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Research Article

Evaluation of clopidogrel on acute and sub-acute models of inflammation in male Wistar rats

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ABSTRACT

Background: Atherosclerosis and its complications remain 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 clopidogrel on acute and sub-acute models of inflammation in male Wistar rats.
Methods: Male Wistar rats (150-200 g) were divided into three groups, i.e. control, aspirin and clopidogrel (n=6 animals in each group). The effect of clopidogrel 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 guidelines. Analysis was done using one-way ANOVA followed by *post-hoc* test of Dunnets. $p<0.05$ was considered as statistically significant.
Results: Clopidogrel showed significant inhibition of rat paw edema in acute model ($p<0.01$) and granuloma dry weight, in sub-acute 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 clopidogrel group when compared to control.
Conclusion: Clopidogrel exhibited a significant anti-inflammatory activity in acute and sub-acute models of inflammation.

Keywords: Clopidogrel, Aspirin, Carrageenan, Inflammation

INTRODUCTION

The World Health Organization has drawn attention to the fact that coronary artery disease is our "modern epidemic."[1] Atherosclerosis and its complications remain the major cause of death and premature disability in developed societies. In addition, current predictions estimate that by the year 2020 cardiovascular diseases, notably atherosclerosis, will become the leading global cause of total disease burden.[2] 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 leukocytes, 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, platelet factor-4, regulated upon activation normal T-cell expressed and secreted (RANTES) and tissue factor. Thus, drugs that simultaneously block thrombotic occlusion and reduce inflammation may have added benefits in the treatment of cardiovascular diseases.[4]

Certain *in vitro* studies have shown that clopidogrel suppresses adenosine diphosphate induced expression of CD-40 ligand on platelet surface, inhibits P-selectin expression, formation of platelet - leukocyte complexes,

decreases production of reactive oxygen species, decreases monocyte expression of tissue factor activity and also reduces high-sensitivity C-reactive protein (hsCRP) levels, a sensitive systemic marker of inflammation.[4] In view of paucity of anti-inflammatory studies of clopidogrel, the present study was planned to evaluate the effect of clopidogrel on acute and sub-acute models of inflammation in male Wistar rats.

METHODS

Animals used

A total of 18 male Wistar rats (150-200 g) were used for the present study. They were fed with standard pellet diet and water *ad libitum*. All animals were acclimatized for 1 week before the experiment session. All experiments were done following the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals. Acute inflammation was produced by injecting carrageenan in the hind paw of Wistar rats and sub-acute inflammation by implanting foreign body (cotton pellets and grass piths) subcutaneously as described below.

Carrageenan induced rat paw edema model

Rats were divided into three groups of six each. Group I (control) received 0.5 ml of 1% gum acacia suspension, orally; Group II (standard) received aspirin 200 mg/kg orally in 1% gum acacia suspension and Group III received clopidogrel 6.75 mg/kg orally in 1% gum acacia suspension.[5,6] Aspirin was taken as the standard anti-inflammatory drug.

After 30 mins, aspirin and 2 hrs after clopidogrel administration, 0.05 ml of 1% w/v carrageenan suspension was injected into the sub-plantar region of left hind paw. The paw edema volume was measured with the help of plethysmograph at 0, ½, 1, 3, 4 and 5 hrs after injecting carrageenan. The percentage inhibition of paw edema in the various treated groups was then calculated using the formula:

$$(V_c - V_t)/V_c \times 100$$

Where, V_c and V_t are mean increase in paw volume in control and treated group respectively.[7]

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 10 mg each and two sterile grass piths (25 mm × 2 mm) through a small incision in all rats. Wounds were then sutured, and animals were caged individually after recovery from anesthesia. Aseptic precautions were ensured throughout the experiment. The treatment was started on the day of implantation and was given for 10 days.[8]

On the 11th day, cotton pellets and grass piths were removed. The grass piths were preserved in 10% formalin for histopathological studies. The cotton pellets, free from extraneous tissue were dried overnight at 60°C to note their dry weight. Mean granuloma dry weight for various groups was calculated and expressed as mg/100 g body weight. The percentage inhibition of granuloma dry weight was calculated using formula:

$$(W_c - W_t)/W_c \times 100$$

Where, W_c and W_t are mean granuloma dry weight in control and treated group respectively.[8,9]

Statistical analysis

The data for all the groups was expressed as mean±standard error of the mean and were analyzed by one-way 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, anti-platelet drug clopidogrel, in the therapeutic equivalent dose, was investigated for its possible anti-inflammatory activity, in acute and sub-acute models of inflammation in male Wistar rats.

