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RESEARCH ARTICLE

Anti-inflammatory activity of lisinopril in albino rats

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ABSTRACT

Background: Angiotensin II (Ang II) is a product of renin angiotensin aldosterone system. Angiotensin-II regulates vascular tone, stimulates the release of pro-inflammatory cytokines, activates nuclear factor-kappa B, increases oxidant stress and functions as an inflammatory molecule. Lisinopril an angiotensin converting enzyme (ACE) inhibitor act by inhibiting ACE, decreases angiotensinogen II activity. Hence the present was to evaluate the anti-inflammatory activity of lisinopril. **Aims and Objectives:** To evaluate the anti-inflammatory activity of lisinopril in albino rats. **Materials and Methods:** Eighteen Wistar albino rats weighing around 150–200 g of either sex were randomly selected from central animal facility and divided into three groups. The control group received normal saline 25 ml/kg, standard group received indomethacin 10 mg/kg and test group received lisinopril (3.6 mg/kg) orally for 6 days. The animals were subjected to carrageenan induced paw oedema and cotton pellet induced granuloma model. **Results:** Lisinopril significantly decreased the mean paw oedema in carrageenan induced paw oedema when compared to control and in cotton pellet induced granuloma lisinopril decreased the mean granuloma weight when compared to control. **Conclusion:** Lisinopril showed anti-inflammatory activity when given for 6 consecutive days per orally in albino rats in carrageenan induced paw oedema and cotton pellet induced granuloma model.

KEY WORDS: Angiotensin II; Carrageenan; Cotton Pellet; Nuclear Factor-kappa B; Reactive Oxygen Species

INTRODUCTION

Inflammation is the final common pathway of various insults, such as infection, trauma, and allergies to the human body. It is a complex reaction in vascularised connective tissue due to exogenous/endogenous stimuli. Inflammation is fundamentally a protective response the ultimate goal of which is to rid the organism of both initial cause of cell injury and its consequences.[1] Pain is often associated with inflammation. Inflammation is a normal response to any noxious stimulus that threatens the host and may vary from localized response to a generalized one. It is a complex process involving release of chemicals from tissues and migrating cells and various mediators such as prostaglandins, leukotrienes and platelet activating factors. Tissue injury induces a series of reactions with the release of pro inflammatory cytokines such as tumour necrosis factor-α (TNF-α), IL-β, IL-6. IL-8, followed by subsequent inflammatory reactions. In inflammatory disease like rheumatoid arthritis, the inflamed tissue produce elevated levels of prostaglandins such as PGE1 and PGE2, increase local blood flow and potentiate the effect of mediators like bradykinin that cause increased vascular permeability.[2]

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Angiotensin II (Ang II) is a key product of renin angiotensin aldosterone system (RAAS). Ang II elicits the cellular response through AT1 and AT2 receptors. Most of the actions of Ang II are mediated through AT1 type. Ang II function and helps in inflammatory cell recruitment.[3] Ang II also

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increases the transcription of monocyte chemotactic protein-1 (MCP-1), macrophage colony stimulating factor, endothelial selectin, intercellular adhesion molecule-1, vascular cell adhesion molecule-1 (VCAM-1), inducible nitric oxide synthase, cyclooxygenase-2.[4-6] Reduction of MCP-1 was observed in AT1 knockout mice.[7] Angiotensin-II regulates vascular tone, stimulates the release of pro-inflammatory cytokines, activates nuclear factor-kappa B (NF-κB), increases oxidant stress and functions as an inflammatory molecule.[3] NFκβ plays an important role in regulation of expression of proinflammatory genes, nitric oxide synthase, angiotensinogen, cell adhesion proteins and other genes involved in inflammation.[8] Tissue levels of NF-κB, results in inflammatory cell infiltration.[9] Elevated tissue NF-κB was correlated with expression of MCP-1 and VCAM-1 adhesion molecule.[10] Ang II enhances reactive oxygen species (ROS) production by activating nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and stimulates the DNA-binding activity of NF-κB in human neutrophils. ROS activates the NFκβ, which in turn increases transcription of pro inflammatory cytokines, adhesion molecules and NADPH molecules. Ang II increases the synthesis and concentration of TNF-α, interleukin-6 (IL6), and chemokine monocyte chemo attractant protein-1. Ang II supresses PPAR-α and PPAR-γ. Increase angiotensin converting enzyme (ACE) activity leads to high levels of Ang II. High levels of Ang II activated NADPH which in turn leads to enhanced formation of ROS and decreased nitric acid levels.[11]

The function of Ang II is participation in inflammatory cell recruitment. Several studies have shown in several models that ACE inhibitors reduce the number of infiltrating cells through various mechanisms. Hence Ang II is regarded as a true cytokine involved in regulation of inflammatory response.

