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Effect of Irbesartan on Acute and Sub-Acute Models of Inflammation in Male Wistar Rats: An Experimental Study

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Abstract

Inflammation is the local response of the living mammalian tissues to injury due to any agent. It is the defense mechanism to eliminate or limit the spread of injurious agent. to evaluate the effect of selected angiotensin receptor blockers i.e. irbesartan in acute and sub-acute models of inflammation, in male Wistar rats. This study was conducted on adult male healthy Wistar rats weighing 175±25 g were obtained from the central animal house, J. N. Medical College, Belgaum and were acclimatized to 12:12 hour light-dark cycle for 10 days prior to the day of experimentation. Acute inflammation was produced by injecting carrageenan in the hind paw and sub-acute inflammation by implanting foreign body subcutaneously. Significant inhibition of rat paw edema in acute model and granuloma dry weight in sub-acute model of inflammation when compared to control. Also anti-inflammatory effect of irbesartan was comparable to aspirin in acute model of inflammation. Whereas in sub-acute model of inflammation, irbesartan were found to have comparable anti-inflammatory effect to aspirin. Histopathological examination of grass pith revealed markedly reduced fibroblasts, fibrous tissue and collagen in aspirin and irbesartan groups when compared to control. These results clearly show that irbesartan possess anti-inflammatory property. In acute model of inflammation, irbesartan have shown significant anti-inflammatory effect when compared to control and their effect was comparable to aspirin.

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Key Words

Irbesartan, anti-inflammatory, sub-acute model of inflammation

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INTRODUCTION

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

Essential hypertension is an important public health challenge because of its high prevalence. In United States, 28.7 % of adults have hypertension and prevalence has increased among aged=60 years-65.4%. Hypertension doubles the risk of cardiovascular diseases, including coronary heart diseases, congestive heart failure, ischemic and hemorrhagic stroke, renal failure and peripheral arterial diseases. It has been estimated that hypertension accounts for 6 % deaths worldwide.1 In India, prevalence of hypertension is 59.9 and 69.9 per 1000 in males and females respectively in urban population while in rural population it is 35.5 and 35.9 per 1000 in males and females respectively[2].

The lesions of atherosclerosis represent a series of highly specific cellular and molecular responses that can be described, as an inflammatory disease[3,4]. Inflammatory cells and pathways contribute to the initiation, progression and complications of atherosclerotic lesion[5].

Monocytes initiate the endothelial inflammation leading to atherosclerosis. Macrophages avidly engulf lipoproteins including oxidized low density lipoprotein (LDL) which augments macrophage activation and cytokine production [e.g.Tumor Necrosis Factor (TNF)]. This further increases leukocyte adhesion and production of chemokines [e.g. Monocyte Chemotactic Protein-1(MCP-1)]. Also, activated T-cells in growing intimal lesions elaborate inflammatory cytokines [e.g. Interferon-γ (IFN-γ)][5].

Chronic inflammation is a common link in between cardiovascular risk factors and hypertension and acts as an independent determinant of arterial blood pressure[6]. Increasing evidence suggests that hypertension-associated vascular disease is an inflammatory process and chronic inflammation may play a key role in the pathophysiology of elevated blood pressure[7]. Patients with essential hypertension have increased concentration of circulating interleukin 1ß (IL-1ß).8 Recent work has shown that the key proinflammatory transcription factor nuclear factor-κB (NF-κB) is activated in proinflammatory states including atherosclerosis[9].

Many steroidal and non steroidal anti-inflammatory drugs (NSAIDs) are in clinical use. Also, some other drugs like penicillamine, allopurinol etc[10]., have been in clinical use to treat inflammatory conditions like rheumatoid arthritis, gout etc.

One of the strategies for the management of atherosclerosis in hypertensive patient is to reduce blood pressure. However, it may be difficult to reduce serum inflammatory cytokines and markers only by reducing blood pressure[6]. Hence the present study was planned to evaluate the effect of irbesartan on acute and sub-acute models of inflammation in male Wistar rats.

MATERIALS AND METHODS

This study was conducted on adult male healthy Wistar rats weighing 175±25 g were obtained from the central animal house, J. N. Medical College, Belgaum and were acclimatized to 12:12 hour light-dark cycle for 10 days prior to the day of experimentation. They were maintained on standard rat chow pellet and water ad libitum. The study was approved by the Institutional Animal Ethics Committee.

