The effect of enalapril on inflammation and IL-1β and IL-8 production in chronic arthritis

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ABSTRACT

Background and the purpose of the study: Angiotensin II (Ang II) other than acting as a vasopressor hormone has pro-inflammatory properties. Since angiotensin-converting enzyme (ACE), is present in inflamed synovial membrane, in this study the effect of enalapril in modulation of inflammation and cytokine production in experimental induced chronic arthritis was investigated.

Methods: Chronic joint inflammation was induced by antigen-induced arthritis method in rabbits and enalapril was given orally (7.5mg/kg/day) two weeks before (prophylaxis group) or two weeks after (treatment group) induction. Serum of arthritis's ACE activity was measured by HPLC, pro-inflammatory cytokines, IL-1β & IL-8 were measured in synovial fluid, and histology of knee joints was assessed in both groups.

Results: Results revealed that enalapril reduced ACE activity in serum significantly (P=0.004), had no effect on IL-8 of synovial fluid and reduced the IL-1β production (P<0.05). Histological results revealed a significant reduction in villous hyperplasia and pannus formation (P<0.05 in both groups). While in prophylaxis group no bone erosion was observed and the cartilage was either intact or slightly invaded by synoviocytes, in non-treated group the cartilage was mostly invaded.

Conclusion: Enalapril reduces production of pro-inflammatory cytokine IL-1β and severity of joint damage in chronic arthritis and may have therapeutics benefits in inflammatory joint diseases

Keywords: Enalapril, Chronic arthritis, Cytokines, ACE inhibitors

INTRODUCTION

Angiotensin converting enzyme (ACE) is a halide activated peptidase which converts angiotensin I to angiotensin II, and catalyses degradation of bradykinin and substance P. Angiotensin II, the main hormone of rennin-angiotensin system (RAS) has also the autocrine and paracrine pro-inflammatory properties(1). Ang II acts not only as a cytokine (2) but also activates the transcription nuclear factor κB (NF-κB) (3).The subsequent production of pro inflammatory cytokines, chemokines and adhesion molecules, recruits inflammatory cells into the tissue. These cells in turn activate the RAS and increase the generation of Ang II locally, thus creating a cycle of tissue injury (4). These inflammatory pathways are critical in maintenance of disease in rheumatoid arthritis (RA), an autoimmune disease with chronic inflammation of synovial lining cells (5). Furthermore, the wide distribution of all components of RAS system in RA (6, 7), indicates that locally formed Ang II in inflamed knee joints has a pathophysiological and therapeutic implication in RA. It has been hypothesized that ACE inhibitors may have anti-inflammatory properties and can be used in treatment of arthritis. The results of previous trials using different ACE inhibitors in RA disease were variable (8-11). While the benefits of captopril have been attributed to its thiol residue, the advantage of using non-thiol ACE inhibitors in treatment of arthritis is yet uncertain. In this study the effect of a non-thiol and commonly used ACE inhibitor, enalapril, in treatment of antigen-induced arthritis in rabbits was investigated.

MATERIALS AND METHODS

Animals and study design

All experimental procedures of this study were in accordance with protocols set by the ethic committee of Kerman university of medical sciences (EC 85/40). Forty eight male New Zealand white rabbits weighting between 2-2.5 kg

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were used. All animals had free access to water and standard rabbit chow. Animals were randomly divided into 8 groups as follows (n=6 in each group):

1- Control group to measure normal level of cytokines: 2- Control group to assess histological parameters: 3- Inflamed group to measure cytokines: 4- Inflamed group to measure histological parameters: 5- Prophylaxis enalapril group to measure cytokines: 6- Prophylaxis enalapril group to assess histological parameters: 7- Treatment enalapril group to measure cytokines: 8- Treatment enalapril group to assess histological parameters

The dose of enalapril was 7.5 mg/kg once daily, which was dissolved in 2 ml of tap water (12). To ascertain that animals receive all drugs, solution was dropped by a dropping tube in their mouths. Control animals received tap water only. In prophylaxis group, enalapril was given for 6 weeks, starting two weeks prior to induction of arthritis. In the treatment group, enalapril was administered two weeks after the induction of arthritis and continued to the end of the study.