Carrageenan induced acute inflammation

The edema volume in milliliters (ml), as measured by mercury displacement using a plethysmograph, for control group at ½ h, 1 hr, 3 hrs, 4 hrs, and 5 hrs, 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 and Figure 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 and Figure 2).

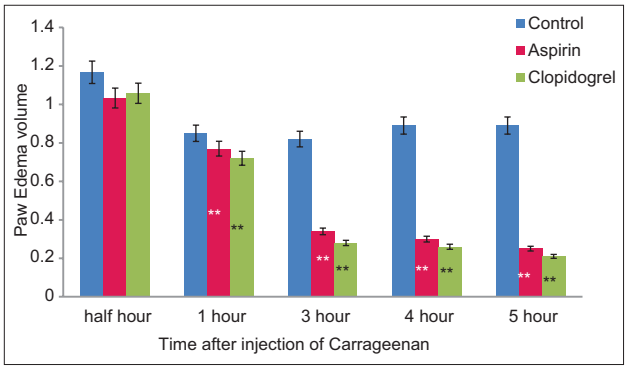

Figure 1: Effect of various treatments on carrageenan-induced paw edema in male Wistar rats. *p<0.05, **p<0.01.

Table 1: Effect of various treatments on carrageenan induced paw edema of male Wistar rats.

Time after carragee-nan injection	Control group paw edema in ml (mean±SEM)	Aspirin group		Clopidogrel group		ANOVA result
		Paw edema in ml (mean±SEM)	Percentage inhibition (%)	Paw edema in ml (mean±SEM)	Percentage inhibition (%)	p value
½ hr	1.17±0.04	1.03±0.06	12.0	1.06±0.02	10.0	>0.05
1 hr	0.85±0.01	0.77±0.02**	10.0	0.72±0.02**	15.29	<0.001
3 hrs	0.82±0.02	0.34±0.01**	58.53	0.28±0.01**	65.85	<0.0001
4 hrs	0.89±0.01	0.30±0.01**	66.29	0.26±0.01**	70.78	<0.0001
5 hrs	0.89±0.01	0.25±0.01**	71.91	0.21±0.01**	76.40	<0.0001

Post-hoc analysis by Dunnet's test: *p< 0.05, **p<0.01. SEM: Standard error of mean

The edema volume in ml in clopidogrel treated group (6.75 mg/kg) at ½ hr, 1 hr, 3 hrs, 4 hrs, and 5 hrs was 1.058±0.015, 0.72±0.02, 0.28±0.01, 0.26±0.01 and 0.21±0.01 respectively (Table 1 and Figure 1) with percentage inhibition 10%, 15.29%, 65.85%, 70.78% and 76.40% respectively suggesting significant inhibition of paw edema (p<0.01), indicating anti-inflammatory activity (Table 1 and Figure 2). The above results clearly indicate the anti-inflammatory activity of clopidogrel in acute model of inflammation.

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, clopidogrel treated group exhibited decreased granuloma weight (p<0.01) with mean value of 11.17±0.16 with percentage inhibition of 51.07% (Table 2 and Figure 3).

The anti-inflammatory activity of clopidogrel as observed in both, acute and sub-acute studies was further confirmed by histopathological studies. The sections of grass pith when stained with hematoxylin 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 clopidogrel treated groups (Figures 4a-c).

