DOI: 10.5958/2319-5886.2015.00104.6

International Journal of Medical Research & Health Sciences

www.ijmrhs.com Volume 4 Issue 3 Coden: IJMRHS Copyright @2015 ISSN: 2319-5886

Received: 17th Mar 2015 Revised: 20th Apr 2015 Accepted: 18th May 2015

Research article

EVALUATIONOF DIPYRIDAMOLE ON ACUTE AND SUBACUTE MODELS OF INFLAMMATION IN MALE WISTAR RATS: AN EXPERIMENTAL STUDY

*****Angadi Netravathi B[1], Hiremath Shrishail V [2], Suranagi Vijayalakshmi V [3]

[1, 2] Department of Pharmacology, [3]Department of Pathology, KLE University's J. N. Medical College, Nehru Nagar, Belagavi,Karnataka, India.

*Corresponding author email: drnetra.angadi@gmail.com

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 dipyridamole 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 dipyridamole (n=6 animals in each group). The effect of dipyridamole, 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:** Dipyridamole 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 dipyridamole group when compared to control. **Conclusion**: Dipyridamole exhibited a significant anti inflammatory activity in acute and sub-acute models of inflammation.

Keywords: Dipyridamole, 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, 3, 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, surfaceexpression 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]

Dipyridamole is a vasodilator that inhibits platelet function by inhibiting adenosine uptake and cGMP phosphodiesterase activity. Certain in vitro studies suggest that dipyridamole inhibits secretion of monocyte chemo attractant Protein-1, matrix metalloproteinase-9 (MMP-9) and tissue factor which in turn leads to inhibition of platelet- monocyte interaction. And also it acts indirectly through adenosine and prostaglandin I-2, inhibiting lymphocyte recruitment, activation and secretion of pro inflammatory mediators suggesting its anti-inflammatory activity. [5, 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 dipyridamole, the present study was planned to evaluate the effect of dipyridamole on acute and sub acute models of inflammation in male Wistar rats.

MATERIALS AND METHODS

Study design: An experimental animal based study

The study was approved by Institutional Animal Ethical Committee of KLE University's J. N. Medical College, Belagavi. 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).

Grouping: Adult male healthy Wistar rats weighing 150- 200 g were obtained from the central animal house, J. N. Medical College, Belagavi and were acclimatized to 12:12 h light - dark cycle for 10 days prior to the day of experimentation. Rats were divided into three groups of six each, on which both acute and subacute studies were carried out with a gap of 2 weeks. Group I received 0.5ml of 1% gum acacia suspension orally(control group), group II received aspirin (Standard group) and group III received dipyridamole (Test group).

Drugs administration: Aspirin was administered in the dose of 200 mg/kg body weight of rat, equivalent to 2222 mg of clinical dose orally in 1% gum acacia suspension. [7, 8]

Dipyridamole was administered in the dose of 27 mg/kg body weight equivalent to 300 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 right hind paws and sub-acute inflammation by randomly implanting a foreign body subcutaneously in axilla and groin as described below.

1. Carrageenan induced rat paw edema [9]

Rats were divided into three groups of six each. (Same animals were used for foreign body induced inflammation)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 dipyridamole27 mg/kg orally in 1% gum acacia suspension.[7,8] Aspirin was taken as the standard anti-inflammatory drug.

Thirty minutes after aspirin and dipyridamole administration, 0.05ml of 1% w/v carrageenan suspension was injected into the sub-plantar region of right hind paw. A mark was put on the hind limb at the malleolus to facilitate uniform dipping at subsequent readings. The paw edema volume in milliliters was measured with the help of a plethysmograph by mercury displacement [9] 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 edema in the various treated groups was then calculated by using the formula,[9]

$$\% \text{ of inhibition of edema} = 1 - \frac{\text{Mean increase in treated group}}{\text{Mean increase in control group}} \times 100$$

2. Foreign Body Induced Granuloma Method[11,12]

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 grass pith with 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, [10]

$$\% \text{ of inhibition of granuloma} = 1 - \frac{\text{Dry weight of granuloma in treated}}{\text{Dry weight of granuloma in control}} \times 100$$

Statistical analysis: The data for all the groups was expressed as Mean ±SEM and were analyzed 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, dipyridamole in therapeutic equivalent dose was investigated for its possible anti inflammatory effect, in acute and sub-acute models of inflammation. Carrageenan induced acute inflammation:

The edema volume in milliliters (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, Fig 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, Fig 2).

