

Effect of Captopril on Neoangiogenesis in Adjuvant Induced Arthritis

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ABSTRACT

Background: Rheumatoid arthritis is an inflammatory disease with an autoimmune origin that affects joints and then progresses to be a systemic disease. Angiogenesis plays an important role in the evolution and progression of this disease. Captopril is an angiotensin enzyme inhibitor that is widely used to control elevation in blood pressure. This drug has anti-inflammatory and antiangiogenic activities; for this reason, we try to investigate its action in RA.

Methods: Rheumatoid arthritis was induced by injection of Complete Freund's Adjuvant inside the footpad of female albino rats. The animals have grouped into four groups: group A considered a normal control group, group B was considered an induction group, group C was treated with methotrexate (MTX) as standard treatment, group D was treated with 300 mg/kg captopril. On day 14 of immunization, treatments began and lasted for 21 days; at the end of the experiment, all animals were sacrificed, and serum was collected. The serum markers that had been evaluated were VEGF and TSP-1.

Results Captopril significantly (p -value ≤ 0.05) decreased the serum level VEGF and significantly (p -value ≤ 0.05) increased serum level of TSP-1.

Conclusion: Captopril has a significantly beneficial role in suppressing serum pro-angiogenic factor VEGF and decreasing serum antiangiogenic factor TSP-1 as a result of decreasing TGF- β 1.

Keywords: Health, TSP-1, VEGF.

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INTRODUCTION

Rheumatoid Arthritis

Definition, Symptoms, and Prevalence

Rheumatoid arthritis (RA) is a chronic, inflammatory disease with an 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. When the disease progresses, it involves other organs tending to be a systemic disease affecting heart, eyes, and skin, lungs, and kidneys.^{1,2} The patients complained 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 is 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 the Middle East, it accounts 13%, and in Western Africa, it affects 14% of the 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

Pathogenesis of RA

Both innate and adaptive immune systems have a role in the evolution and progression of RA. The innate effector cells, including mast cells, macrophages and natural killer cells, are found in the synovial membrane, while neutrophils reside fundamentally in synovial fluid. Macrophage colony-stimulating factor, granulocyte colony-stimulating factor, and granulocyte-macrophage colony-stimulating factor (GM-CSF) enhance these cells' maturation and their efflux from the bone marrow and accumulation in the synovium.⁷ Macrophages play a central role in synovitis; they act through the 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 Macrophages, upon the activation, can also produce thrombospondin-1, which is an antiangiogenic

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mediator.⁸ After antigen presentation by antigen-presenting cells APCs (macrophages, dendritic cells, and B cells), the adaptive immunity started. APC will activate a special type of T cells called T helper cells (Th); these cells will help B cells to produce antibodies.⁹ As RA is an autoimmune disease, autoantibodies are essential in its development. B cells will produce many autoantibodies while, RF and ACPA are the most important among them.¹⁰

Angiogenesis in RA

Definition

Angiogenesis is a multistage process of the formation of new capillaries from preexisting blood vessels. Inflammation, injury or hypoxia of the tissues can induce angiogenesis. Angiogenesis occurs normally and continuously in many tissues during life, and it is essential for proper functioning of the body.¹¹

As we mentioned, angiogenesis is a multistage process, and specific factors control each stage. This process begins when VEGF and/or FGF (fibroblast growth factor) binds to their receptors VEGFR and FGFR, respectively, in endothelial cells. After their binding, they stimulate them to release matrix metalloproteinase enzymes (MMPs) that degrade the basement membrane and enhance endothelial cell proliferation and migration to the vascular tubules formed by integrins' effect.¹²

Finally, the newly formed blood vessel is stabilized by angiopoietin 1 which facilitates the incorporation of pericytes into the basement membrane to support the blood flow process.¹³

In RA, the inflammatory conditions shift the balance between pro-angiogenic and antiangiogenic factors toward angiogenesis. This will lead to further infiltration of inflammatory cells such as macrophages, neutrophils, and lymphocytes to the synovium and booster synovitis and joint destruction.¹⁴

