

Effect of Captopril on Toll Like Receptor Expression in Adjuvant Induced Arthritis

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Abstract:

Rheumatoid arthritis (RA) is an inflammatory disease with autoimmune origin that affect joints firstly and then progress to be a systemic disease. Toll like receptor (TLR) play an important role in the evolution and progression of this disease. Captopril is an angiotensin

enzyme inhibitor (ACEI) that is widely used to control elevation in the blood pressure. This drug has anti-inflammatory activities, for this reason we try to investigate its action in RA. In this study we found that captopril decreases both expression and intensity of TLR2.

Key words: Rheumatoid arthritis, Captopril, Toll like receptor.

تأثير دواء الكابتوبريل على المستقبلات الشبيهة بالتول في الجرذان المستحثة بالتهاب
المفاصل الروماتيزمي
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الخلاصة:

التهاب المفاصل الروماتيزمي هو مرض التهابي ناتج عن كسر التحمل المناعي للجسم، يبدأ المرض في المفاصل ثم يتطور بعد ذلك ليشمل أعضاء أخرى من الجسم. تلعب المستقبلات الشبيهة بالتول دوراً مهماً في تحفيز و تفاقم هذا المرض. الكابتوبريل هو دواء يعمل كمثبط للانزيم المحول للانجيوتنسين، فهو دواء مستخدم منذ فترة طويلة لعلاج ارتفاع ضغط الدم. من جهة أخرى فإن الكابتوبريل لديه خصائص أخرى فهو يتميز بقدرته على العمل كمضاد للالتهابات وهذا ما جعل الكابتوبريل هدفاً مهماً لدراسة تأثيره على المستقبلات الشبيهة بالتول في مرض التهاب المفاصل الروماتيزمي حيث وجد في دراستنا انه يقلل الالتهاب عن طريق تقليل المستقبلات الشبيهة بالتول.
الكلمات المفتاحية: التهاب المفاصل الروماتيزمي، الكابتوبريل، المستقبلات الشبيهة بالتول.

Introduction

Rheumatoid arthritis (RA) is a chronic, inflammatory disease with autoimmune origin that affects joints in a symmetrical pattern. Polyarthritis and tenosynovitis are the main pathological pictures of the disease starting from small joints and then ascending to the larger one, and when the disease progress it involves other organs tending to be a systemic disease affecting heart, eyes, skin, lungs, and kidneys [1] [2].

The patients complaining morning stiffness, malaise, fatigue, and fever [3] [4]. The global annual prevalence of RA is confined between 0.24 and 1% with the 2:1 female to male ratio which appear to be related to hormonal factors. The prevalence of RA is more in urban than in rural populations. RA incidence varies around the world in different regions and

countries, for example in North Africa and in Middle East it accounts 13% and in Western Africa it affects 14% of population, while there is a decrease in the prevalence of RA in Central and Southern parts of Africa and it is account only 4-12% [5]. In Iraq the incidence of RA in Babylon is about 3% [6]. Both innate and adaptive immune system have a role in the evolution and progression of RA [7]. The innate effector cells, including mast cells, macrophages and natural killer cells activation is mediated by toll like receptors (TLRs) that have the ability to recognize wide range of damage associated molecular patterns (DAMP) and pathogen associated molecular patterns (PAMP) that include bacterial, viral, and putative endogenous ligands [8]. Macrophages can induce synovitis, they act through release of cytokines (e.g., TNF- α interleukin-1, 6, 12, 15, 18, and 23), reactive oxygen species (ROS), and reactive nitrogen species (RNS) [4]. Furthermore, macrophages can act through the production of prostanoids, VEGF, FGF, GM-CSF, chemokines like CC and CXC and matrix-degrading enzymes like MMPs and through phagocytosis, and antigen presentation [7]. While mast cells responsible for the production of histamine which is vasodilator and responsible for edema and joint swelling [9]. TLRs were upregulated and activated by angiotensin II and they found that ACEI decrease their activation in diabetic nephropathy. However, the exact mechanism of this activation is not fully clear yet, the inflammatory action of Ang II that enhance proinflammatory cytokines production may involve in the activation of TLRs [10].

Materials and Methods:

Captopril preparation: Captopril powder as raw material is obtained from State Company for Drugs Industry and Medical Appliance/ Samarra (SDI). Captopril solution for intraperitoneal IP injection is prepared by dissolving a given amount of captopril powder in distilled water (DW) [11]. The solution was prepared prior to injection. In our experiment we were use 300 mg /kg/ day [12]. So, each 200 g rat should receive 60 mg of captopril daily. We dissolve 1200 g of captopril in 8 ml DW. Then, each rat was injected by 0.4 ml which contain 60 mg intraperitoneally.

