Anti-inflammatory Effects of Ramipril In Experimentally Induced Rheumatoid Arthritis

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Abstract:Objective: To evaluate anti-inflammatory effects of ramipril in experimentally induced rheumatoid arthritis (RA).Materials and Methods: Adjuvant induced arthritis model is used in this study.Albino-Wistar rats of either sex were used. Arthritis was induced by single intradermal injection of Freund's complete adjuvant (FCA) suspended in oil inplantar region of right hind paw. Rats were divided in to three groups (n=8) namely disease control, standard and test group. Drug treatment was carried out for 21 days. Effect of test drug on acute inflammatory phase was evaluated on day 5 by assessing right hind paw edema. After 21 days animals were sacrificed and evaluated for left hind paw edema, weight changes, histo-pathological synovitis grading in left hind limbs and secondary lesion score.Results: Results showed that ramiprilsignificantly reduced right hind paw edema on day 5 ($p<0.05$). Ramipril also showed statistically significant weight gain ($p<0.05$), reduction of histo-pathological synovitis grade ($p<0.05$) as well as secondary lesion score ($p<0.05$).Conclusion:Our study suggests that ramiprilmay be used as an adjuvant anti-inflammatory agent in patients with RA. However this speculation needs to be confirmed clinically.[Munde G et al NJIRM 2013; 4(3) : 1-7]

KEY Words: Anti-inflammatory agents, Adjuvant induced arthritis, Experimental rheumatoid arthritis,Ramipril

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Introduction: Rheumatoid arthritis affects 1% of population worldwide [1,2].It is a chronic inflammatory disorder of the joint characterized by inflammation of the synovial membrane, pain, and restricted joint movement. It is poly-articular joint disease characterized by massive synovial proliferation and sub-intimal infiltration of inflammatory cells, which along with angiogenesis leads to the formation of a very aggressive tissue called 'pannus'. Expansion of the pannus induces bone erosion and cartilage thinning, leading to the loss of joint function [3].

Angiotensin-converting enzyme (ACE) inhibitors have proven clinicalefficacy in the treatment of hypertension, congestive cardiac failure,ischaemic heart disease, and renal disease. ACE convertsangiotensin I to angiotensin II, and catalyses the degradationof bradykinin and substance P. Angiotensin II plays an important role in the regulation of blood pressure and fluid homeostasis. Two distinct subclasses of the angiotensin II receptors, AT_1 and AT_2, have been described. This octapeptidehormone also has number of other effects and, in particular,autocrine and paracrine proinflammatory properties [4].

Angiotensin II has significant pro-inflammatory actions in the vascular wall, inducing the production of reactive oxygen species (ROS), inflammatory cytokines and adhesion molecules[5]. AngiotensinII signalling through AT1 receptor leads to activation ofthe transcription factor nuclear factor κB (NF-κB) with subsequent production of pro-inflammatory cytokines like TNF-α, IL-1 and IL-6, ROS and adhesion molecules[6,7].The presence and up-regulation of angiotensin converting enzyme (ACE)[8] and AT1 receptors[9] has been described in synovial samples obtained from RA patients.

An earlier study has found that ACE inhibitor captopril was clinically beneficial in the treatment of RA[10]. But the clinical benefit was attributed subsequently to the presence of a thiol-group in the compound structure (chemically similar to penicillamine) and not to ACE inhibition per se. This was supported by Bird et al who showed that the non thiol ACE inhibitor pentopril was not effective in the treatment of RA [11]. However, Dalbeth et al demonstrated therapeutic benefit of non thiol ACE inhibitor quinapril in collagen induced mouse model of arthritis [12].

In view of the different studies, our study was undertaken to find out the anti-inflammatory role of ramipril in experimental RA.

Material And Methods:Animals: Albino Wistar rats of either sex, 8-10 weeks old and weighing 150-200

grams were procured fromHaffkineBiopharma Corporation, Parel. Animals were fed with standard pellet diet and water *ad libitum*. They were housed in animal laboratory at temperature (25°C+2°C), relative humidity (45%-55%) and 12 hours light dark cycle was maintained. The experimental protocol was reviewed and approved by Institutional Animal Ethics Committee (IAEC), BYL Nair Ch. Hospital and T.N.M.C., Mumbai. All experiments carried out according to guidelines suggested by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Drugs and Chemicals: Ramiprilused in pure form (Cipla Pharmaceuticals, Mumbai), indomethacin 50 mg capsule(Drug store of BYL Nair Ch. Hospital, Mumbai) and Freund's Complete Adjuvant (Sigma Chemicals Ltd., Mumbai) were used.Ramipril(0.45 mg/kg) and indomethacin (4.5 mg/kg) was suspended in 0.3% carboxy-methyl cellulose (CMC) in distilled water for oral administration to each rat.