ACE inhibitors like lisinopril act by inhibiting ACE, decreases angiotensinogen II activity and increases the bradykinin and (met) enkaphalin. ACE inhibitors are commonly used in the treatment of hypertension, heart failure, diabetic nephropathy, nondiabetic renal disease and post myocardial infarction.[2] Lisinopril is known to have several pleotrophic pharmacological effects. Several studies confirm that activation of RAAS, through the production of Ang II, is closely related to local vascular inflammation. Over the blood pressure lowering effects of anti-hypertensive treatments, several ancillary effects of lisinopril may be found.

Hence the present study is undertaken to evaluate the possible anti-inflammatory effect of lisinopril in albino rats.

Hypothesis

Hence it is hypothesized that lisinopril mediate anti-inflammatory effect *via* reducing the action of angiotensin II.

MATERIALS AND METHODS

The study was conducted after the approval of Institutional Ethical Committee. Adult healthy albino rats of Wistar strain of either sex, weighing between 200 and 250 g aged 3–4 months were selected from the central animal facility. The rats were inbred in the central animal house, under suitable conditions of housing, temperature, ventilation and nutrition. Rats were housed 2–3 per stainless cage under conventional conditions. They were kept at a constant temperature of 26 ± 2°C and relative humidity of 30–70% under a 12 h dark/light cycle. The animals were fed with standard diet and water *ad libitum*. The rats were acclimatized to the laboratory conditions for 7 days prior to test before assigning animals to treatment group. The doses of drugs were based on human daily dose converted to that of rats according to Paget and Barnes (1962).

Drugs and Chemicals

Indomethacin (Sun Pharmaceutical Industries Ltd., India), lisinopril (Cipla, India), Carrageenan (TCI Chemicals, India), Vernier calliper purchased from Precision India Ltd. The rats were divided into 3 groups containing six animals (n = 6) in each group (control, standard and test group).

1. Group-1 (Control): Normal saline 25 ml/kg
2. Group-2 (Standard): Indomethacin 10 mg/kg
3. Group-3 (Test 1): Lisinopril (3.6 mg/kg).

Carrageenan Induced Rat Paw Oedema[12-14]

1 h after the drug administration, paw oedema was induced by injecting 0.1 ml of 1% carrageenan into sub plantar tissue of the right hind paw of each rat of each group. The right hind paw volume will be measured immediately by using the vernier calliper (0-h-volume) and at the end of 4 h. The mean paw oedema in each group of animals treated with drugs groups and control group were noted. The anti-inflammatory activity was calculated as percentage inhibition of oedema in the animals treated with lisinopril in comparison to the carrageenan control group.

The percentage (%) inhibition of oedema is calculated using the formula:

$$\text{Percentage inhibition} = \frac{Vc - Vt}{Vt} \times 100$$

Where, Vc is paw volume in control.
Vt is paw volume in test drug.

Cotton Wool Pellet Induced Granulomas[15,16]

Cotton pellets weighing 10 ± 1 mg were sterilized in an autoclave for 30 min 120°C. 1 h after the drug administration on 1st day, under mild ether anaesthesia 4 incisions were

made in both axilla and groins. Four pellets were implanted subcutaneously into the ventral region, two on either side. Cotton wool pellets were introduced in it and sutured back with a black silk. Later drugs will be administered once a day for 6 consecutive days. On 8th day the animals were anaesthetised using ether and the pellets together with the granuloma tissue were carefully removed. The wet pellets were weighed for the determination of the wet weight and then dried in an incubator at 60°C for 18 h until a constant weight is obtained and then the dried pellets were weighed to determine the constant dry weight.

Exudate amount (mg) = wet weight of pellet-constant dry weight.

Granulation tissue formation (mg) = constant dry weight - weight of the cotton pellet (10 mg)

Statistical Analysis

The results was analysed by calculating-mean, standard deviation, *t*-test and analysis of variance (ANOVA) at different time intervals within the same group, followed by independent sample *t*-test between the two groups. One way ANOVA was used for multiple group comparisons followed by *post-hoc* Tukey's test for statistical significance between groups. IBM SPSS statistics ©IBM Corporation and Other(s) 1989, 2012 software was used for statistical analysis purpose. $P < 0.05$ was considered as significant.