Experimental animal selection, housing, and feeding: Twenty-eight female albino rats weighing 200-230 g, aged 12 to 14 weeks, obtained from College of Veterinary Medicine / University of Duhok. These animals were positioned under controlled conditioned in well ventilated environment of $25\pm5^{\circ}$ C in light/ dark cycle by artificial light system. They freely reach to food and water in the animal house in College of Pharmacy/ Mustansiriyah University. This part of study is started in November 2020 and finished in January 2021. An ethical committee from the College of Pharmacy/ Mustansiriyah University was obtained before starting this study.

Experimental design: Animals was grouped in four groups; each group consist of 7 rats. Group A is the control group, rats in this group are normal animals. Group B is the induction group. While group C is the induced group receiving MTX, and group D is the induced group receiving captopril. Animal grouping are detailed in table (1).

Table (1): Animals grouping and study design.

Group	Group specification	Number of animals
Group A	Control group (normal animals)	7
Group B	Induction group	7
Group C	Methotrexate treated animals	7
Group D	Captopril treated animals	7

Induction of rheumatoid arthritis:

Freund's Complete Adjuvant (FCA) is used to induce RA in rat. FCA is obtained from Santa-Cruz Biotechnology, Inc. It contains 1mg of heat-killed dried Mycobacterium tuberculosis and liquid paraffin in each 1ml of solution as the manufacturer leaflet states. Animals in the groups B, C, and D was injected in the footpad by (0.2, 0.1, and 0.05) ml FCA on the day (0, 5, and 10) respectively. This induction method was confirmed by depending on CRP values and histopathological features. These animals were cared and at the day 14 captopril and standard therapy MTX were used ^[13]. At the day 14, group A (control group) and group B (induction group) were injected by 0.5 ml IP normal saline daily. Group C animals were treated with 1 mg/kg/day MTX, it was prepared by taking 1 ml from the vial containing 10 mg/ml and dilute it in 10 ml distilled water, the diluted solution was contained 1 mg/ ml. Each 200 g rat was injected by daily dose of 0.2 ml of the diluted MTX solution which contain 0.2 mg MTX intraperitoneally ^[14]. While group D was received 60 mg/ day (0.4 ml) IP of the captopril solution. At the 35-day, Ketamine (50 mg/ kg) IP was used to anesthetize our rats ^[15]. Then, knee joints were resected surgically in order to be studied by immunohistochemistry studies.

Immunohistochemistry staining for TLR2 expression:

When animals are sacrificed foot palms and knee joints are removed by surgical excision. Foot palms and knee joints are then following the processing techniques as below ^[16]:

a) Fixation: Soon after surgical excision, samples are stocked in 10% formalin in order to inactivate the degradative enzymes. This step last for 48 hours.

b) Softening: After 48 hours, samples are placed in 10% Nitric Acid for 15 minutes. This step is important to soften the bony structures in our samples to facilitate their cutting.

c) Cutting: In this step our samples were cut in a suitable size and from a selected parts to prepare them to the next step.

d) Dehydration: Samples then are placed in alcohol (50, 70, 90, and 100) % respectively for 2 hours in each of which, after that, samples are kept in absolute alcohol overnight. Dehydration is important step to remove water from our samples.

e) Infiltration: Melted paraffin is used in this step. Samples are immersed in melted paraffin in special molds to get wax blocks. These blocks then kept in a freezer (- 20 ° C) overnight in order to harden.

Procedure for staining:

Blocks that prepared in the previous steps were used in this procedure following manufacturer leaflet (17):

1. Trimming: Semi-automated microtome was used to trim blocks in 30–40 µm thickness sections. These sections then placed in jars containing 1-2 ml of 0.1M phosphate buffer system (PBS).

2. Antigen retrieval was done depending on manufacturer leaflet.

3. Peroxidase was added with slight agitation for 15 minutes.

4. PBS was used for washing. This process was repeated three times for 15 minutes.

5. Blocking buffer (10% normal serum plus 1% BSA in TBS) was added and incubate at room temperature for 2 hours with slight agitation.

6. Repeat step 4.

7. Diluted primary antibody was added and incubated at 4° C overnight.

8. Repeat step 4.

9. Solution containing 0.3% peroxidase in TBS was used for washing last for 15 minutes.

10. Sections were incubated at room temperature for 1 hour after the addition of secondary antibodies.

11. Repeat step 4.

12. Hematoxylin was added as counterstain.

13. Then tap water was used for slides washing.

14. Slides were kept for reading.

Assessment of the staining signal: Light microscope connected to digital camera was used to picturing our slides at 40X magnification many times. Anti-TLR2 assessment is performed by using IHC staining score system according to the equation as below ^[18]:

(Number of positive stained cells /total number of cells) * 100

Staining intensity was evaluated by using criteria scale from 0 – 1 (18)

- (0) Indicates negative stain.
- (1) Indicates weak stain signal.
- (2) Indicates moderate stain signal.
- (3) Indicates strong stain signal.

