 2, Graph-3), with percentage inhibition of 48.18% when compared to control (Table 2).

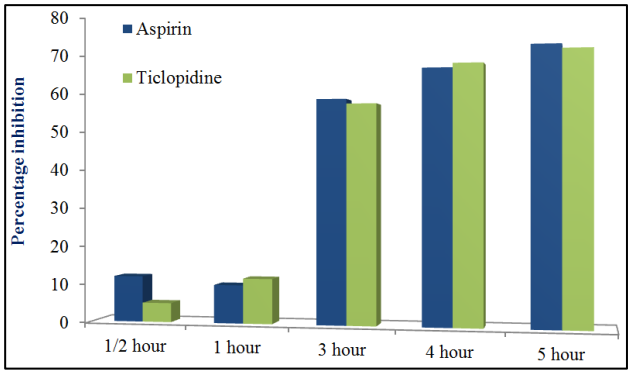


Figure 2: Percentage inhibition of carrageenan induced paw edema.

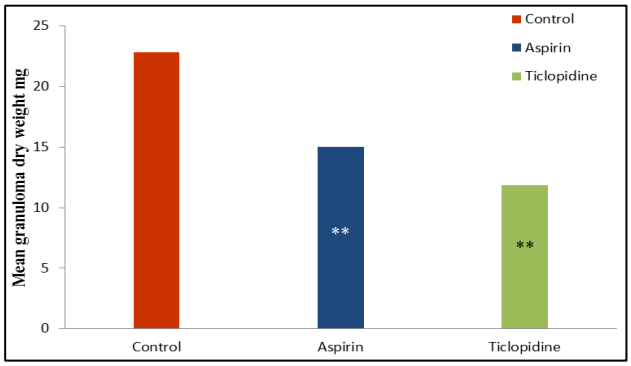


Figure 3: Mean Granuloma dry weight of cotton pellets.

The anti-inflammatory activity of ticlopidine as observed in both, acute and sub-acute studies was further confirmed by histopathological studies. The sections of grass pith when stained with haematoxylin and eosin showed abundant fibrous tissue in the control group, but revealed reduced number of fibroblasts, decreased granulation tissue, collagen content and fibrous tissue in aspirin and ticlopidine treated groups (Figures 1 - 3).

DISCUSSION

Antiplatelet agents are the mainstay of preventive care because they decrease the incidence of end-stage vessel occlusion that is responsible for most cardiovascular events. In addition to thrombosis, however, it is now appreciated that inflammation contributes to the development of atherosclerosis and its complications. In some cases, inflammatory pathways promote thrombosis, and conversely, thrombotic events often exacerbate inflammatory reactions. Thus, drugs that simultaneously block thrombotic occlusion and reduce inflammation may have added benefits in the treatment of cardiovascular disease.[13-15]

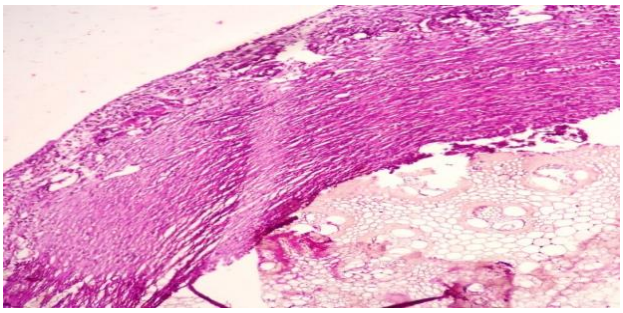


Figure 4: Control group.

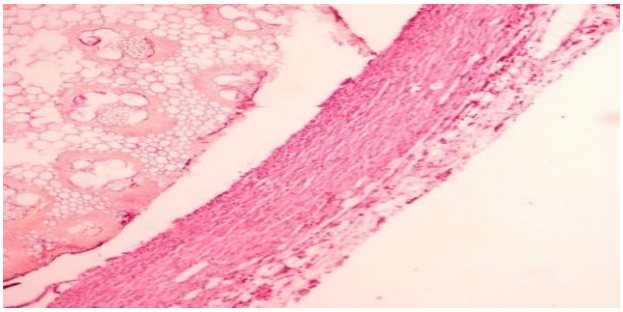


Figure 5: Aspirin group.

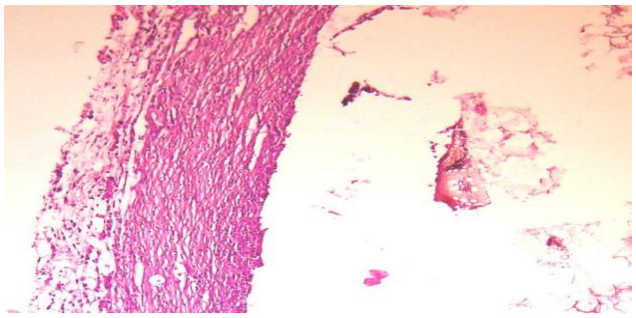


Figure 6: Ticlopidine group.

Note: As compared to control group, Aspirin and Ticlopidine group showed decreased number of fibroblasts, decreased granulation tissue, collagen content and fibrous tissue (Hematoxylin & Eosin Stain – X 10).

To prevent cardiovascular disease and its complications, patients typically receive antiplatelet therapy to suppress thrombotic events; however, the inflammatory arm of treatment has not received as much attention. Ticlopidine, an antiplatelet drug, is clinically used for the secondary prevention of transient ischemic attacks (TIA), ischemic stroke, and coronary heart diseases, which are closely associated with atherosclerosis.

Carrageenan induced rat hind paw edema method was used to assess acute Anti-inflammatory activity. In our study ticlopidine showed significant inhibition of paw edema in carrageenan induced paw edema model when

compared to control group. In sub-acute model of inflammation; ticlopidine exhibited significant decrease in the granuloma weight when compared to control group in cotton pellet granuloma method. In grass pith induced granuloma method, the sections of grass pith when stained with haematoxylin and eosin showed abundant fibrous tissue in the control group, but revealed reduced number of fibroblasts, decreased granulation tissue collagen content and fibrous tissue in ticlopidine group. In vitro studies have shown that ticlopidine attenuates progression of atherosclerosis in apolipoprotein E and low density lipoprotein receptor double knockout (apoE/LDLR-/-) mice and improve the endothelial function in mice.[6] The size of the atherosclerotic plaques and the number of macrophages were significantly reduced by ticlopidine treated mice as compared to their non-treated counterparts.[6] Thus, ticlopidine treatment limited the vascular inflammatory response of atherosclerosis in addition to the antiplatelet effects. Other antiplatelet drugs like Clopidogrel and Dipyridamole have shown anti-inflammatory activity in some studies.[16,17] Studies have shown that ticlopidine decreases the mRNA and protein levels of TNF- α stimulated MCP-1, IL-8, and vascular cell adhesion molecule-1 (VCAM-1) in human umbilical vein endothelial cells (HUVECs).[18]

Since inflammation drives all phases of atherosclerosis, including initiation, progression and thrombotic complications of the lesion. Drugs that simultaneously block thrombotic occlusion and reduce inflammation may have added benefit in the treatment of cardiovascular disease by virtue of its anti-inflammatory activity in addition to its anti-platelet activity.

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Ethical approval: The study was approved by the Institutional Animal Ethics Committee

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