Proangiogenic VEGF and Antiangiogenic TSP-1

Inflammatory mediators such as TNF, IL-1, IL-6, and IL-18 are released from TLR-driven M1 macrophages. These cytokines, combined with hypoxia, will activate macrophages and FLS to secrete VEGF.¹⁵ VEGF has a major role in the angiogenesis process. It enhances endothelial cell proliferation, migration, and vascular tube formation and inhibits endothelial cell apoptosis. In RA the increasing rate of leukocytes infiltration during inflammation will lead to hypoxia due to the increase in metabolic demand of these cells. These hypoxic conditions cause cytoplasmic accumulation of hypoxia-inducible factor-1 α (HIF-1 α). Then, these HIF-1 α will translocate in the nucleus and associate with HIF- β leading to VEGF expression and production from FLS and macrophages.¹⁶

Thrombospondin-1 (TSP-1) is a glycoprotein released from platelets in response to thrombin stimulation.¹⁷ TSP-1 binds to integrins receptor and CD36 that expressed on endothelial cells and inhibit their migration. TSP-1 promotes endothelial cells apoptosis by caspase-dependent pathway and decreases vascular permeability by inhibiting the nitric oxide pathway.¹⁸

Transforming growth factor- β 1 (TGF- β 1), an angiogenic growth factor, upregulates TSP-1 expression.¹⁹

Its effect on VEGF can see the obvious antiangiogenic effect of TSP-1. TSP-1 inhibits VEGF release by inhibiting MMP9 activation also it can bind to VEGF directly and enhance VEGF clearance from extracellular space.²⁰

Captopril

Captopril (D- 3- mercapto- 2- methylpropranoyl- L- proline) is the first orally active angiotensin-converting enzyme (ACE) inhibitor. ACE is the enzyme responsible for the conversion of angiotensin I (Ang I) to angiotensin II (AngII). It has been used effectively for many years to control hypertension. Besides its blood lowering capacity, Captopril has various pharmacological properties. It has immune regulating and anti-inflammatory properties. Also, it possesses anti-proliferative and antiangiogenic effects.²¹ Recent study shows that Ang II and ACE serum levels are higher in RA patients than in normal. Ang II have proinflammatory action and when it binds to its receptor AT1 expressed on inflammatory cells it will enhance production of proinflammatory cytokines such as TNF, VEGF, IL-1 β , IL-6 and inhibit FLS apoptosis via the NF- κ B pathway. Furthermore, Ang II enhances leukocytes infiltration to the joint by increasing the production of chemokines. So, Ang II will participate in articular destruction and joint damage.^{22,23} Ang II can induce upregulation of TGF- β 1, which in turn induce TSP-1 production from FLS.^{19,24} Captopril can inhibit angiogenesis by two pathway. Firstly, as we mentioned previously, its anti-inflammatory action can decrease many proinflammatory mediators such as TNF, IL-6, and VEGF that are essential for angiogenesis. Secondly, the captopril thiol group makes it potent inhibitor of metalloproteinases enzymes MMPs beside ACE. This MMPs enzyme inhibitory effect will block endothelial cell migration, an important step in angiogenesis.²⁵ Evidence shows that Captopril accelerates wound healing by its anti-inflammatory effects.²⁶ While in benign prostatic hypertrophy (BPH), captopril show an important effect against prostate enlargement by its anti-proliferative and antiangiogenic effects. It acts by decreasing VEGF and TNF levels, and it can also decrease the level of MMP, which is essential in angiogenesis.²¹

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.²⁷ The solution was prepared prior to injection. In our experiment, we used 300 mg/kg/day.²⁸ 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 with 0.4 mL, which contained 60 mg intraperitoneally.