DISCUSSION

In the present study, the effect of clopidogrel on inflammation was studied using inhibition of carrageenan-induced inflammation model which is one of the most feasible methods to screen anti-inflammatory agents. Carrageenan-induced inflammation is a useful experimental model of acute inflammation for detecting orally active anti-inflammatory agents. Granuloma formation method was first described by D'Arcy et al. 1960 wherein, sterilized cotton pellets weighing 7-10 mg are implanted subcutaneously, in male albino rats, under anesthesia. Treatment is given daily throughout the study. The granulomas are dissected out on the 5th day for quantification. The cotton pellets are weighed after overnight drying at 60°C.[9]

Table 2: Effect of various treatments on granuloma dry weight in sub-acute model.

Drug treatment	Mean granuloma dry weight mg (mean±SEM)	Percentage inhibition
Control	22.83±1.138	-
Aspirin	15±0.81**	34.29
Clopidogrel	11.17±0.16**	51.07

Post-hoc analysis by Dunnet's test: *p<0.05, **p<0.01. SEM: Standard error of mean

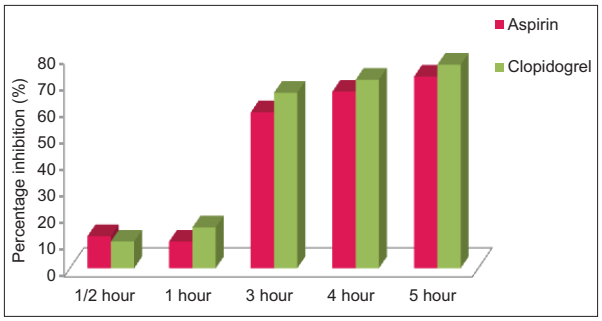

Figure 2: Percentage inhibition of rat paw edema in acute model

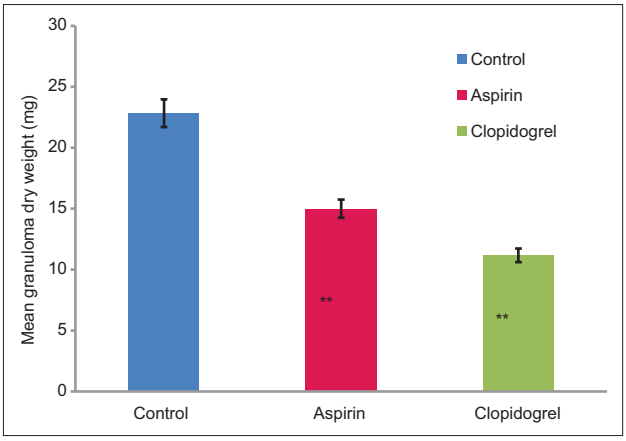

Figure 3: Effect of clopidogrel on granuloma dry weight in subacute model. *p<0.05, **p<0.01.