RESULTS

Table 1 shows the effect of lisinopril and Indomethacin drug in carrageenan induced paw oedema using vernier callipers. Lisinopril at the dose of 3.6 mg/kg per orally prevented carrageenan induced paw oedema with the percentage inhibition of 42.63%, while, Indomethacin at the dose of 10 mg/kg per orally produced 62.23% when compared to control. Table 2 shows the effect of lisinopril and indomethacin drug as compared to control in cotton pellet induced granuloma model. Mean weight of granuloma of lisinopril is 56.39 mg less when compared to control, and Indomethacin group showed 78.45 mg less compared to control.

DISCUSSION

Table 1 shows at the end of 4 h the mean difference of paw oedema is 9.85 cm of control, 3.72 cm of indomethacin and 5.65 cm of test drug lisinopril. The carrageenan induced paw oedema model is used to evaluate the effect of non-steroidal anti-inflammatory agents because it inhibits the cyclooxygenase pathway. As shown in Table 1 there is significant ($P < 0.05$) percentage inhibition of paw oedema of lisinopril group is 42.63% when compared to control. Table 2 shows indomethacin at the dose of 10 mg/kg showed the mean granuloma weight of 57.37 mg and lisinopril at the dose of 3.6 mg/kg showed the mean granuloma weight of 79.4361 mg. When compared to control the percentage decrease in granulation tissue formation of lisinopril is 41.51% and that of indomethacin is 57.76%. There is significant ($P < 0.05$) decrease in mean granuloma weight of lisinopril when compared to control.

Carrageenan induced rat paw oedema is the suitable model to screen acute inflammation. The accumulation of paw oedema is biphasic. The first phase begins immediately after the injection of carrageenan and diminishes in an hour. The mediators of inflammation in the first phase is histamine and 5HT. The second phase is related to release of prostaglandins, begins at the end of 1st h and persist through the 3rd h. The first phase accounts for 40% of the total oedema volume. In cotton pellet induced granuloma there are three phases, a transudative phase during the first 3 h, exudative phase between 3 and 72 h and proliferative phase between 3 and 6 days.

Table 1: Effect of lisinopril and indomethacin as compared to control group at different hour in carrageenan induced paw edema using vernier caliper

Groups	Dose	Change in paw edema		
		Mean paw edema (cm) at 0 h	Mean paw edema (cm) at 4 h	Difference in mean paw edema (cm)
Control	25 ml/kg	1.48±0.10	11.33±0.48	9.85±0.38
Indomethacin	10 mg/kg	1.47±0.04	5.19±0.26	3.72±0.22
Lisinopril	3.6 mg/kg	1.50±0.19	7.15±0.52	5.65±0.33*

The values are expressed as mean±SD, where n=6 rats, *P<0.05. SD: Standard deviation

Table 2: Shows mean granuloma weight in lisinopril and indomethacin as compared to control group

Groups	Dose	Mean weight of granuloma (mg) (mean±SD)
Control	25 ml/kg	135.82±3.18
Indomethacin	10 mg/kg	57.37±2.65
Lisinopril	3.6 mg/kg	79.431±3.20*

The values are expressed as mean±SD where n=6 rats, *P<0.05. SD: Standard deviation

In a study which involved the testing of hepatoprotective effect of lisinopril against hepatic ischemic and reperfusion injury in rats, it was found that lisinopril to have anti-inflammatory effects *via* suppression of the pro-inflammatory cytokines such as TNF-α production. Lisinopril provided protection against hepatic ischemia/reperfusion injury in rats. The protective effect is due to reduction of oxidative stress induced-lipid peroxidation level and enhancement of nitric oxide availability.[17]

In another study it was found out that lisinopril supresses production of interleukin-12 and interferon γ by human peripheral blood mononuclear cells thus contributing to immunomodulatory effect of lisinopril and which has beneficial effect in inflammatory or autoimmune conditions involving interleukin 12.[18]

Thus, the above findings of our study indicates that lisinopril acts as an anti-inflammatory drug in albino rats. Carrageenan induced rat paw edema and cotton pellet induced granuloma method are used to assess the inflammatory activity of lisinopril.

Our study is limited to blockage of ACE. In order to strictly study the effects of blockage of RAAS on inflammation, further studies may explore different strategies by simultaneously acting on ACE and AT1 receptor.

CONCLUSION

The test drug lisinopril showed significant decrease in mean paw edema and mean granuloma weight when compared to that of control. The anti-inflammatory activity justifies the hypothesis stated above. Thus, to conclude lisinopril showed the anti-inflammatory activity in albino rats by decreasing the Ang II, which inturn decreases the ROS and NF-κB.