Experimental Animal Selection, Housing, and Feeding

Thirty-five female albino rats weighing 200–230 g, aged 12 to 14 weeks, were obtained from the College of Veterinary

Medicine, University of Duhok. These animals were positioned under controlled conditions in well-ventilated environment of $25\pm 5^{\circ}\text{C}$ in a light/dark cycle by an artificial light system. They freely reach to food and water in the animal house in College of Pharmacy, Mustansiriyah University. This part of the study is started in November 2020 and finished in January 2021. Before starting this study, an ethical committee from the College of Pharmacy, Mustansiriyah University was obtained.

Experimental Design

Animals was grouped in 5 groups, each group consisting 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.

Induction of RA

Freund's Complete Adjuvant (FCA) is used to induce RA in rat. FCA is obtained from Santa-Cruz Biotechnology, Inc. The manufacturer leaflet states that it contains 1 mg of heat-killed dried *Mycobacterium tuberculosis* and liquid paraffin in each 1-mL of solution. Animals in group B, C, D, and E were injected in the footpad by (0.2, 0.1, and 0.05) mL FCA on the day (0, 5, and 10). This induction method was confirmed by depending on CRP values and histopathological features. These animals have been cared for, and at day 14, Captopril and standard therapy MTX were used.²⁹ At 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 contained 1 mg/mL. Each 200 g rat was injected by a daily dose of 0.2 mL of the diluted MTX solution, which contained 0.2 mg MTX intraperitoneally.³⁰ While group D 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.³¹ After that, blood was collected from the

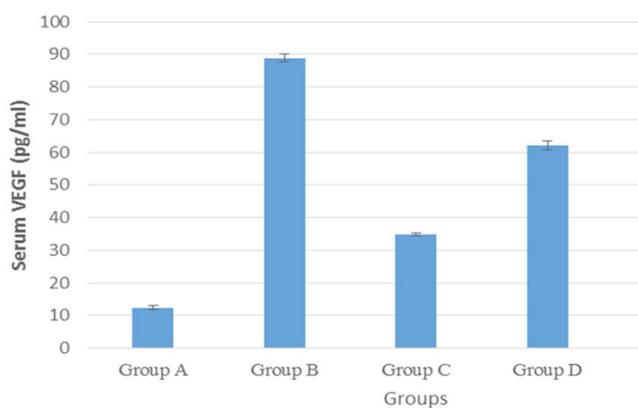


Figure 1: Changes in serum level of vascular endothelial growth factor in different treatment strategies. VEGF: Vascular Endothelial Growth Factor. Group A: negative control group, normal control. Group B: induction group. Group C: Group treated with MTX. Group D: group treated with Captopril. Data expressed as means \pm SEM.

heart, poured into a gel tube, and centrifuged at 1000 rpm/min. For 15 minutes to separate serum. Then, serum was transferred to Eppendorf tubes and kept in a freezer at (-20°C) to be used then to evaluate serum markers.³²

Serum Markers

When serum was collected, it was used to evaluate serum markers, VEGF (Komabiotech, Inc. China) and TSP-1 (Elabscience, Inc. USA).

Statistical Analysis

All data analysis (which expressed as mean \pm standard error of the mean) was performed using SPSS (V16.0), analysis of variance (ANOVA) test, in which the P-value was less than 0.05, and the statistical significance has been considered.

RESULTS

Serum VEGF

Animals in all treated groups showed a significant decrease (p -value ≤ 0.05) in VEGF levels (group C and D). Captopril-treated animals (group D) showed a decrease in VEGF level compared to the induction group animals (group B) which was (62.16 ± 1.4 pg/mL and 88.9 ± 1.27 pg/mL) respectively. Results obtained from animals that were treated with MTX (group C) exhibit a strong decline in VEGF level (34.85 ± 0.36 pg/mL) as shown in Figure 1 and Table 2.

Serum TSP-1

The serum level of TSP-1 was significantly increased (P -value ≤ 0.05) in group B compared to group A which was (5.88 ± 0.11 ng/mL and 1.27 ± 0.11 ng/mL, respectively). There was a significant decrease (p -value ≤ 0.05) in all treated animals in group C, and D, which was arisen up to (2.32 ± 0.2 ng/mL and 3.44 ± 0.16 ng/mL, respectively). Tables 3 and Figure 2 show these findings.