On the day of 28 of arthritis induction, rabbits were killed by cutting the neck vessels under deep anesthesia with sodium thiopental.

Induction of arthritis

Rabbits were first sensitized by intradermal injection. For this purpose, 1ml of methylated bovine serum albumin (MBSA, 4mg/ml in distilled water) homogenized with complete Freund's adjuvant (FCA, 1:1) was injected at five sites of the back neck of the animal (0.2 ml in each site) which shaved. For making this solution, MBSA solution was added drop- by- drop to FCA and vigorously shacked until a semi-viscous homogenized solution was made. To test the stability of antigen, a drop of the solution was added to the surface of a beaker containing distilled water. In the case that the drop was spread on water, a few drops of FCA was added to the solution and the mixture was shaked again until the drop did not spread over the water. The injection of antigen was repeated at the day of 14 (second booster), and then at the day of 21, and sensitization of the animal was confirmed by subcutaneous injection of MBSA solution (0.2 mg/ml) in a shaved area of leg skin. The skin thickness was measured before and 24 hours after injection using a caliper. An increase in skin thickness of at least 100% was a criterion for sensitization to antigen. Sensitized animals at the day of 28 received intra-articular injection of 0.5 ml solution of MBSA (2mg/ml) using a 1 ml syringe. The needle (28 G) was inserted through the mid-patellar tendon into the joint cavity. Half of the solution was injected deeper into the posterior space and the other half into anterior space. Control groups received 0.5 ml of sterile saline.

Measurement of knee-joint diameter

The knee-joint diameter was regularly measured during the next four weeks post intraarticular antigen injection, where chronic inflammation had developed (13). For this purpose, the medio-lateral diameter of the joint in the maximum diameter point was measured by a caliper.

Detection of synovial fluid cytokines

At the day of 28 post intra-articular injection of MBSA, the synovial fluid was lavaged from each knee of cytokine groups using 1 ml of heparinized buffered saline. The samples were centrifuged and supernatants were stored at -70 °C prior to assay. Synovial fluid IL-1β and IL-8 were detected by enzyme-linked immunosorbent assay (Elisa). Briefly, in coating stage the samples were leaved overnight in micro plates in which a special anti-rabbit antibody had been covered the surface of the wells. After blocking stage which took 2 hours, the tracer (biotin Conjugated antibody) and samples were added. In the next stage Streptoavidin Peroxoidase was added to wells and they were left for 30 minutes at room temperature. Tetramethylbenzedine (TMB) was added then as Chromogen. The stop solution was 1 M H_2SO_4. The reaction produced a color which was read in Elisa reader at 450 nm. The dilution ratio was 1:10 for IL-8 and 1:5 for IL-1β.

Measurement of plasma ACE activity

Under deep anesthesia, blood samples were collected directly from the heart of the animals before killing. Serum ACE activity was determined by high performance liquid chromatography (HPLC) (Waters, UV visible, model 486) (14).

Histological evaluation

Knee joints of the histology groups were harvested post mortem, fixed in 10% formalin, decalcified in 10% nitric acid for up to 24 h, then processed and embedded in paraffin wax. Sections of 5μm thick were stained with haematoxylin-eosin (H & E) for light microscope study. Slide preparations were investigated by two pathologists blinded to animal groups by Olympus and Seitz microscopes. The histopathological findings were scored according to the following arbitrary scores (13): synovial hyperplasia: 0; one to three layers of cells (synoviocytes), 1; four-six layers of cells, 2; seven or more layers of cells; villous hyperplasia: 0; absence , 1; few, dispersed

and short, 2; prominent and tall, 3; prominent and diffuse; mononuclear cellular infiltrates: 0; absence, 1; mild (25% of the field), 2; (50% of the field), 3; (>50% of the field), 4; diffuse with lymphoid follicle formation.

Statistical analysis

Results were expressed as mean ± S.E.M. The data were analyzed by student t test or by using one way analysis of variance (ANOVA) followed by Tukey's post hoc test. P<0.05 was considered statistically significant.