Acute inflammation was produced by injecting carrageenan in the hind paw and sub-acute inflammation by implanting foreign body subcutaneously as described below.

Carrageenan Induced Rat Paw Edema: Rats were divided into five groups of six each (n=6). They were starved overnight with water ad libitum prior to the day of experiment. Control group received 0.5 ml of 1% gum acacia suspension, while the other groups received calculated clinical equivalent dose of one of the drug, aspirin, irbesartan in 1% gum acacia suspension. Aspirin was taken as the standard anti-inflammatory drug. Irbesartan were administered two hours prior to the induction of edema.

0.05 ml of 1% carrageenan in normal saline was injected into the sub plantar region of one of the hind paw[11]. A mark was put on the hind limb at the malleolus to facilitate uniform dipping at subsequent readings. The paw edema volume in milliliter was measured with the help of 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. The difference between 0 hour and subsequent reading was taken as actual edema volume.

Foreign Body Induced Granuloma Method[12]: Rats were divided into five groups of six each (n=6). After clipping the hair in axillae and groin, under thiopentone anesthesia, two sterile cotton pellets weighing l0 mg (Fig 4) and two sterile grass piths measuring 25x2 mm (were implanted randomly, subcutaneously, through a small incision. Wounds were then sutured and animals were caged individually after recovery from anesthesia. 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.

On the eleventh day, the rats were sacrificed with an overdose of anesthesia to remove the cotton pellets and grass piths. The grass piths were preserved in 10 % formalin for histopathological studies. 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 (10 mg) from the weights noted. Mean granuloma dry weight for various groups was calculated and expressed as mg/100 g body weight.

The clinical doses[12] for various drugs were converted to rat equivalent doses with the help of table devised by Paget and Barnes[13].

Irbesartan: (Cipla, Mumbai) It was administered in the dose of 27 mg/kg body weight equivalent to 300 mg of clinical dose orally.

Aspirin I.P: (Cipla, Mumbai) It was administered in the dose of 200 mg/kg body weight equivalent to 2222 mg of clinical dose orally.

Carrageenan: (Sigma Co. St. Louis.) Carrageenan is mixture of polysaccharide composed of sulphated galactose units and is derived from Irish Seamoss. It was administered as a suspension in 1% warm normal saline given in the volume of 0.05 ml per rat paw.

The granulation tissue preserved in 10 % formalin was processed in the Department of Pathology, J. N. Medical College, Belgaum and sections were stained with haematoxylin and eosin and the granulation tissue in each group was studied microscopically.

*Similar groups (n=6 in each group) were included for sub-acute studies and drugs were given once daily for 10 days. Irbesartan was administered two hours prior to carrageenan injection.

Statistical Analysis: The results were analyzed by one way ANOVA (Analysis of variance) followed by Dunnet's test. ANOVA followed by Bonferroni's test was used to compare study groups viz. irbesartan with standard i.e. aspirin. Statistical analysis was done using Graph Pad Prism 4 software and $p<0.05$ was considered significant.

RESULTS AND DISCUSSIONS

The mean paw edema volumes in milliliter's (ml), as measured by mercury displacement using a plethysmograph, for control group at ½ h, 1 h, 3 h, 4 h, and 5 h intervals were 0.991±0.041, 1.258±0.023, 1.458±0.02, 1.325±0.051 and 1.2±0.022 respectively. The corresponding mean paw edema volumes in aspirin treated group were 1.108±0.02, 1.117±0.016, 1.092±0.008, 0.983±0.021 and 0.975±0.021 respectively, with the calculated percentage inhibitions of 30.24%, 71.06%, 84.15%, 98.32% and 95.54% respectively. Aspirin treated group showed statistically significant inhibition of paw edema volume ($p<0.01$) when compared to control.

The paw edema volumes in ml in irbesartan treated group at ½ h, 1 h, 3 h, 4 h and 5 h were 0.891±0.023, 1.142±0.037, 1.125±0.052, 1.025±0.035 and 0.983±0.042 respectively, with the calculated percentage inhibitions of 0.0%, 5.508%, 25.85%, 14.17% and 14.41% respectively (Table 6, Graph 2). Inhibition of paw edema volume in irbesartan treatment group was statistically significant ($p<0.05$), when compared to control at 1 h, 3 h, 4 h, 5 h.