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

Table 2: Serum level of vascular endothelial growth factor in all groups.

Animals groups	Number of animals	Serum VEGF (pg/mL)
Group A	7	12.4 ± 0.55
Group B	7	88.9 ± 1.27
Group C	7	34.85 ± 0.36
Group D	7	62.16 ± 1.4

Table 3: Serum level of thrombospondin-1 in all groups.

Animals groups	Number of animals	Serum TSP-1 (ng/mL)
Group A	7	1.27 ± 0.11
Group B	7	5.88 ± 0.11
Group C	7	2.32 ± 0.2
Group D	7	3.44 ± 0.16

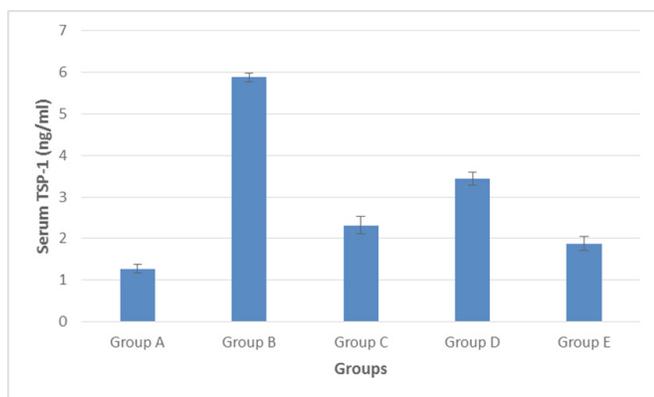


Figure 2: Serum levels of TSP-1 in different animal groups. TSP-1: Thrombospondin-1. Group A: negative control group, normal control. Group B: control positive group. Group C: Group treated with MTX. Group D: group treated with Captopril. Data expressed as means \pm SEM.

DISCUSSION

Animal Model Selection and Induction Protocol

Rat is widely used in the case of RA study because of homogeneity of genetic background with humans, low cost, and easily to be handling.³³ There are many animal models for rheumatoid arthritis such as collagen induced arthritis (CIA), adjuvant induced arthritis (AIA), streptococcal cell wall induced arthritis (SCWIA), and others.³⁴ Here, in our experiment we were use AIA as an animal model for RA induction and this done by injecting Freund's complete adjuvant (FCA) in the lower paw as discussed in chapter two. AIA can cause joint swelling, lymphocyte infiltration and cartilage degradation similar to that occurs in human. During the early few days of induction, we can notice an elevation of IL-17, IFN and TNF- α . As the severity of inflammation progresses in the joint, levels of IL-4, IL-6, and TGF- β elevation can be noticed. TNF- α , IL-1 β , IL-21 and IL-17 are all involved in the pathogenesis of RA.³³ TNF- α , IL-1, and IL-2 and other proinflammatory cytokine in articular tissues induce collagen production from synovial cells leading to cartilage and bone destruction.³⁵ This destructive effect is came from the role of these proinflammatory cytokines in increasing production of MMPs from FLS, monocytes, and endothelial cells.¹⁵ These cytokines also induce the production of chemokines such as CC and CXC responsible for inflammatory cells infiltration, upregulate TLRs expression on macrophages, and support angiogenesis through upregulation of angiogenic factors such as VEGF.^{7,15,36} If someone ask how could FCA cause all of these things and how it can induce inflammatory response, the answer is that FCA contain dried heat killed *Mycobacterium* and the active components that give mycobacteria immunogenic activity is N-acetylmuramyl-L-alanine-D-isoglutamine (MDP), this molecule activates macrophages and dendritic cells. Besides stimulating inflammation, FCA can cause aggregation and precipitation of soluble protein antigens to form particles that enhance their uptake by APCs. After antigen presentation FCA promotes Th1 to induce B cell synthesis of IgG rather than IgM,

and follows delayed hypersensitivity reactions (type IV).³⁷ All of these properties make AIA the most convenient model for our study, since we try to investigate the antiangiogenic effect and TLRs expression inhibitory action of Captopril.

