

To evaluate the anti-inflammatory activity of ramipril in albino rats**Siddamma Amoghmath^{1*}, Suresha R. N.², Jayanthi M. K.²**¹Department of Pharmacology, Gadag Institute of Medical Sciences, Gadag, Karnataka, India²Department of Pharmacology, JSS Medical College and Hospital, Mysuru, Karnataka, India**Received:** 12 November 2017**Revised:** 14 November 2017**Accepted:** 24 November 2017***Correspondence to:**Dr. Siddamma Amoghmath,
Email: siddama.a@gmail.com**Copyright:** © the author(s), publisher and licensee Medip Academy. This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.**ABSTRACT****Background:** Angiotensin II (Ang II) is a product of Renin angiotensin aldosterone system (RAAS). 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. Ramipril an ACE inhibitor act by inhibiting angiotensin converting enzyme, decreases angiotensinogen II activity. Hence the present was to evaluate the anti-inflammatory activity of Ramipril.**Methods:** Eighteen Wistar albino rats weighing around 150-200gms of either sex were randomly selected from central animal facility and divided into three groups. The control group received normal saline 25ml/kg, standard group received Indomethacin 10mg/kg and test group received Ramipril (0.9mg/kg) orally for six days. The animals were subjected to carrageenan induced paw oedema and cotton pellet induced granuloma model.**Results:** Ramipril significantly decreased the mean paw oedema in carrageenan induced paw oedema when compared to control and in cotton pellet induced granuloma Ramipril decreased the mean granuloma weight when compared to control.**Conclusions:** Ramipril 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.**Keywords:** Angiotensin II, Carrageenan, Cotton pellet, NF-κβ, ROS**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 TNF-α, IL-β, IL-6, IL-8, followed by subsequent inflammatory reactions. In inflammatory disease like Rheumatoid arthritis, the inflamed tissue produces 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

Angiotensin II (Ang II) is a key product of Renin angiotensin aldosterone system (RAAS). 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 Ang II initiates

the inflammatory process by the release of reactive oxygen species (ROS). ROS activates the NF- κ B, which in turn increases transcription of pro inflammatory cytokines, adhesion molecules and NADPH molecules. Ang II also increases the transcription of monocyte chemotactic protein-1(MCP-1), macrophage colony stimulating factor (M-CSF), endothelial selectin (E-selectin), intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule -1(VCAM-1), inducible nitric oxide synthase, cyclooxygenase-2.^{4,6} Angiotensin-II enhances ROS production by activating NADPH oxidase and stimulates the DNA-binding activity of NF- κ B in human neutrophils. Tissue levels of NF- κ B, results in inflammatory cell infiltration.⁷ Angiotensin-II increases the synthesis and concentration of tumour necrosis factor- α (TNF- α), interleukin-6 (IL6), and chemokine monocyte chemo attractant protein-1. Ang II suppresses PPAR- α and PPAR- γ . Increase 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.⁸

Angiotensin converting enzyme inhibitors like Ramipril act by inhibiting angiotensin converting enzyme, decreases angiotensinogen II activity and increases the bradykinin and (met) enkephalin. ACE inhibitors are commonly used in the treatment of hypertension, heart failure, diabetic nephropathy, nondiabetic renal disease and post myocardial infarction.

Hypothesis: Hence it is hypothesized that Ramipril mediate anti-inflammatory effect via reducing the action of Angiotensin II.

METHODS

The study was conducted after the approval of IAEC (Institutional Ethical Committee). Adult healthy albino rats of Wistar strain of either sex, weighing between 200-250gm 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 two to three per stainless cage under conventional conditions. They were kept at a constant temperature of $26\pm 2^{\circ}\text{C}$ 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 seven 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), *Ramipril* (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).

- *Group-1 (Control):* Normal saline 25ml/kg
- *Group-2 (Standard):* Indomethacin 10mg/kg
- *Group-3 (Test 1):* Ramipril (0.9mg/kg)

Carrageenan induced rat paw oedema^{9,10}

One hour after the drug administration, paw oedema was induced by injecting 0.1ml 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 (zero - hour-volume) and at the end of 4 hours. 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 Ramipril in comparison to the carrageenan control group.