Dipyridamole in the dose of 27 mg/kg showed significant inhibition ($P < 0.01$) of paw edema at ½ h, 1h, 3h, 4h, and 5h, with mean edema volume of 1.008 ± 0.03, 0.77 ± 0.02, 0.43 ± 0.02, 0.36 ± 0.02 and 0.35 ± 0.01 respectively (Table- 1, Fig-1) and percentage inhibition 14%, 10%, 47.56%, 59.55%, and 60.67% respectively (Table- 1, Graph- 2).The above results clearly show the anti-inflammatory effect of dipyridamole in acute model of inflammation when compared to control.

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, dipyridamole treated group exhibited statistically significant decrease in granuloma weight ($P<0.01$) with mean value of 13.50 ± 0.50 (Table - 2), with percentage inhibition of 40.86% when compared to control (Table - 2).

The anti-inflammatory activity of dipyridamole 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 dipyridamole treated groups. (Figures 3-5)

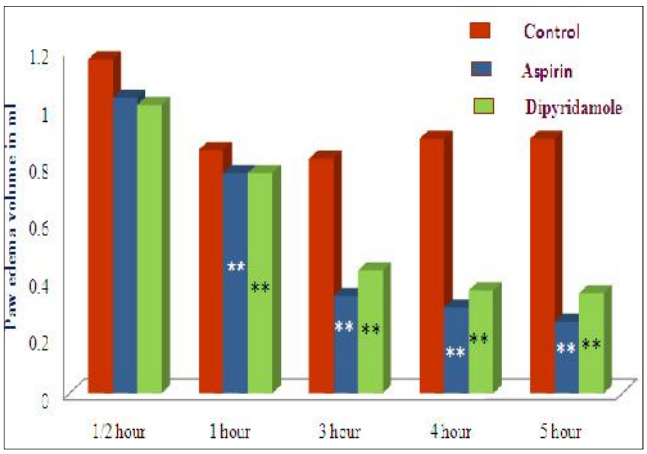

Fig1: Carrageenan induced paw edema when compared with control group.

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

Table 1: Effect of aspirin and dipyridamole treatment on carrageenan induced paw edema.

Time after carrageenan injection	Control Paw edema in ml (Mean± SEM)	Aspirin		Dipyridamole		ANOVA Result
		Paw edema in ml (Mean ± SEM)	inhibition %	Paw edema in ml (Mean ± SEM)	inhibition %	P value
½ hr	1.167±0.04	1.033± 0.06	12	1.008± 0.03*	14	>0.05
1 hr	0.85 ± 0.01	0.77± 0.02**	10	0.77± 0.02*	10	<0.003
3 hr	0.82± 0.02	0.34± 0.01**	58.53	0.43± 0.02**	47.56	<0.0001
4 hr	0.89 ± 0.01	0.30± 0.01**	66.29	0.36± 0.02**	59.55	<0.0001
5 hr	0.89 ± 0.01	0.25 ± 0.01**	71.9	0.35± 0.01**	60.67	<0.0001

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

Table 2: Effect of aspirin and dipyridamole treatments on granuloma dry weight

Drug treatment	Granuloma dry weight mg/100 gm b/w	% inhibition
Control	22.80±1.13	--
Aspririn	15±0.81**	34.29
Dipyridamole	13.50±0.50**	40.86