Rat is widely used in case of RA study because of homogeneity of genetic background with human, low cost, and easily to be handling. There was no animal model that similar to RA in human completely but we try to select the least dissimilar model like Adjuvant Induced Arthritis (AIA) in order to perform our study. This model is performed by injecting Freund's Complete Adjuvant (FCA) in the lower paw as explained in chapter two. AIA is characterized by rapid onset of the inflammation and it can progress to involve more joint that distal from the site of the injection of FCA. After about 10–14 days of induction the manifestation of RA can be predicted such as T lymphocytes infiltration, joint swelling, cartilage degradation, and bone resorption which are similar to that occurs in human. In the early stages of inflammation we can find many inflammatory mediators in the joint that involved in the pathogenesis of RA such as IL-17, IFN and TNF- α and in the later stages IL-4, IL-6, and TGF- β are also detected.³³

Captopril Effects on VEGF and TSP-1 in RA

Angiogenesis is a multistage process of formation new capillaries from preexisting blood vessels. Inflammation, injury or hypoxia of the tissues can induce angiogenesis like that occurs during RA.¹¹ Many factors control this process some of them are pro-angiogenic others are antiangiogenic, in normal conditions these factors are in equilibrium state and any disruption in this state will shift it toward angiogenesis.¹⁴ VEGF and TSP-1 were used in our study to investigate the effect of Captopril on the balance between them and its effect on angiogenesis in general. Angiogenesis was occurs during RA and play a major role in pathogenesis of this disease.³⁸ Inflammatory mediators such as TNF, IL-1, IL-6, and IL-18 and hypoxic state in RA will induce macrophages and FLS to secrete VEGF.¹⁵ Increase leukocytes infiltration during inflammation will cause hypoxic state due to increase metabolic demand this will cause accumulation of HIF-1 α in the cell cytoplasm which in turn will translocate in the nucleus and enhance VEGF production from macrophages and FLS. VEGF can increase angiogenesis by its ability to enhance endothelial cell proliferation, migration and vascular tube formation as well as inhibit endothelial cell apoptosis.¹⁶ On the other hand when we talking about TSP-1, it binds to integrins receptor and CD36 that expressed on endothelial cells and inhibit their migration. TSP-1 promote endothelial cells apoptosis by caspase dependent pathway and decrease vascular permeability by inhibition of nitric oxide pathway.¹⁸ In addition TSP-1 inhibit VEGF release by inhibiting MMP9 activation, also it can bind to VEGF directly and enhance VEGF clearance from extracellular space.²⁴ TSP-1 level was increased during RA as a response to the inflammatory conditions.³⁹ Captopril have antiangiogenic effect since it can reduce the production of VEGF and by inhibiting MMPs and this was noticed in our experiment.²⁵ Animals of the induction group (group B) shows

the greatest elevation in serum VEGF levels and this is due to the direct inflammatory effect of FCA due to the excessive production VEGF from the inflammatory cells.³³

We found Captopril had an inhibitory effect on serum VEGF levels in the group D, it significantly (p -value ≤ 0.05) decrease serum VEGF levels compared with the group B. This finding it is completely agree with Rha *et al* which found Captopril decrease VEGF levels.⁴⁰ MTX group (group C) will exhibit a significant decrease in VEGF levels (p -value ≤ 0.05) in comparison with group B. MTX inhibit production of VEGF from the inflammatory cells and prevent VEGF from binding to its receptor.⁴¹ Roekevisch *et al.* shows that MTX have antiangiogenic effect and it can decrease VEGF and this is agree with our results.⁴² When we talking about TSP-1, we found that group B (induction group) show significant increase (p -value ≤ 0.05) in the serum level of TSP-1 and this is due to the inflammatory condition occurs during RA. Macrophages and endothelial cells enhance production of TSP-1 as a compensatory mechanism in order to prevent excessive damage during the immune response.⁸ Captopril significantly decrease the level of TSP-1 (p -value ≤ 0.05) and this reduction in serum level is due to the suppression effect of Captopril on Ang II formation. Ang II induce production of TGF- β 1 which in turn induce production of TSP-1.^{19,24} This finding was agree with Lanz *et al.*, they approved that TSP-1 elevated under the effect of Ang II during brain inflammation.⁴³