REFERENCES

1. Kumar V. Robbins Basic Pathology. In: Kumar V, Abbas AK, Aster JC, editors. Acute and Chronic Inflammation. New Delhi: Saunders; 2013. p. 29-40.
2. Katzung BG. Basic and Clinical Pharmacology. In: Furst DE, Ulrich RD, editors. Cissy Varkey Altamirano. Non Steroidal anti Inflammatory Drugs. Mumbai: McGraw hill; 2009. p. 181-5.
3. Ruiz-Ortega M, Ruperez M, Lorenzo O, Esteban V, Blanco J, Mezzano S, *et al.* Angiotensin II regulates the synthesis of proinflammatory cytokines and chemokines in the kidney. Kidney Int Suppl 2002;82:S12-22.
4. EL Bekay R, Alvarez M, Monteseirin A, Alba G, Chacon P, Vega A, *et al*. Oxidative stress is a crutial mediator of the angiotensin II signal in human neutrophils of mitogen activated protein kinase, calcineurin and the transcription factor NFκβ. Blood 2003;102:662-71.
5. Tham DM, Martin-McNulty B, Wang YX, Wilson DW, Vergona R, Sullivan ME, *et al.* Angiotensin II is associated with activation of NF-kappaB-mediated genes and downregulation of PPARs. Physiol Genomics 2002;11:21-30.
6. Pueyo ME, Gonzalez W, Nicoletti A, Savoie F, Amal JF, Michel JB. Angiotensin II stimulates endothelial cell adhesion molecule I *via* nuclear factor κβ activation induced by intracellular oxidative stress. Arterioscler Thromb Vasc Biol 2000;20:645-51.
7. Hisada Y, Sugaya T, Yamanouchi M, Uchida H, Fujimura H, Sakurai H, *et al*. Angiotensis II plays a pathogenic role in immune mediated renal injury in mice. J Clin Invest 1990;103:627-35.
8. Barnes PJ, Karin M. Nuclear factor- κB. A pivotal transcription factor in chronic inflammatory disease. N Engl J Med 1997;336:1066-71.
9. Das UN. Angiotensin-II behaves as an endogenous pro-inflammatory molecule. J Assoc Physicians India 2005;53:472-6.
10. Presa MH, Bustos C, Ortego M, Tunon J, Ortega MR, Egido J. Angiotensin converting enzyme inhibition prevents arterial NF-κB activation, MCP-1 expression and macrophage infiltration in the rabbit model of early accelerated atherosclerosis. Circulation 1997;95:1532-41.
11. Hayek T, Pavlotzky E, Hamoud S, Coleman R, Keidar S, Aviram M, *et al.* Tissue angiotensin-converting-enzyme (ACE) deficiency leads to a reduction in oxidative stress and in atherosclerosis: Studies in ACE-knockout mice Type 2. Arterioscler Thromb Vasc Biol 2003;23:2090-6.
12. Winter CA, Porter CC. Effect of alteration in side chains upon anti-inflammatory and liver glycogen activities in hydrocortisone ester. J Am Pharmacol Soc 1957;46:515-9.
13. Di Rosa M, Giroud JP, Willoughby DA. Studies on the mediators of the acute inflammatory response induced in rats in different sites by carrageenan and turpentine. J Pathol 1971;104:15-29.
14. Teotino VM, Polofrig G, Gandini A, Bella D. Antipyretic activity of thio derivatives of 2, 3-dihydro 4H-1, 3-Benzoxacin-4-1 synthesis and pharmacological property. J Med Chem 1963;6:2489.
15. Garcia Leme J, Hamamura L, Leite MP, Rocha e Silva M. Pharmacological analysis of the acute inflammatory process induced in the rat's paw by local injection of carrageenin and by heating. Br J Pharmacol 1973;48:88-96.
16. Hanson JM, Morley J, Soria-Herrera C. Anti-inflammatory property of 401 (MCD-peptide), a peptide from the venom of the bee *Apis mellifera* (L.). Br J Pharmacol 1974;50:383-92.
17. Morsy MA. Protective effect of lisinopril on hepatic ischemia/reperfusion injury in rats. Indian J Pharmacol 2011;43:652-5.
18. Cris SC, Goodmanc DB, Elvira SV. Captopril and lisinopril supress production of interleukin-12 by human peripheral blood mononuclear cells. Immunol Lett 1998;62:25-31.

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