Results:

Immunohistochemistry study: There was a slight expression of TLRs in normal joint of the control group (group A), since they

are normally expressed in skin and synovium as shown in figure (1-A). The expression was increased markedly in group B (induction group) and the intensity of staining is clearly increased as a result of increase inflammatory cells infiltration to the synovium as shown in figure (1-B). It was noticed that MTX decrease the expression of TLR2 (group C) compared with the group B (20.63 % and 77.15 % respectively) as in table (2), also the intensity of staining was decreased in the MTX group (group C), as in figure (1-C). Captopril also has the ability to reduce the expression of TLR2 in the captopril group (group D) which was (36.43 %) as shown in table (2). The intensity of expression was also decreased as shown in figure (3-D).

Table (2): Numbers and percent of immune cells that stained with anti- TLR2.

Groups	Number of total cells	Number of stained cells	Percentage of stained cells
Group B	168.85 ± 5.31	130.28 ± 3.48	77.15 %
Group C	122.28 ± 3.44	25.23 ± 2.18	20.63 %
Group D	147.57 ± 2	53.76 ± 1.24	36.43 %

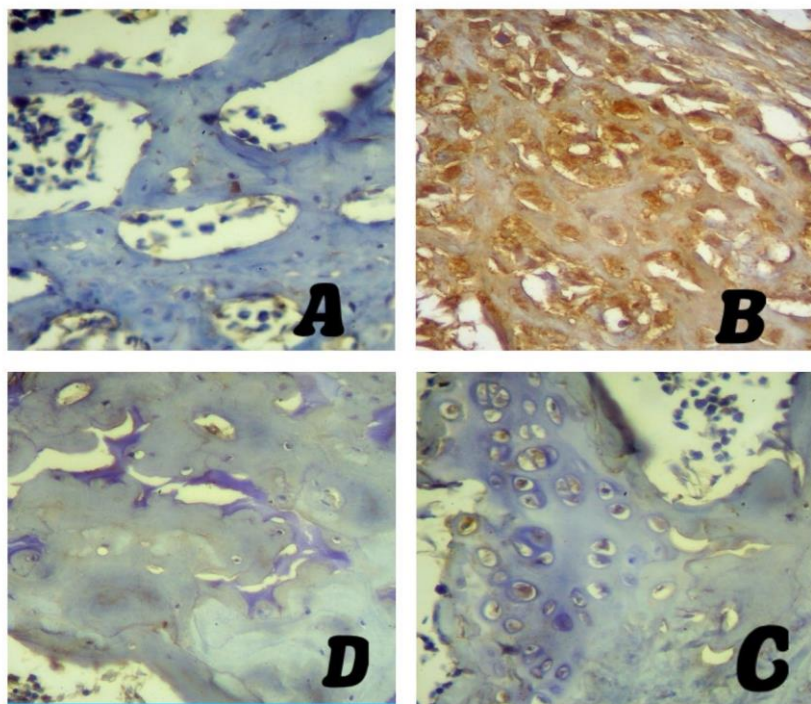


Figure (1): Immunohistochemistry staining of TLR2 expressed in knee joint of different groups. A: Control group, stain intensity: 1. B: Induction group, stain intensity 3. C: Methotrexate group, stain intensity 2. D: Captopril group, stain intensity 2.

Discussion:

Captopril effect on TLR2 expression:

Toll like receptors (TLRs) play an important role in the initiation and continuation of RA by their ability to activate antigen presenting cells (APCs), T cells, and B cells leading to release of pro inflammatory mediators. Also they enhance the expansion of autoreactive lymphocytes to detect the autoantibodies (RF and ACPA) [19]. TLRs increase the production of IL-6, IFN- γ , IFN β , and TNF- α and enhance angiogenesis through its ability to increase MMP3, CXCL10 and CCL5 [12]. TLRs mediate osteoclast survival and activity and this is because their activity in increase the production of (TNF)- α , interleukin (IL)-1, IL-6 and IL-17 which released from macrophages, monocytes and synovial fibroblast. These proinflammatory cytokines will increase expression of RANKL which in turn will enhance macrophages differentiation into osteoclast. Osteoclasts will aggravate joint destruction by causing bone resorption [20]. Feng *et al.* prove that TLRs were

upregulated and activated by Ang II and they found that ACEI decrease this activation in diabetic nephropathy, however the exact mechanism of this activation is not fully clear yet, the inflammatory action of Ang II that enhance proinflammatory cytokines production may involve in the activation of TLRs [10]. This finding was agreed with our result, we found that captopril reduce both percentage of stained cells and intensity of staining in the sections obtained from captopril group (group D) compared to induction group (group B) during IHC study as shown in table (2) and figure (1). Expression of TLR2 in the section of MTX group (group C) is also decreased in both percentage of stained cells and staining intensity compared the induction group. MTX decrease the expression of TLR in the synovial tissues, since they inhibit inflammatory cells such as T cells and microphages migration to the joint during inflammation according to Takakubo *et al.* [21].

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