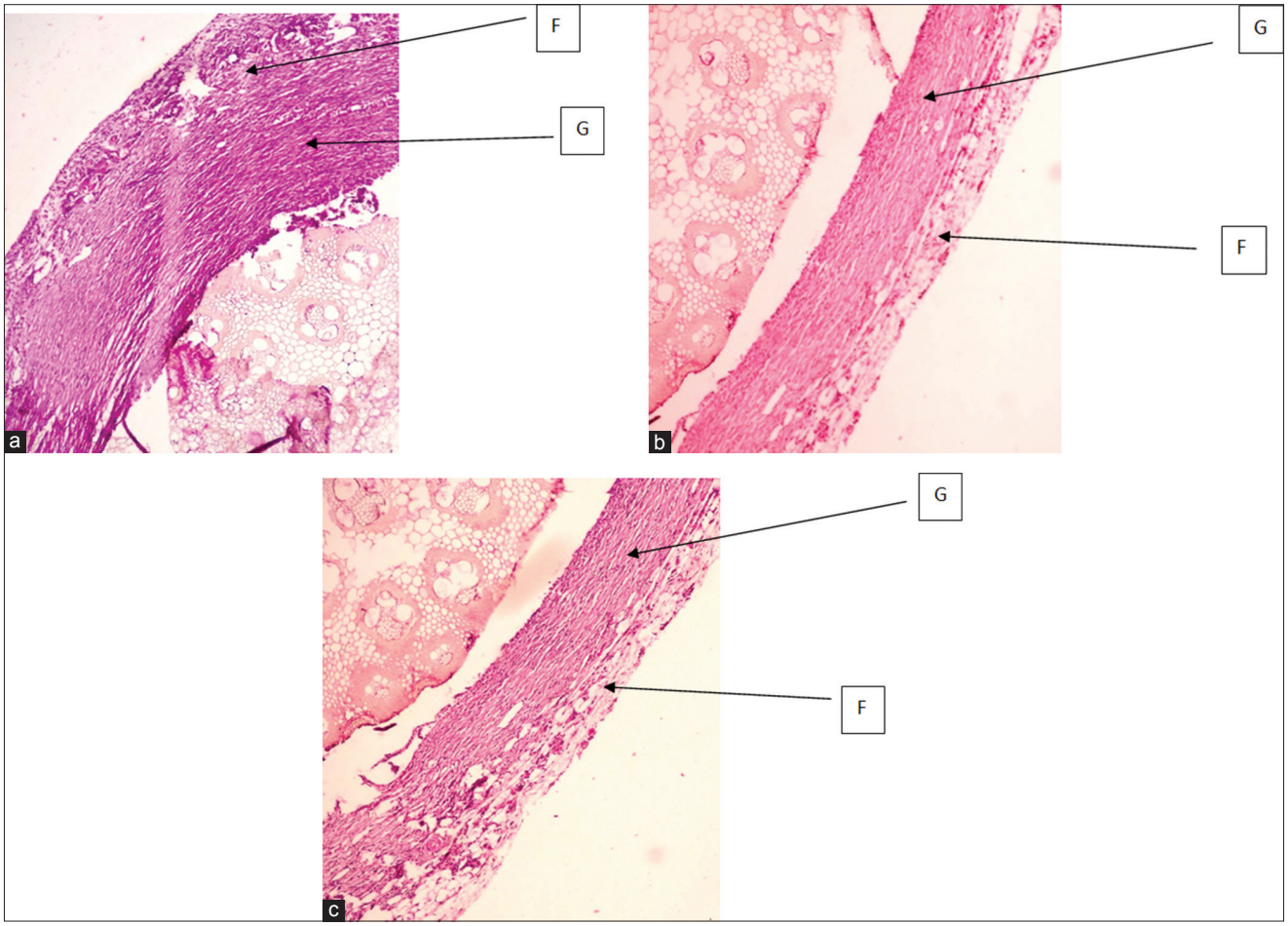

Figure 4: Photomicrographs of grass pith with granulation tissue (a) Control group, (b) aspirin group, (c) Clopidogrel group, G: Granulation tissue, F: Fibrous tissue. Note: As compared to control group, aspirin and clopidogrel group showed decreased number of fibroblasts, decreased granulation tissue, collagen content, and fibrous tissue. (Hematoxylin and eosin stain - ×10).

However, the technique has been suitably modified using another suitable form of foreign bodies like grass piths, plastic rods, etc. and prolonging the study for 10 days.[10] The grass piths are immersed in 10% formalin for subsequent microscopic studies.

Results of the present study clearly indicate that clopidogrel showed significant anti-inflammatory activity when compared with control in acute and sub-acute models of inflammation. *In vitro* studies have shown that, anti-inflammatory activity of clopidogrel can be attributed to its potential to inhibit the production of monocyte chemoattractant protein-1, macrophage inflammatory protein-1α, hsCRP, RANTES, matrix metalloproteinase-9, vascular cell adhesion molecule-1, reactive oxygen species and decreases monocyte expression of tissue factor activity.[4,11-14]

Anti-platelet 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.[3,15] Use of anti-platelet drugs in the treatment of atherosclerosis and its complications can reduce the inflammatory complications, by virtue of their anti-inflammatory activity, in addition to their anti-platelet activity.

CONCLUSION

Our study result shows that clopidogrel suppresses the carrageenan - induced paw edema and granuloma formation, thereby acts as an anti-inflammatory agent. This may be due to inhibition of mediators of inflammation. But these findings need to be verified by further clinical studies.

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