RESULTS

Effect of enalapril on knee joint diameter

Fig 1 shows the time course of changes in joint diameter in four groups of animals. A sharp rise in joint diameter was observed in inflamed groups with the peak at the day of 2. Enalapril treatment reduced the rise in diameter, where in prophylaxis group, this reduction was significant between the days 1 and 4 (Fig. 1). In all inflamed groups, the joint diameter gradually returned to normal level towards the day of 28.

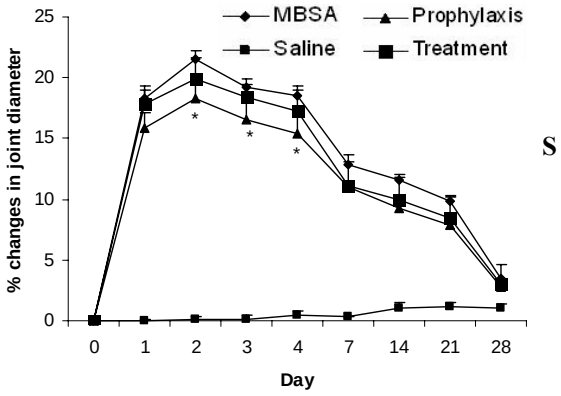

Figure 1. The effect of enalapril on knee joint diameter in the rabbit. Joint diameter was increased significantly in all antigen induced inflammatory groups compared to control (saline) group, however the increase in diameter in Prophylaxis group was significantly lower than non-treated (MBSA) group between the days of 2-4.

Effect of enalapril on plasma ACE activity

Animals receiving enalapril showed significant lower serum ACE activity compared to control group (*P*=0.004) (Fig. 2). As the ACE activity was not different between the prophylaxis and treatment groups, the data of these two groups were pooled.

Effect of enalapril on synovial fluid cytokines

In order to establish whether reduction in inflammation after enalapril administration was due to reduction in cytokine production, IL-1β and IL-8 concentrations were measured in synovial fluid. A significant reduction in IL-1 β synovial fluid was observed in both prophylaxis and treatment groups (P<0.05). Enalapril did not affect IL-8 production. (Fig. 3)

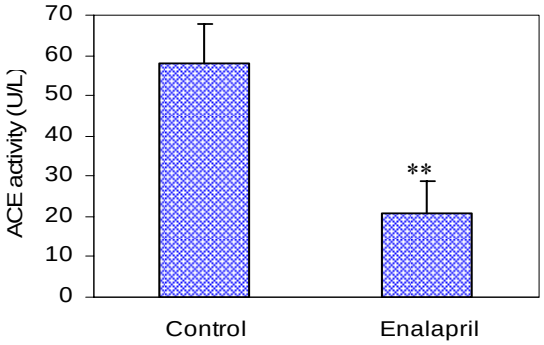

Figure 2. Effect of enalapril administration (7.5mg/kg /day through drinking water for two weeks) on serum ACE activity. Control animals received tap water only, n=12 for enalapril group, n=6 for control group (Student unpaired t test). ** = P<0.01

Histological findings

The severity of inflammation was ameliorated by administration of enalapril (Figs 4 & 5). While there was a reduction in all four histological parameters (Fig 4), it was only statistically significant for villous hyperplasia (P<0.05 for both prophylaxis and treatment groups) and pannus formation (P<0.01 in prophylaxis and P<0.05 in treatment groups).

DISCUSSION

The results of this study indicate that, non thiol ACE inhibitors, enalapril, have significant anti-inflammatory properties and can reduce the severity of arthritis. This effect is more pronounced when it is used for prophylaxis. These findings are in agreement with previous reports on the benefits of captopril in treatment of arthritis in human (8) or rabbit (13).