There was also significant decrease (p -value ≤ 0.05) in TSP-1 level in group C (MTX group) in comparison with group B and this is due to the reducing effect of MTX on TGF- β 1.⁴⁴ This results indicate that Captopril inhibit angiogenesis by its inhibitory effect on VEGF and TGF- β 1 and not by its effect on TSP-1 since we notice that captopril decrease the serum level in the animals receiving Captopril.

REFERENCE

- Chua-Aguilera CJ, Möller B, Yawalkar N. Skin Manifestations of Rheumatoid Arthritis, Juvenile Idiopathic Arthritis, and Spondyloarthritis. *Clinic Rev Allerg Immunol.* 2017;53(3):371–93.
- Shaw M, Collins BF, Ho LA, Raghu G. Rheumatoid arthritis-associated lung disease. *Eur Respir Rev.* 2015;24(135):1–16.
- Schmalz G, Noack S, Patschan S, Patschan D, Müller GA, Rupprecht A, *et al.* Disease activity, morning stiffness and missing teeth are associated with oral health-related quality of life in individuals with rheumatoid arthritis. *Clin Oral Invest.* 2020;24(10):3559–66.
- Alam J, Jantan I, Bukhari SNA. Rheumatoid arthritis: Recent advances on its etiology, role of cytokines and pharmacotherapy. *Biomedicine & Pharmacotherapy.* 2017;92:615–33.
- Almoallim H, Al Saleh J, Badsha H, Ahmed HM, Habjoka S, Menassa JA, *et al.* A Review of the Prevalence and Unmet Needs in the Management of Rheumatoid Arthritis in Africa and the Middle East. *Rheumatol Ther.* 2020.
- Alkazzaz AMH. Incidence of Rheumatoid Arthritis [2001 to 2011]. 2013;(4):5.
- McInnes IB, Schett G. The Pathogenesis of Rheumatoid Arthritis. *New England Journal of Medicine.* 2011;365(23):2205–19.
- Lopez-Dee Z, Pidcock K, Gutierrez LS. Thrombospondin-1: Multiple Paths to Inflammation. *Mediators of Inflammation.* 2011;11.
- Rao DA, Gurish MF, Marshall JL, Slowikowski K, Fonseka CY, Liu Y, *et al.* Pathologically expanded peripheral T helper cell subset drives B cells in rheumatoid arthritis. *Nature.* 2017;542(7639):110–4.
- Volkov M, Schie KA van, Woude D van der. Autoantibodies and B Cells: The ABC of rheumatoid arthritis pathophysiology. *Immunological Reviews.* 2020;294(1):148–63.
- Swidrowska-Jaros J, Smolewska E. A fresh look at angiogenesis in juvenile idiopathic arthritis. *cejoj.* 2018;43(3):325–30.
- Liu G, Chen T, Ding Z, Wang Y, Wei Y, Wei X. Inhibition of FGF-FGFR and VEGF-VEGFR signalling in cancer treatment. *Cell Proliferation.* 2021;54(4).
