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Research Article

Effect of telmisartan on sub-acute model of inflammation in male Wistar rats - an experimental study

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ABSTRACT

Background: 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. The objective of the study was to investigate the influence of telmisartan on sub-acute model of inflammation in adult male Wistar rats.
Methods: After obtaining ethical clearance from Institutional Animal Ethics Committee, animals were allotted to the three groups i.e. control, aspirin and telmisartan (n=6 animals in each group). The effect of telmisartan on inflammation was studied using sub-acute (Cotton pellet granuloma and histopathologic examination of grass piths) models. Experiment was conducted according to the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines. Analysis was done using one way ANOVA followed by post hoc tests of Dunnett's and Bonferroni's. $P<0.05$ was considered as statistically significant.
Results: In the present study telmisartan showed significant anti-inflammatory activity in sub-acute models of inflammation.
Conclusions: In view of role of inflammation in the pathogenesis of atherosclerosis and their complications, treatment by telmisartan can reduce complications by virtue of its anti-inflammatory activity, in addition to its antihypertensive effect. Also this study may help to open new avenues for therapeutic indications of telmisartan.

Key words: Telmisartan, Aspirin, Sub-acute inflammation, Foreign body granuloma

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]

In United States, 28.7% of adults have hypertension and prevalence has increased among aged ≥60 years to 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 and 35.5 and 35.9 per 1000 in male and females respectively in rural population.[2] Chronic inflammation is a common link between cardiovascular risk factors and

hypertension and acts as independent determinant of arterial blood pressure.[3] Patients with essential hypertension have increased concentration of circulating interleukin 1β (IL-1β).[4]

Recent work has shown that the key proinflammatory transcription factor nuclear factor-κB (NF-κB) is activated in proinflammatory states including atherosclerosis.[5] Inflammatory cells and pathways contribute to the initiation, progression and complications of atherosclerotic lesion.

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 chemokine's (e.g. monocyte chemotactic protein-1). Also, activated T-cells in growing intimal lesions elaborate inflammatory cytokines [e.g. Interferon-γ (IFN-γ)].[6]

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.[4]

Hence it could be hypothesized that drugs with antihypertensive and anti-inflammatory activity would be of dual benefit in the treatment of atherosclerosis in hypertensive patients. In our previous study we showed that telmisartan has anti-inflammatory activity in acute model of inflammation, now in the present study was planned to investigate the anti-inflammatory activity of telmisartan in sub-acute models of inflammation.[7]

METHODS

Adult male healthy Wistar rats weighing 175±25 g were obtained from the central animal house, J. N. Medical College, Belagavi, India 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 and water *ad libitum.*

The study was approved by the IAEC (Institutional Animal Ethics Committee) constituted as per the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision on Experiments on Animals). Aspirin (Cipla Limited, Mumbai) was administered in the dose of 200 mg/kg body weight of rat, equivalent to 2222 mg of clinical dose orally.[8,9] Telmisartan (Cipla Limited, Mumbai) was administered in the dose of 7.20 mg/Kg body weight of rat, equivalent to 80 mg of clinical dose orally.[8,9] Sub-acute inflammation was induced by randomly implanting a foreign body subcutaneously in axilla and groin as described below.

Foreign body induced granuloma method

Three groups (n=6 in each group) were included for sub-acute studies and drugs were given once daily for 10 days. After clipping the hair in axillae and groin, under thiopentone anaesthesia (40mg/Kg), two sterile cotton pellets weighing l0mg and two sterile grass piths (25x2mm) were randomly implanted subcutaneously, through a small incision. 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 with an overdose of anaesthesia to remove the cotton pellets and grass piths. The grass piths were preserved in 10% formalin for histopathological studies and were processed in the Department of Pathology, J.N. Medical College, Belagavi, India.