The clinical anti-inflammatory effects of captopril have been attributed to its thiol residue (8).To test this hypothesis, other investigators used non- thiol pentopril and in a clinical trial in 15 patients with RA it did not show any beneficial effect on disease (9). However, the number of cases in that reported study was small and in order to confirm the efficacy of a drug, at least 100 RA patients is required for a placebo- controlled double blind study. Surprisingly, in another study, in which a co-treatment of enalapril with L-NAME was used, enalapril reversed the anti-inflammatory action of L-NAME (11). The dose of enalapril in this study (0.12 mg/kg/day for two weeks prior to the induction of arthritis) was several times lower than the dose which is used in human and it is doubtful, whether at this dose of enalapril inhibits the ACE activity. However, the authors did not

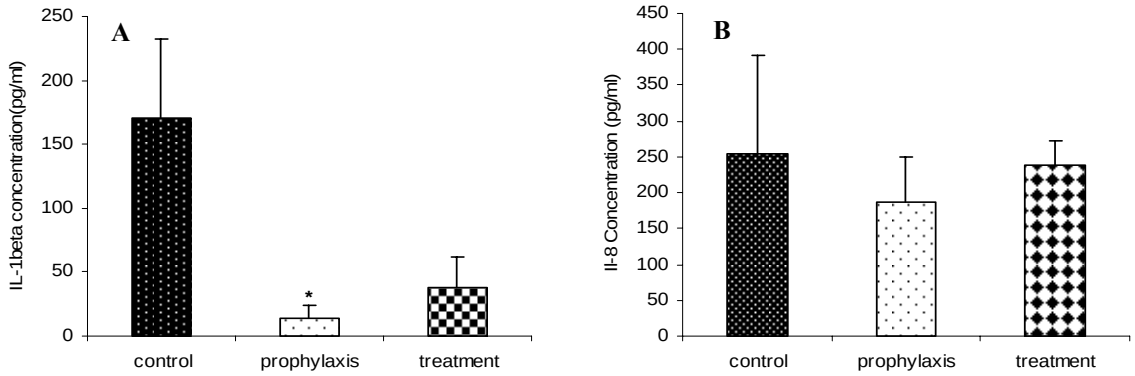

Figure 3. The effect of enalapril as prophylaxis or treatment on Il-1beta (A) and IL-8 (B) concentrations. Enalapril significantly reduced IL-1 beta production ($P<0.05$) but had no effects on IL-8 production. (n=6 in each group)

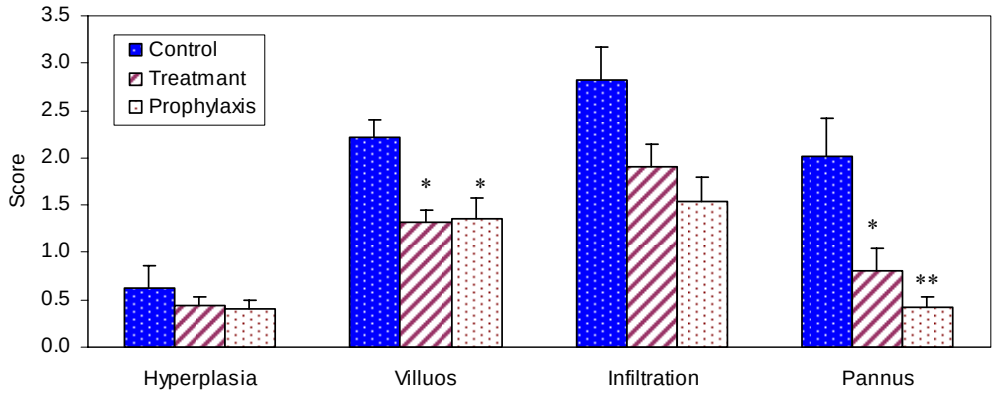

Figure 4. The effect of enalapril on histological parameters of inflammation in chronic arthritis. (n=6 for each group)

attribute the reversal of inflammation to ACE inhibition, but to bradykinin (BK) accumulation at the site of injury because the effect was completely blocked by administration of BK antagonists. A recent study has revealed significant anti-inflammatory effect for Quinapril on antigen-induced arthritis in rabbits (10).