- Payne LB, Zhao H, James CC, Darden J, McGuire D, Taylor S, *et al.* The pericyte microenvironment during vascular development. *Microcirculation [Internet].* 2019;26(8).
- Rahat MA, Safieh M, Simanovich E, Pasand E, Gazitt T, Haddad A, *et al.* The role of EMMPRIN/CD147 in regulating angiogenesis in patients with psoriatic arthritis. *Arthritis Research & Therapy.* 2020;22(1):240.
- Elshabrawy HA, Chen Z, Volin MV, Ravella S, Virupannavar S, Shahrara S. The pathogenic role of angiogenesis in rheumatoid arthritis. *Angiogenesis.* 2015;18(4):433–48.
- Nakagawa T, Li JH, Garcia G, Mu W, Piek E, Böttinger EP, *et al.* TGF- β induces proangiogenic and antiangiogenic factors via parallel but distinct Smad pathways. *Kidney International.* 2004;66(2):605–13.
- Aburima A, Berger M, Spurgeon BEJ, Webb BA, Wraith KS, Febbraio M, *et al.* Thrombospondin-1 promotes hemostasis through modulation of cAMP signaling in blood platelets. *Blood.* 2021;137(5):678–89.
- Kazerounian S, Lawler J. Integration of pro- and anti-angiogenic signals by endothelial cells. *J Cell Commun Signal.* 2018;12(1):171–9.
- Liu Y, Lv H, Tan R, An X, Niu X-H, Liu Y-J, *et al.* Platelets Promote Ang II (Angiotensin II)-Induced Atrial Fibrillation by Releasing TGF- β 1 (Transforming Growth Factor- β 1) and Interacting With Fibroblasts. *Hypertension.* 2020;76(6):1856–67.
- Zhao C, Isenberg JS, Popel AS. Transcriptional and Post-Transcriptional Regulation of Thrombospondin-1 Expression: A Computational Model. *PLOS Computational Biology.* 2017;13(1).
- Mostafa F, Mantawy EM, Azab SS, El-Demerdash E. The angiotensin converting enzyme inhibitor captopril attenuates testosterone-induced benign prostatic hyperplasia in rats; a mechanistic approach. *European Journal of Pharmacology.* 2019 Dec;865:172729.
- Pattacini L, Casali B, Boiardi L, Pipitone N, Albertazzi L, Salvarani C. Angiotensin II protects fibroblast-like synoviocytes from apoptosis via the AT1-NF- κ B pathway. *Rheumatology.* 2007;46(8):1252–7.
- Braz NFT, Pinto MRC, Vieira ÉLM, Souza AJ, Teixeira AL, Simões-e-Silva AC, *et al.* Renin-angiotensin system molecules are associated with subclinical atherosclerosis and disease activity in rheumatoid arthritis. *Modern Rheumatology.* 2021;31(1):119–26.
- Suzuki T, Iwamoto N, Yamasaki S, Nishino A, Nakashima Y, Horai Y, *et al.* Upregulation of Thrombospondin 1 Expression in Synovial Tissues and Plasma of Rheumatoid Arthritis: Role of