Sections were stained with haematoxylin and eosin, and the granulation tissue in each group was studied microscopically. The cotton 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. The percentage inhibition of granuloma dry weight was calculated using the formula,

Percentage Inhibition of 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±standard error of the mean (SEM) and analyzed by one way ANOVA (Analysis of Variance) followed by Dunnet's test. ANOVA followed by Bonferroni's test was used to compare the study groups (Graph Pad Prism Software, Inc.). $P<0.05$ was considered statistically significant.

RESULTS

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

The mean dry weight of ten day old granuloma, expressed as mg percent (mg/100 g) 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±0.6270 and percentage inhibition of

31.65%. Similarly, telmisartan treated group exhibited statistically significant decrease in granuloma weight (P<0.01) with mean value of 12.57±0.5941, with percentage inhibition of 15.52% when compared to control (Table 1).

Table 1: Effect of aspirin and telmisartan treatments on granuloma dry weight when compared with control group.

Drug Treatment	Mean granuloma dry weight mg/100gm body weight (Mean±SEM)	Percentage inhibition
Control	14.88±0.3992	---
Aspirin	10.17±0.6270**	31.65
Telmisartan	12.57±0.5941**	15.52

ANOVA: $F_{2,15} = 19.31$, P<0.0001, Post hoc analysis by Dunnet's: **P<0.01.

Further, mean granuloma dry weight of telmisartan group was compared with mean granuloma dry weight of aspirin group. There was statistically significant difference in mean granuloma dry weight of telmisartan group when compared to mean granuloma dry weight of aspirin (P<0.05) group. It shows that the anti-inflammatory effect of telmisartan is inferior to aspirin (Table 2).

Table 2: Effect of telmisartan treatment on granuloma dry weight when compared with aspirin group.

Drug Treatment	Mean granuloma dry weight mg/100gm body weight (Mean±SEM)
Control	14.88±0.3992
Aspirin	10.17±0.6270
Telmisartan	12.57±0.5941*

ANOVA: F 2,15 =19.31, P<0.0001, Post hoc analysis by Bonferroni's Test: *P<0.05

Histopathological examination of granulation tissue

The anti-inflammatory effect of telmisartan as observed in above sub-acute study was further confirmed by histopathological studies.

The sections of granulation tissues when stained with haematoxylin and eosin showed increased fibroblasts, thick fibrous tissue and abundant granulation tissue in the control group (Figure 3a and 3b) whereas aspirin (Figure 3c and 3d) and telmisartan (Figure 3e and 3f) treated groups revealed less number of fibroblasts, scanty collagen tissue and decreased thickness of fibrous tissue.

Photomicrograph of control group shows dense acute inflammatory infiltrate with granulation tissue in the wall of the lesion (Figure 3a and 3b), of aspirin group (Figure 3c and 3d) and telmisartan group (Figure 3e and 3f) showing scanty acute inflammatory infiltrate and reduced fibroblastic proliferation in the wall of lesion.

Photomicrographs of granulation tissue (H and E stain)

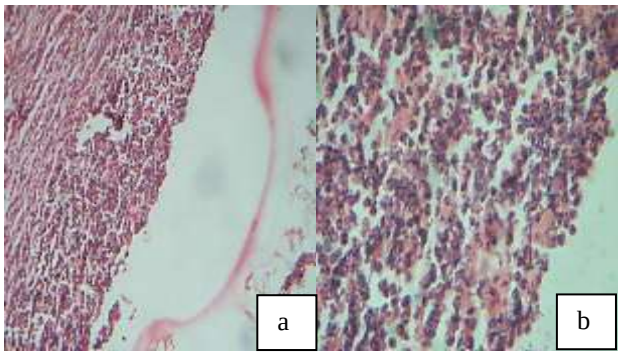

Figure 3a and b: Control group (x10) and (x40).