In the present study it was shown that enalapril attenuates the severity of inflammation in rabbits (Fig 1, 4 and 5). The dose of enalapril used in this study was 7.5 mg/kg, which is 10 times higher than the usual dose of this drug in human. However, this dose did not alter rabbit physical activities, and only caused significant hypotension in animals. This dose of enalapril induced a significant reduction in plasma ACE activity (64%, Fig 2), suggesting that angiotensin II production has also been inhibited in joints. In order to establish clinical efficacy of enalapril in human arthritis, further studies are required. The results imply that Ang II production has pro-inflammatory properties, where this concept has been confirmed by several studies revealing that Ang II promotes the activation of the transcription factor NF-κB which in turn activates the inflammatory pathways (3,15-17).

In the present study, pre- treatment with enalapril significantly reduced the production of Il-1β but had no effect on 1L-8 productions (Fig 3). It is well established that, IL-8 is the most proximal cytokines, which is detected only during the acute stage of arthritis (18). IL-8 plays a causative role in PMN infiltration during early phase of arthritis (19,20). Apparently IL-8 has no effect on breakdown of proteoglycan of articular cartilage (21). In contrast, IL-1β which is a prominent inflammatory cytokine in RA plays an important role in bone destruction (22). Based on these findings, our histological results are in accordance with ineffectiveness of enalapril on 1L-8 as the infiltration rate of PMNs was not affected (Fig 4). Enalapril also reduced pannus formation significantly probably by reduction of IL-1β in synovial fluid (Figs 4 and 5).

Although suppression of inflammation in this study was significant, it was not complete even in the prophylaxis group in which enalapril was administered two weeks before induction of arthritis. One possibility is that other pro-inflammatory pathways also take part in joint inflammation. Another possible explanation is incomplete blockade of Ang II formation that may

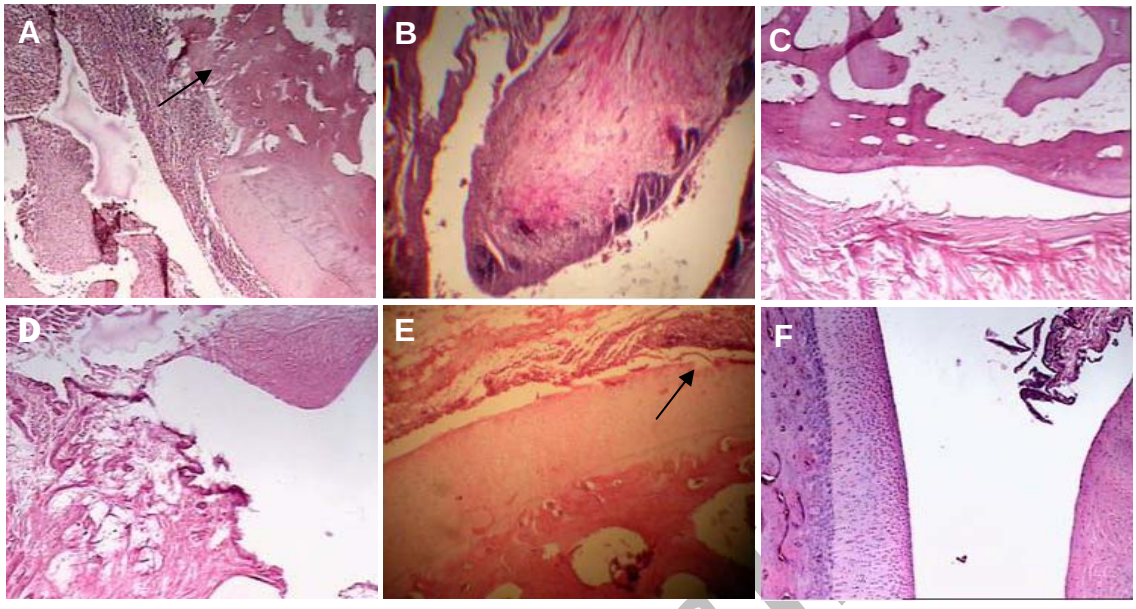

Figure 5. The effect of enalapril on Knee joint histology. The invasion of synoviocytes to bone (A) and long villous (B) were observed in chronically inflamed joints, Intact bone (C) and short and scattered villous (D) were the characteristics of prophylaxis group. In some cases of treatment group, invasion of synoviocytes was just limited to surface of cartilage (E). F: Intact surface of synovium in normal joint.
Each slide belongs to one separate animal. The arrow in A and E shows the invasion of synoviocytes to bone and cartilage respectively.

occur via chymase pathway. It is possible that during long term blockade of ACE pathway, there is an up regulation in Ang II production through chymase pathway (ACE independent Ang II production pathway). In accordance with this hypothesis, in ACE- knock out mice, local formation of Ang II remained unchanged due to the increase in chymase activity (23).