The above results clearly show the anti-inflammatory effect of irbesartan in acute model of inflammation when compared to control. Further anti-inflammatory effect of irbesartan was compared with anti-inflammatory effect of aspirin. It was found that anti-inflammatory effect of irbesartan was comparable to aspirin ($p>0.05$) in acute model of inflammation.

Sub-acute Inflammation (Foreign Body Induced Granuloma Method): The mean dry weight of ten day old granuloma, expressed as mg percent (mg/100 g)

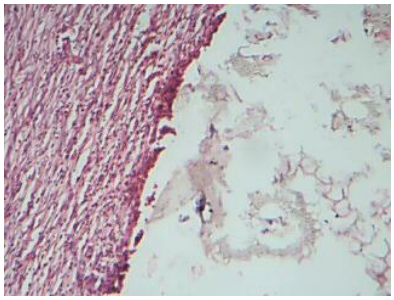

Fig. 1: Photomicrograph showing scanty inflammatory infiltrate and reduced fibroblastic proliferation in the wall of lesion (H and E STAIN)

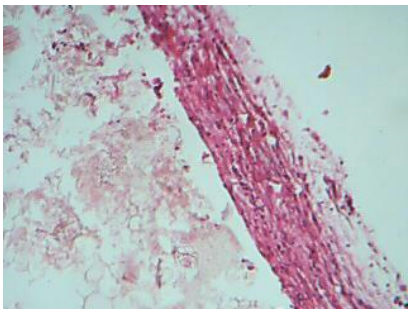

Fig.2: Photomicrograph showing scanty acute inflammatory infiltrate with reduced fibroblastic activity in the wall of lesion and thin fibrous wall (H and E STAIN)

Table 1: Effect of aspirin and irbesartan treatments on carrageenan induced paw edema when compared with control

		Aspirin			Irbesartan		
Time after carragee -nan injection	Control(Paw edema in ml ± SEM)	Paw edema in ml (±SEM)	Percentage inhibition (%)	Percentage inhibition (%)	Paw edema in ml (±SEM)	Percentage in hibition (%)	p-value
½ h	0.991 ± 0.041	1.108 ± 0.020	30.24	20.03	0.891 ± 0.023	00.00	<0.0001
1 h	1.258 ± 0.023	1.117 ± 0.016**	71.06	63.44	1.142 ± 0.037*	3.70	0.025
3 h	1.458 ± 0.020	1.092 ± 0.008**	84.15	74.99	1.125 ± 0.052**	36.80	<0.0001
4 h	1.325 ± 0.051	0.983 ± 0.021**	98.32	84.94	1.025 ± 0.035**	40.00	<0.0001
5 h	1.200 ± 0.022	0.975 ±0.021**	95.54	97.78	0.983 ± 0.042**	31.12	<0.0001

Table 2: Effect of irbesartan treatments on carrageenan induced paw edema when compared with aspirin group

	Aspirin		Irbesartan		ANOVA Result	
Time after carrageenan injection	Paw edema in ml (SEM)	Percentage inhibition (%)	Paw edema in ml (SEM)	Percentage inhibition (%)	$F_{3, 20}$	p-value
½ h	1.108 ± 0.020	30.24	0.891 ± 0.023**	00.00	14.19	<0.0001
1 h	1.117 ± 0.016	71.06	1.142 ± 0.037	3.70	5.508	0.025
3 h	1.092 ± 0.008	84.15	1.125 ± 0.052	36.80	25.85	<0.0001
4 h	0.983 ± 0.021	98.32	1.025 ± 0.035	40.00	14.17	<0.0001
5 h	0.975 ± 0.021	95.54	0.983 ± 0.042	31.12	14.41	<0.0001

Table 3: Effect of aspirin and irbesartan treatments on granuloma dry weight when compared with control group

S. No	Drug Treatment	Mean granuloma dry weight mg/100 g body weight (Mean ± SEM)	Percentage inhibition (%)
1.	Control	14.88 ± 0.3992	-
2.	Aspirin	10.17 ± 0.6270**	31.65
5.	Irbesartan	9.887 ± 0.4351**	33.55

Table 4: Effect of telmisartan, valsartan, and irbesartan treatments on granuloma dry weight when compared with aspirin group