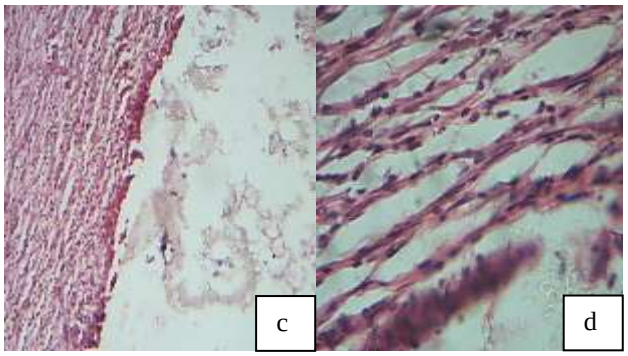

Figure 3c and d: Aspirin group (x10) and (x40).

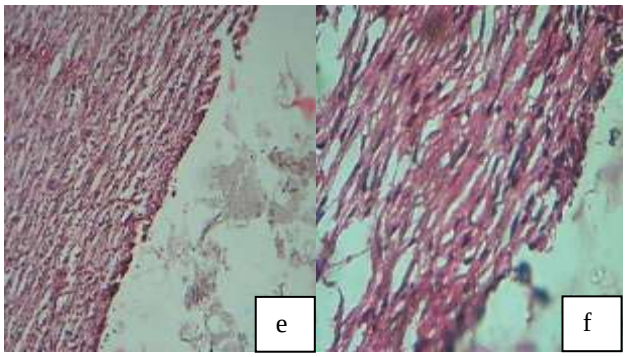

Figure 3e and f: Telmisartan group (x10) and (x40)

DISCUSSION

The ability of angiotensin receptor blockers (ARBs) to reduce mortality and morbidity of cardiovascular diseases has been ascribed not only to antihypertensive activity but also to a number of additional protective effects like inhibition of smooth muscle cells growth and left ventricular hypertrophy as well as improvement in endothelial dysfunction.[11-13]

Recently, various *in vitro* studies have suggested that ARBs may possess anti-inflammatory activity. TNF-α and angiotensin II play important roles in atherogenesis through enhancement of vascular inflammation.[14] ARBs

have been found to decrease TNF-α and IL-6 levels in a dose dependent manner.[15]

Telmisartan has been found to inhibit TNF-α induced IL-6 expression at the transcriptional level through the activation of peroxisome proliferator-activated receptor-γ (PPAR-γ). The transrepression effects of telmisartan on NF-κB and C/EBPβ activity are responsible for the IL-6 suppression.[16] Also telmisartan has been shown to modulate pleiotropically, TNF-α induced vascular cell adhesion molecule-1 (VCAM-1) expression and oxidative damage in vascular endothelium, possibly by acting as a hydroxyl radical scavenger. These anti-inflammatory and antioxidant properties may contribute to the therapeutic effect.[17]

This suggests that telmisartan may attenuate the inflammatory process induced by TNF-α in addition to the blockade of angiotensin II type 1 receptor. ARBs have been found to antagonize the effect of angiotensin by blockade of angiotensin II binding to the macrophage receptors and therefore may also exert anti-inflammatory effects.[18] In concordance with our previous study , where we investigated anti-inflammatory activity of telmisartan in acute model of inflammation[7], in our present study also telmisartan showed significant anti-inflammatory activity in sub-acute model of inflammation. There is further need to do the study of inflammatory markers in hypertensive patients who are on telmisartan therapy.

CONCLUSION

Present study clearly showed anti-inflammatory effect of telmisartan in sub-acute model of inflammation in male Wistar rats. This anti-inflammatory effect of telmisartan beyond its class effects as angiotensin II receptor blocker might make this compound a very powerful inhibitor of atherosclerosis. This study shows that in addition to antihypertensive property, telmisartan may also possess anti-inflammatory properties that could be of additional benefit in the treatment of atherosclerosis and hypertension and their complications like coronary heart diseases, congestive heart failure, ischemic and hemorrhagic stroke, renal failure and peripheral arterial diseases.

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