Study design: The study comprised of three groups of eight animals each (n=8) and the treatment given is described below:
Disease control group:1 ml suspension of 0.3% CMC in distilled water was fed daily to each rat for 21 days.
Test group:1 ml suspension of ramipril (0.45 mg/kg) in 0.3% CMC in distilled water was fed daily to each rat for 21 days.
Standard group:1 ml suspension of indomethacin (4.5 mg/kg) in 0.3% CMC in distilled waterwas fed daily to each rat for 21 days.

On day 0 of the experiment, the baseline measurements recorded were right and left hind paw edema and animal body weights. Experimental arthritis was induced on day 1. The animals were fed with the drugs orally daily for 21 days.On day 5, the right hind paw edema was measured in all the groups.On day 21, the left hind paw edema was measured. Rats were sacrificed and left hind limb joints were sent for histopathology. Secondary lesion score was noted.

Induction of adjuvant induced arthritis [13]**:** The subplantar injection of 0.1 ml of FCA suspended in oil in the right hind paw results in primary, nonimmune, localized inflammatory response in the right hind paw. This local swelling (primary lesion) begin in the injected paw within 24 hrs, reach peak on 3rd -5th day and subsides by day 8-9. This local swelling on day 5 corresponds to acute inflammatory reaction, indicating the influence of therapeutic agents on this phase.

This is followed by the secondary immune systemic response after a delay of 11-12 days after injection of FCA. Secondary lesions are characterized by inflammation of non-injected sites (left hinpaw, forepaws, ears, nose and tail), and by decrease of weight. The secondary lesions are present till day 21. Day 21 was used for the influence of drugs on this phase of RA.

Histopathology: On day 21 the animals were sacrificed by ether anaesthesia.Left hind-paws with ankle joints obtained from rats were harvested post-mortem, fixed in 10% buffered formalin, decalcifiedin 5% nitric acid for up to 48 hours, routinely processed and embeddedin paraffin wax. Sagittal sections (5 µm) were taken from the paraffin blocks and stainedwith haematoxylin–eosin (H & E) stain. The joints were studied by blinded examiner from the Pathology Department (BYL Nair Ch. Hospital, Mumbai).

Histopathologicalsynovitis grading [14]**:**The synovial inflammation was scored in 3 categories as follows: First: hyperplasia/enlargement of synovial lining cell layer.Second: activation of resident cells/synovial stroma.Third: inflammatory infiltration.Each category was scored from 0 to 3 as follows: 0 = nil, 1= mild, 2 = moderate, and 3 = severe.

Synovitis graded as grade 0 (nil), grade 1 (mild), grade 2 (moderate) and grade 3 (severe) with summaries ranging from 0 to 9 as: 0 to 1 corresponds to grade 0, 2 to 3corresponds to grade 1, 4 to 6 corresponds to grade 2 and 7 to 9 corresponds to grade 3.

Assessment of rat paw edema: Two methods were used to assess rat paw edema.

Mercury Plethysmography:A mark was put on the right and left hind limb at the malleolus to facilitate uniform dipping at subsequent readings. The rat was held with left hand and the hind limb was dipped in the mercury column up to the mark. In this study, paw volume (in ml) was expressed as displacement of mercury in plethysmograph tube in cm.

Micrometer screw gauge: Micrometer screw gauge uses an auxiliary scale (measuring hundredths of mm) which is marked on a thimble with rotating vernier scale. In order to measure the diameter of the rat paw, hind paw was placed between the jaws, and the thimble was rotated using the ratchet until the object was secured. The maximum diameter of the paw was measured in mm.

Secondary lesion score [15]**:** Secondary lesions scored as; Ears, Nose and Tail : absence of nodules (0), Presence of nodules (1). For each: Fore paws : absence of inflammation (0), Inflammation of atleast one joint (1). Hind paws : absence of inflammation (0), Slight inflammation (1), Moderate inflammation (2), Marked inflammation(3).

Secondary lesions were graded as: Grade 0 (nil), grade 1(mild), grade 2 (moderate) and grade 3 (severe)with summaries ranging from 0 to 7. 0 to 1 corresponds to grade 0, 2 to 3 corresponds to grade 1, 4 to 5 corresponds to grade 2 and 6 to 7 corresponds to grade 3.