S. No	Drug Treatment	Mean granuloma dry weight mg/100 g body weight (Mean ±SEM)
1.	Aspirin	10.17 ± 0.6270
4.	Irbesartan	9.887 ± 0.4351

ANOVA: F 3, 20 = 20.39, p < 0.0001, Post hoc analysis by Bonferroni's Test: *p < 0.05

body weight of rat, in control group was 14.88±0.3992. In aspirin treated group, it was significantly decreased (p<0.01) with the mean value of 10.17±0.627 and percentage inhibition of 31.65%. Similarly, irbesartan treated groups exhibited statistically significant decrease in granuloma dry weight (p<0.01) with mean value of 12.57±0.5941, 10.30±0.3052 and 9.887±0.4351 with percentage inhibition of 15.52% and 30.77 % and 33.55 % respectively when compared to control. (Table-8, Graph-3 and 4)

Further mean granuloma dry weight of irbesartan group was compared with mean granuloma dry weight of aspirin group. There was no statistically significant difference in mean granuloma dry weight of irbesartan group when compared to mean granuloma dry weight of aspirin (p>0.05) group. It shows that the anti-inflammatory effect of irbesartan was comparable to aspirin in sub-acute model of inflammation.

The anti-inflammatory effect of irbesartan as observed in both, acute and sub-acute studies was further confirmed by histopathological studies. The sections of granulation tissues when stained with haematoxylin and eosin showed dense acute inflammatory infiltrates, increased fibroblasts, thick fibrous tissue and abundant granulation tissue in the control group. Aspirin and irbesartan treated groups revealed scanty acute inflammatory infiltrates, less number of fibroblasts, scanty collagen tissue and decreased thickness of fibrous tissue .

Present study clearly indicate that irbesartan show significant anti-inflammatory activity in acute and sub-acute models of inflammation when compared to control group. In acute model of inflammation, it was found that the anti-inflammatory effect of irbesartan was comparable to that of aspirin. In sub-acute model of inflammation it was found that the anti-inflammatory effect of irbesartan was comparable with aspirin.

Observations of the present study are in agreement with the earlier reports stating that selected angiotensin receptor blockers irbesartan may have anti-inflammatory activity.

Anti-inflammatory effect of irbesartan may be attributed to its inhibitory action on production of IFN-γ and IFN-a by T-cells. It also effectively inhibits AP-1 transcriptional activity, inhibits activation of JNK and p38 MAPK140 and activates PPAR-γ[14].

The RAS plays a crucial role in circulatory homeostasis. In patients with atherosclerosis or diabetes or hypertension, angiotensin II can contribute to the development and progression of disease. The endocrine and the autocrine/paracrine effects of angiotensin II, including vasoconstriction, enhanced susceptibility to thrombosis, superoxide production, vascular smooth muscle growth, myocyte hypertrophy, fibrosis, remodelling of tissues and stimulation of a number of other hormonal mediators, represent solid candidate mechanisms driving cardiovascular and renal pathology[15].

Since ARBs have anti-inflammatory activity, they can provide clinical benefit across cardiovascular disease spectrum-from control of cardiovascular risk

factors mostly hypertension, to the early stages of cardiovascular disease and/or renal damage, through to patients in whom cardiovascular disease is already present. Also ARBs should be considered as appropriate therapy for patients with arterial hypertension regardless of the stage of disease. The capacity of ARBs to reduce blood pressure is like that of any other class of antihypertensive agent and there is a considerable evidence base for their ability to provide protective effects beyond blood pressure control for the heart, brain and kidney

CONCLUSION

In the current study, irbesartan have shown significant anti-inflammatory activity in acute and sub-acute models of inflammation. In acute model of inflammation, irbesartan have shown significant anti-inflammatory effect when compared to control and their effect was comparable to aspirin. In sub-acute model of inflammation, anti-inflammatory effect of irbesartan was comparable with aspirin.

Use of irbesartan in treating hypertension can reduce the inflammatory complications, by virtue of their anti-inflammatory activity, in addition to blood pressure lowering property. Also use of irbesartan for treating hypertension can reduce cardiovascular endpoints in variety of disease conditions including diabetes mellitus, acute myocardial infarction and coronary artery disease, left ventricular dysfunction, heart failure, renal disease and stroke.

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