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

$$\text{Percentage Inhibition} = \frac{V_c - V_t}{V_t} \times 100$$

Where, V_c is paw volume in control; V_t is paw volume in test drug

Cotton wool pellet induced granulomas¹¹

Cotton pellets weighing $10\pm 1\text{mg}$ were sterilized in an autoclave for 30 minutes 120°C . One hour after the drug administration on first 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 18h 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 (10mg).

Statistical analysis

The results were 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.

Table 1: Effect of Ramipril and Indomethacin as compared to control group at different hour in carrageenan induced paw oedema using vernier calliper.

Groups	Dose	Change in paw oedema (cm)		
		Mean paw oedema (0 hr.)	Mean paw oedema (4 hr.)	Difference in mean paw oedema
Control	25ml/kg	1.48±0.10	11.3±0.48	9.85±0.38
Indomethacin	10mg/kg	1.20±0.07	5.19±0.26	3.99±0.19
Ramipril	0.9mg/kg	1.46±0.14	7.03±0.69	5.57±0.55*

The values are expressed as Mean±SD, where n=6 rats, *p value <0.05

Ramipril at the dose of 0.9mg/kg per orally prevented carrageenan induced paw oedema with the percentage inhibition of 43.45%, while, Indomethacin at the dose of 10mg/kg per orally produced 59.49% when compared to control.

Table 2 Shows the effect of Ramipril and Indomethacin drug as compared to control in cotton pellet induced granuloma model. Mean weight of granuloma of Ramipril is 52.72mg less when compared to control, and Indomethacin group showed 75.51mg less compared to control.

Table 2: Mean granuloma weight in all treated groups.

Groups	Dose	Mean weight of granuloma (mg) (Mean±SD)
Control	25ml/kg	130.33±3.37
Indomethacin	10mg/kg	54.82±2.72
Ramipril	0.9mg/kg	77.61±1.43*

The values are expressed as Mean±SD where n=6 rats, *p value<0.05.

DISCUSSION

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 5-HT. The second phase is related to release of prostaglandins, begins at the end of first hour and persist through the 3rd hour. The first phase accounts for 40% of the total oedema volume. Table 1 Shows the mean difference of paw oedema is 9.85cm of control, 3.99cm of Indomethacin and 5.57cm of test drug Ramipril. The carrageenan induced paw oedema model is used to evaluate the effect of non-steroidal anti-inflammatory agents because it inhibits the

RESULTS

Table 1 Shows the effect of Ramipril and Indomethacin drug in carrageenan induced paw oedema using vernier callipers.

cyclooxygenase pathway. As shown in Table 1 There is significant ($p < 0.05$) percentage inhibition of paw oedema of Ramipril group is 43.45% when compared to control.

In cotton pellet induced granuloma there are three phases, a transudative phase during the first 3 hours, exudative phase between 3 to 72 hours and proliferative phase between 3 to six days. Indomethacin at the dose of 10mg/kg showed the mean granuloma weight of 54.82mg and Ramipril at the dose of 0.9mg/kg showed the mean granuloma weight of 77.61mg. When compared to control the percentage decrease in granulation tissue formation of Ramipril is 40.45% and that of Indomethacin is 57.93%. Table 2 shows there is significant ($p < 0.05$) decrease in mean granuloma weight of Ramipril when compared to control.

The above findings indicate that Ramipril acts as an anti-inflammatory drug in albino rats.¹² Carrageenan induced rat paw edema and cotton pellet induced granuloma method are used to assess the inflammatory activity of Ramipril.

CONCLUSION

The test drug Ramipril 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 Ramipril showed the anti-inflammatory activity in albino rats by decreasing the Ang II, which in turn decreases the reactive oxygen species (ROS) and nuclear factor-kappa B (NF-κB).

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