The up-regulation of chymase expression also occurs during diabetic nephropathy (24). Chymase can be synthesized and stored in mast, endothelial and mesenchymal cells (25). Activated synovial mast cells can also produce chymase (26). Therefore, a combination of ACE inhibitors, Ang II receptor blockers and chymase inhibitors are suggested in RA patients for a complete anti-inflammatory action. This combination therapy may have potential therapeutic effect on arthritis.

Although in this study the duration of enalapril administration was different in prophylaxis and treatment groups and one may concludes that the better results in prophylaxis group are due to the longer duration of treatment in this group, this may not be the case because the half life of Ang II in circulation is 30 seconds and in tissues is around 15-30 minutes (27). This means that after the first day of drug administration the level of Ang II approaches to minimum and drug administration in treatment group for 2 week is also sufficient.

In conclusion, this study showed that enalapril reduces the severity of inflammation and joint damages in chronic arthritis.

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REFERENCES

1. Suzuki Y, Ruiz-Ortega M, Lorenzo O, Ruperez M, EstebanV, Egido J. Inflammation and angiotensin II. *Int J Biochem cell Biol* . 2003; 35: 881-900
2. Sadoshima J. Cytokine actions of angiotensin II. Circ Res. 2000; 86: 1187-1199
3. Kranzhofer R, Browatzki M, Schmidt J, Kubler W. Angiotensin II activates the pro inflammatory transcription factor nuclear factor- kappa B in human monocytes. *Biochem Biophys Res Commun*. 1999; 257: 826-8
4. Ruiz-Ortega M, Lorenzo O, Suzuki Y, Ruperez M and Egido j. Proinflammatory actions of angiotensins. *Curr Opin Nephrol Hypertens*. 2001;10: 321-329
5. Feldmann M, Brennan FM, Maini RN. Role of cytokines in rheumatoid arthritis. *Annu Rev Immunol*. 1996; 14: 397-440