Statistical analysis: Results are expressed as mean + SEM. One Way ANOVA followed by Tukey's post hoc test was used to analyse rat paw edema and difference in body weight between day 1 and day 21. Kruskal-Wallis test was used to analysehistopathological grades and grades of secondary lesions.*P* value less than 0.05 was considered as statistically significant.

Results: We assessed the rat paw edema by mercury-plethysmography as well as by micrometer screw gauge. On day 5 (Figure 1& 2) as well as on day 21 (Figure 3 & 4) ramipril 0.45 mg/kg significantly reduced the rat hind-paw edema on right side as compared to disease control group (p<0.001) by both methods.

Figure 1: Right Hind Paw Edema Measured On Day 0 And Day 5 By Mercury Plethysmography

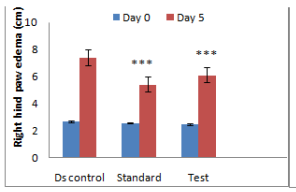
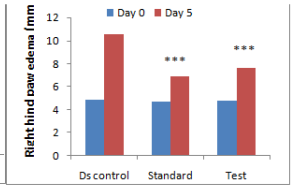


Figure 2: Right Hind Paw Edema Measured On Day 0 And Day 5 By Micrometer Screw Gauge.



Each bar is expressed as mean ± SEM. (n=8); ***p<0.001 as compared with control group; (One way ANOVA followed by Tukey's post hoc test)

Figure 3: Left Hind Paw Edema Measured On Day 0 And Day 21 By Mercury Plethysmography.

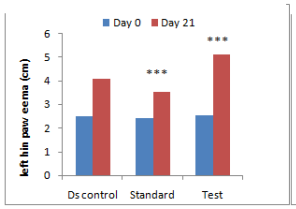
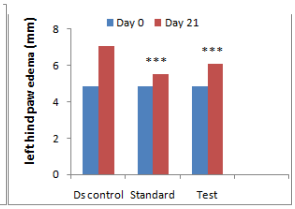


Figure 4: Left Hind Paw Edema Measured On Day 0 And Day 21 By Mercury Plethysmography



Each bar is expressed as mean ± SEM. (n=8); ***p<0.001 as compared with control group; (One way ANOVA followed by Tukey's post hoc test)

On comparing the change in the body weight from day 0 to day 21, the weight gain in indomethacin and ramipril group was significantly greater than disease control group (P<0.001) (**Table 1**).

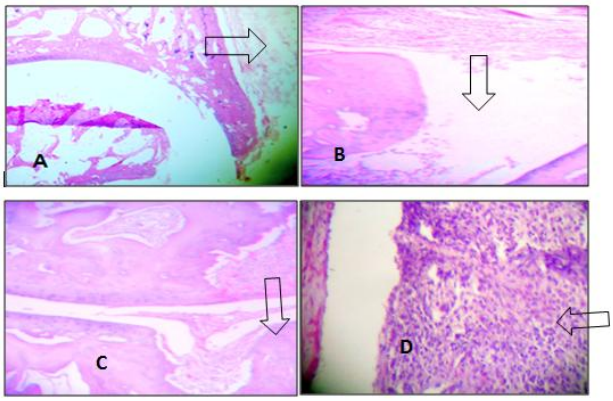
Table 1: Effect of ramiprilon histopathological grade, grade of secondary lesions and change in body weight in adjuvant induced arthritis in rats.

Groups	HSP grade	Grade of Secondary lesions	Change in body weight (day 21-day 0)
Ds control	2 ± 0.76	2.75 ± 0.74	4.62 ± 3.41
Standard	0125 ± 035***	0.5 ± 0.63***	13.75 ± 3.66***
Test	0.62 ± 0.74***	1.25 ± 0.91***	11.43 ± 3.44***

All values expressed as mean ± SEM (n=8). ***P<0.001 as compared to disease control group. (Kruskal Wallis test for HSP and secondary lesion grading. One Way ANOVA followed by Tukey's post hoc test for change in body weight). HSP: histopathology

Histo-pathological grade of synovitis and secondary lesion score were also significantly reduced by ramipril 0.45 mg/kgas compared to disease control group (P<0.001) (Table 1). The synovitis was graded in to four grades. The absence of infiltration of inflammatory cells was considered as grade 0, mild infiltration of inflammatory cells was grade 1, moderate infiltration of inflammatory cells was grade 2 and marked infiltration of inflammatory cells was grade 3 (Figure 5).

Figure 5: Histopathological grades of synovial inflammation:



Ankle joints of rats were send for histopathology to study synovial inflammation in different groups (n=8), (**A**) Inflammatory grade 0 with no inflammatory infiltration. (**B**) Inflammatory grade 1 with minimal infiltration of inflammatory cells. (**C**) Inflammatory grade 2 with moderate infiltration of inflammatory cells. (**D**) Inflammatory grade 3 with marked infiltration of inflammatory cells.