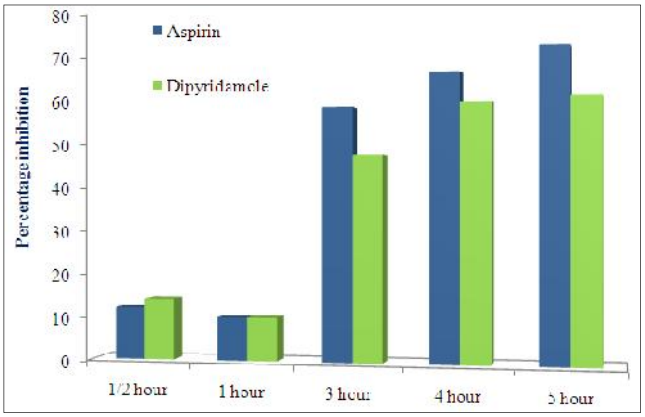

Fig 2: Percentage inhibition of carrageenan induced paw edema

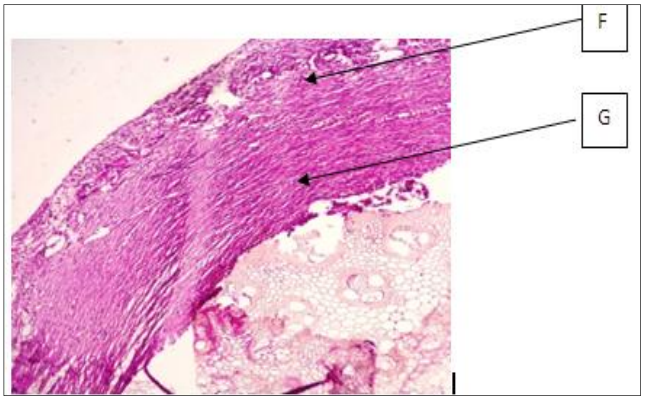

Fig3: Control group grass pith with granulation tissue containing abundant granulation tissue, fibroblasts, collagen content and fibrous tissue. (Hematoxylin& Eosin Stain – X 10) G- Granulation tissue, F- Fibrous tissue (G and Fstands for G- Granulation tissue, F- Fibrous tissue)

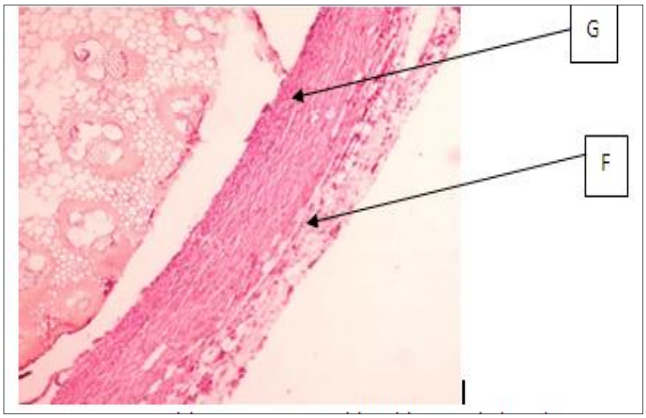

Fig 4: Aspirin group grass pith with granulation tissue containing decreased number of fibroblasts, decreased granulation tissue, collagen content and fibrous tissue. (Hematoxylin& Eosin Stain – X 10), G- Granulation tissue, F- Fibrous tissue

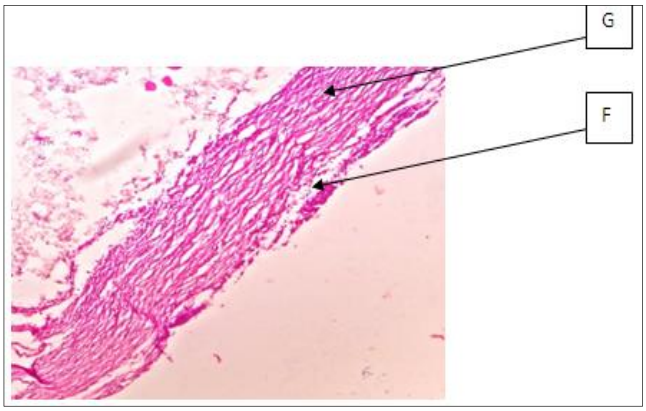

Fig 5: Dipyridamole group grass pith with granulation tissue containing decreased number of fibroblasts, decreased granulation tissue, collagen content and fibrous tissue. (Hematoxylin& Eosin Stain – X 10)G- Granulation tissue, F- Fibrous tissue

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

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 diseases. [13, 14, 15]

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. In the European Stroke Prevention Study-2 (ESPS-2), aspirin plus extended-release dipyridamole showed a 23.1% reduction in the relative risk of stroke events compared with aspirin alone, indicating that the addition of dipyridamole improves patient outcomes. [16]

Carrageenan induced rat hind paw edema method was used to assess acute inflammatory activity. In our study dipyridamole showed significant inhibition of paw edema in carrageenan induced paw edema model when compared to control group. In sub acute model of inflammation; dipyridamole 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 dipyridamole group.