6. Walsh DA, Catravas J, Wharton J. Angiotensin converting enzyme in human synovium: increased stromal [(125) I]351A binding in rheumatoid arthritis. *Ann Rheum Dis* . 2000; 59: 125-31
7. Walsh DA, Suzuki T, Knock GA, Blake DR, Polak JM, Wharton J. AT1 receptor characteristics of angiotensin analogue binding in human synovium. *Br J Pharmacol* 1994; 112: 435-42
8. Martin MF, Surrall KE, Mckenna F, Dixon JS, Bird HA, Wright V. Captopril: a new treatment for rheumatoid arthritis? *Lancet*. 1984, 16; 1 (8390): 1325-1328
9. Bird HA, Le Gallez P, Dixon JS, Catalano MA, Traficante A, Liauw LA, Sussman H, Rotman H, Wright V. A clinical and biochemical assessment of a non-thiol ACE Iinhibitor (pentopril;CGS-13945)in active rheumatoid arthritis. *J Rheumatol*. 1990; 17(5): 603-8
10. Dalbeth N, Edwards J, Fairchild S, Callan M, Hall F.C. The non- thiol angiotensin- converting enzyme inhibitor quinapril suppresses inflammatory arthritis. *Rheumatology* 2005; 44: 24-31
11. Palacios FA, Novaes G.S, Guzzo M.L, Laurindo I.M. Interrelationship of the kinin system, nitric oxide and eicosanoids in the antigen-induced arthritis in rabbits. *Mediators inflamm*. 1999; 8: 245-251
12. Wojakowski W, Gminski J, Siemianowicz K, Goss M,Machalski M. The influence of angiotensin converting enzyme inhibitors on lipid peroxidation in sera and aorta of rabbits in diet-induced hypercholesterolemia. *Int J Mol Med*. 2000; 6(5): 591-4
13. Habu M, Tominaga K, Sukedai M, Alstergren P, Ohkawara S, Kopp S, Fukuda J. Immunohistochemical study of interleukin-1beta and interleukin-1 receptor antagonist in an antigen – induced arthritis of the rabbit tempomandibular joint. *J Oral Pathol Med* 2002; 31: 45-54
14. Horiuchi M, Fujimura K, Terashima T. Method for determination of angiotensin- converting enzyme activity in blood and tissue by high performance liquid chromatography. *J Chromatogr*. 1982; 233: 123-30
15. Agha AM, Mansour M. Effects of captopril on interleukin-6 leukotriene B(4), and oxidative stress markers in serum and inflammatory exudate of arthritic rats: evidence of antiinflammatory activity. *Toxicol Appl pharmacol*. 2000; 168: 123-30
16. Dagenais NJ, Jamali F. Protective effects of angiotensin II interruption: evidence for anti-inflammatory actions? *Pharmacotherapy*.2005; 25(9): 1213-29
17. Das UN. Is angiotensin- II an endogenous pro-inflammatory molecule? *Med Sci Monit*. 2005; 11(5): RA155-162
18. Sukedai M, Tominaga K, Habu M, Matsukawa A, Nishihara T, Fukuda, J. Involvement of tumor necrosis factor –alpha and interleukin-8 in antigen-induced arthritis of the rabbit tempomandibular joint. *J Oral Pathol Med*. 2004; 33(2): 102-10
19. Akahoshi T, Endo H, Kondo H, Kashiwazaki S, Kasahara T, Mukaida N, Harada A, Matsushima K. Essential involvement of interleukin-8 in neutrophil recruitment in rabbits with acute experimental arthritis induced by lipopolysaccharide and interleukin-1. *Lymphokine Cytokine Res, 1994*; 13(2): 113-6
20. Harada A, Sekido N, Akahoshi T, Wada T, Mukaida N, Matsushima K.Essential involvement of interleukin-8 in acute inflammation. *J Leukoc Biol* .1994, 56(5): 559-64
21. Endo H, Akahoshi T, Takagishi K, Kashiwasaki S, Matsushima K. Elevation of interleukin-8 (IL-8) levels in joint fluids of patients with rheumatoid arthritis and the induction by IL-8 of leukocyte infiltration and synovitis in rabbit joints. *Lymphokine Cytokine Res*. 1991; 10(4): 245-52
22. Kay J and Calabrese L .The role of interleukin-1 in the pathogenesis of rheumatoid arthritis. *Rheumatology*, 2004; 43 (Suppl 3): iii2-iii9
23. Wei CC, Tian B, Perry G Meng QC, Chen YF, Oparil S, Dell'Italia LJ. Differential Ang II generation in plasma and tissue of mice with decreased expression of the ACE gene. *Am J Physiol (Heart Circ Physiol)*, 2002; 282: H2254-H2258
24. Huang XR, Chen WY, Truong LD, Lan HY. Chymase is upregulated in diabetic nephropathy: implications for an alternative path way of angiotensin II-mediated diabetic renal and vascular disease. *J Am Soc Nephrol*. 2003; 14:1738-47
25. Nishimoto M, Takai S, Kim S Jin D, Yuda A, Sakaguchi M, Yamada M, Sawada Y, Konko K, Asada K, Iwa OH, Sasaki S, Miyazaki M. Significance of chymase- dependent angiotensin-II forming pathway in the development of vascular proliferation. *Circulation* 2001; 104: 1274-79
26. He S, Gaca MD, Walls AF. The activation of synovial mast cells: modulation of histamine release by tryptase and chymase and their inhibitors. *Eur J Pharmacol*. 2001; 412 (3): 223-9
27. William F. Ganong,. Review of Medical Physiology. McGraw-Hill, Boston, 21st Ed., 2003, pages 458-470.