- Transforming Growth Factor- β 1 toward Fibroblast-like Synovial Cells. *J Rheumatol*. 2015;42(6):943–7.
25. Volpert OV, Ward WF, Lingen MW, Chesler L, Solt DB, Johnson MD, *et al*. Captopril inhibits angiogenesis and slows the growth of experimental tumors in rats. *J Clin Invest*. 1996;98(3):671–9.
 26. Valachova K. Skin wound healing with composite biomembranes loaded by tiopronin or captopril. *Journal of Biotechnology*. 2020;5.
 27. Spertini F, Brunner H R, Waeber B, Gavras H. The opposing effects of chronic angiotensin-converting enzyme blockade by captopril on the responses to exogenous angiotensin II and vasopressin vs. norepinephrine in rats. *Circulation Research*. 1981;48(5):612–8.
 28. Liu H-M, Wang K-J. Therapeutic effect of Captopril on rheumatoid arthritis in rats. *Asian Pacific Journal of Tropical Medicine*. 2014;7(12):996–9.
 29. Saadon A, Alabbassi M, Nadia D, Mohammed H. Effect of Methanol Watery Extract of *Cuscuta Chinensis* in Rheumatoid Arthritis Induced Rats. *Journal of Pharmaceutical Research International*. 2020;12:1596–607.
 30. Wahba MGF, Messiha BAS, Abo-Saif AA. Protective effects of fenofibrate and resveratrol in an aggressive model of rheumatoid arthritis in rats. *Pharmaceutical Biology*. 2016;54(9):1705–15.
 31. Struck MB, Andrutis KA, Ramirez HE, Battles AH. Effect of a Short-term Fast on Ketamine–Xylazine Anesthesia in Rats. *Journal of the American Association for Laboratory Animal Science*. 2011;50(3):344–8.
 32. Wang X, He X, Zhang C-F, Guo C-R, Wang C-Z, Yuan C-S. Anti-arthritis effect of berberine on adjuvant-induced rheumatoid arthritis in rats. *Biomedicine & Pharmacotherapy*. 2017;89:887–93.
 33. Choudhary N, Bhatt LK, Prabhavalkar KS. Experimental animal models for rheumatoid arthritis. *Immunopharmacology and Immunotoxicology*. 2018;40(3):193–200.
 34. Damerau A, Gaber T. Modeling Rheumatoid Arthritis In Vitro: From Experimental Feasibility to Physiological Proximity. *IJMS*. 2020;21(21):7916.
 35. Liu Y, Lin H, Zou R, Wu J, Han R, Raymond LN, *et al*. Suppression of complete Freund's adjuvant-induced adjuvant arthritis by cobratoxin. *Acta Pharmacol Sin*. 2009;30(2):219–27.
 36. Rana AK, Li Y, Dang Q, Yang F. Monocytes in rheumatoid arthritis: Circulating precursors of macrophages and osteoclasts and, their heterogeneity and plasticity role in RA pathogenesis. *International Immunopharmacology*. 2018;65:348–59.
 37. Martiñón S, Cisneros A, Villicaña S, Hernández-Miramontes R, Mixcoha E, Calderón-Vargas P. Chemical and Immunological Characteristics of Aluminum-Based, Oil-Water Emulsion, and Bacterial-Origin Adjuvants. *Journal of Immunology Research*. 2019;2019:e3974127.
 38. Turner JD, Filer A. The role of the synovial fibroblast in rheumatoid arthritis pathogenesis. *Current Opinion in Rheumatology*. 2015;27(2):175–82.
 39. Xing T, Wang Y, Ding W, Li Y, Hu X, Wang C, *et al*. Thrombospondin-1 Production Regulates the Inflammatory Cytokine Secretion in THP-1 Cells Through NF- κ B Signaling Pathway. *Inflammation*. 2017;40(5):1606–21.
 40. Rha EY, Kim JW, Kim JH, Yoo G. Angiotensin-Converting Enzyme Inhibitor, Captopril, Improves Scar Healing in Hypertensive Rats. *Int J Med Sci*. 2021;18(4):975–83.
 41. Pu J, Fang F-F, Li X-Q, Shu Z-H, Jiang Y-P, Han T, *et al*. Matrine Exerts a Strong Anti-Arthritic Effect on Type II Collagen-Induced Arthritis in Rats by Inhibiting Inflammatory Responses. *International Journal of Molecular Sciences*. 2016;17(9):1410.
 42. Roekevisch E, Szegedi K, Hack DP, Schram ME, Res PCJM, Bos JD, *et al*. Effect of immunosuppressive treatment on biomarkers in adult atopic dermatitis patients. *Journal of the European Academy of Dermatology and Venereology*. 2020;34(7):1545–54.
 43. Lanz TV, Ding Z, Ho PP, Luo J, Agrawal AN, Srinagesh H, *et al*. Angiotensin II sustains brain inflammation in mice via TGF- β . *J Clin Invest*. 2010;120(8):2782–94.
 44. Zdanowska N, Owczarczyk-Saczonek A, Czerwińska J, Nowakowski JJ, Kozera-Żywczyk A, Owczarek W, *et al*. Adalimumab and methotrexate affect the concentrations of regulatory cytokines (interleukin-10, transforming growth factor- β 1, and interleukin-35) in patients with plaque psoriasis. *Dermatologic Therapy*. 2020 Nov;33(6).