In vitro studies have shown that anti-inflammatory activity of dipyridamole may be attributed to its potential to attenuate nuclear translocation of nuclear factor kappa beta (NF- kB) [5] and property to block the synthesis of monocyte Chemo attractant Protein-1(MCP-1) at the transcriptional level. Dipyridamole also blocks Interleukin–8(IL-8) and matrix metalloproteinase-9 (MMP-9) generation by lipopolysaccharide-treated monocytes. [17, 18, 19] 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.

CONCLUSION

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

ACKNOWLEDGEMENT

The authors would like to thank the staff of Department of Pharmacology, KLE University's J. N. Medical College, Belagavi for their support.

Conflict of Interest: Nil

REFERENCES

1. Park.K. Park`s textbook of preventive and social medicine. 21st ed. Jabalpur (M. P.): M/s BanarsidasBhanot Publishers; 2011. 320
2. Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J. Harrison's principles of internal medicine. 18th edition. New York: McGraw Hill Publishers; 2012; 183.
3. Libby P, Okamoto Y, Rocha VZ, Folco E. Inflammation in atherosclerosis. Transition from theory to practice. Circ J 2010; 74:213–20.
4. Steinhubl SR, Badimon JJ, Bhatt DL, Herbert JM and Lüscher TF. Clinical evidence for anti-inflammatory effects of antiplatelet therapy in patients with atherothrombotic disease. Vasc Med 2007; 12:113–22.
5. Kim HH and Liao JK. Translational therapeutics of dipyridamole. Arterioscler ThrombVascBiol 2008; 28(3):39–42.
6. Fitzgerald GA. Dipyridamole. N Engl J Med 1987; 316:1247-57.
7. Laurence DR, Bacharach AL. Evaluation of Drug Activities: Pharmacometrics. New York and London: Academic Press Inc; 1964;2: 815

8. Sweetman SC. Martindale The Complete Drug Reference. 36th Edition. London: Pharmaceutical Press; 2009; 1409,20.
9. Winter CA, Risley EA, Nuss GW. Carrageenin-induced edema in hindpaw of the rat as an assay for antiiaflammatory drugs. ProcSocexpBiolMed1962; 111:544-7.
10. Gupta SK. Drug screening methods. 2nd edition. New Delhi, Jayapeebrothers medical publishers (P) Ltd; 2009: 488-9.
11. Turner RA. Screening Methods in Pharmacology. New York and London:Academic Press Inc; 1965.
12. Patil PA, Kulkarni DR. Effect of anti proliferative agents on healing of dead space wounds in rats. Ind J Med Res 1984; 79:445-7.
13. Molero L, Lopez-Farre A, Mateos-Caceres PJ, Fernandez- Sanchez R, Luisa Maestro M, Silva J, et al. Effect of clopidogrel on the expression of inflammatory markers in rabbit ischemic coronary artery. Br J Pharmacology 2005; 146:419–24.
14. Klinkhardt, U, Bauersachs R, Adams J, Graff J, Lindhoff-Last E, Harder S. Clopidogrel but not aspirin reduces P-selectin expression and formation of platelet–leukocyte aggregates in patients with atherosclerotic vascular disease. ClinPharmacolTher 2003; 73:232–41.
15. Ross R. Atherosclerosis – an inflammatory disease. N Engl J Med 1999; 340:115–26.
16. Diener HC, Cunha L, Forbes C, Sivenius J, Smets P, Lowenthal A. European Stroke Prevention Study 2. Dipyridamole and acetylsalicylic acid in the secondary prevention of stroke. Journal of the Neurological Sciences 1996; 143: 1-13.
17. Weyrich AS, Denis MM, Kuhlmann-Eyre JR, Spencer ED, Dixon DA, Marathe GK, et al. Dipyridamole selectively inhibits inflammatory gene expression in platelet-monocyte aggregates. Circulation 2005; 111:633–42.
18. Harker LA, Fuster V. Pharmacology of Platelet Inhibitors. J Am CollCardiol 1986; 8:21.
19. Chakrabarti S, Vitseva O, Iyu D, Varghese S, Freedman JE. The effect of dipyridamole on vascular cell-derived reactive oxygen species. J PharmacolExpTher .2005; 315:494–500.