Discussion: Some of the studiesconducted for determining the role of ACE inhibitors as anti-inflammatory agents in RA showed anti-inflammatory activity[12]while some showed no anti-inflammatory activity.[16] The present study was undertaken to clarify those conflicting anti-inflammatory results about the role of AT1 receptor blockers in RA.

Ramiprilis widely prescribed antihypertensive drugs with favourable safety profile. In our study, dose of ramipril(0.45 mg/kg)used in rats was extrapolated from human doseof ramipril 5 mg[17]normally used as anti-hypertensive therapy in humans [18].

Freund's adjuvant induced arthritis in rats is the most commonly used model for experimental induction of RA [19]. This model involves immunological process for induction of arthritis which closely resembles the process of rheumatoid arthritis in human beings [20].

We assessed rat paw edema by mercury plethysmography as well as by micrometer screw gauge. On day 5 ramipril 0.45 mg/kg significantly reduced the rat paw edema on right side as compared to disease control group (p<0.001) by both methods (Figure 1 & 2). These findings suggest that ramipril 0.45 mg/kg is effective anti-inflammatory agent in acute phase of inflammation in experimental RA.

In contrast to this Caspritz et al.[21] showed that paw edema in the rat by carrageenin and kaolin partially caused by Hageman factor activation was potentiated by ramipril, due to its inhibition of kininase II which results in increased bradykinin levels.

On day 21 ramipril 0.45 mg/kgsignificantly reduced the rat paw edema on left side as compared to disease control group (p<0.001) by both methods (Figure 3 & 4).Histo-pathological grade of synovitis and secondary lesion score were also significantly reduced by ramipril 0.45 mg/kg as compared to disease control group (p<0.001) (Table 1). Above findings suggest that, Ramipril 0.45 mg/kg was effective anti-inflammatory agent in experimental rheumatoid arthritis

Rats with arthritic syndrome invariably lose weight [22,23].In our study, on comparing the change in the body weight from day 1 to day 21, the weight gain in indomethacin and ramiprilgroup was significantly greater than disease control group (p<0.05).

Similar results were obtained by Dalbeth et al.[12] with quinapril. They demonstrated the inhibition of

disease activity in collagen induced arthritis (CIA) in mice with quinapril 10 mg/kg/day. In parallel human in vitro experiments, ACE inhibition suppressed the LPS-stimulated production of TNF-α by monocytes.

Flammer et al.[24]conducted a clinical study and showed that ACE inhibitionwith 10 mg/day of ramipril for 8 weeks on top of current anti-inflammatorytreatment markedly improved endothelial function and markers of inflammation and oxidative stress in patientswith rheumatoid arthritis.They proposed that the excess in cardiovascular risk in patientswith RA provides a strong rationale for earlytherapeutical interventions.

Peeterset al.[25]proposed that despite inhibitory effects on lipopolysaccharide (LPS)-stimulated production of pro-inflammatory cytokines TNF and IL-1 in vitro, it is unlikely that captopril or valsartan could be used in anti-cytokine therapeutic strategies in vivo, since these effects are exerted mainly at high concentrations of the drugs which cannot be achieved at therapeutic concentrations. They administration of one dose of captopril (50mg) or valsartan (80mg) in therapeutic dosages to patients with essential hypertension did not influence LPS-stimulated production of cytokines by whole blood.

The anti-inflammatory actions of ramipril could be attributed to inhibitory effect on Angiotensin II production. Angiotensin II has significant pro-inflammatory actions in the vascular wall, inducing the production of ROS, inflammatory cytokines and adhesion molecules [5]. Angiotensinogen is synthesised by inflammatory cells [26]. The presence and up-regulation of angiotensin converting enzyme (ACE)[8] and AT1 receptors[9] has been described in synovial samples obtained from RA patients. AngiotensinII signalling through the AT1 and AT2 receptors leads to activation ofthe transcription factor nuclear factor κB (NF-κB) with subsequent production of pro-inflammatory cytokines like TNF-α, IL-1 and IL-6, reactive oxygen species and adhesion molecules [6,7].Our view can be supported by the assumptions made by Mindlen et al.[27] and Stella et al.[28].

In conclusion our study shows that ramipril is significant anti-inflammatory agent in experimental RA. Ramipril may be used as an adjuvant anti-inflammatory agent in patients with RA. However this speculation needs to be confirmed clinically.

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Conflict of interest: None