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ARTICLES

THE POTENCY OF ALPHA LIPOIC ACID AS ANTI INFLAMMATORY ON THE COMPLETE FREUND'S ADJUVANT-INDUCED RHEUMATOID ARTHRITIS IN RAT MODEL

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ABSTRAK

Rheumatoid arthritis (RA) adalah penyakit autoimun yang ditandai dengan peradangan kronis dari jaringan sinovial pada sendi. Penelitian ini dirancang untuk menyelidiki efek dari alpha lipoic sebagai antioksidan pada tikus dengan lengkap Freund adjuvant (CFA) -diinduksi RA dengan suntikan artikular intra dari adjuvant lengkap Freund (CFA). ALA diberikan secara oral sekali sehari selama 7 hari pada 30, 60 dan 120 mg dosis seminggu setelah CFA injeksi. Tingkat keparahan arthritis dievaluasi dengan diameter bersama dan waktu latency pada stimulasi thermal. Joint diameter dan waktu latency pada stimulasi thermal akan diukur pada hari 0, 3, 5, 7, 10, 12 dan 14. Pengukuran malondialdehid (MDA) tingkat dalam plasma dilakukan dengan menggunakan metode asam thiobarbituric (TBA) untuk menilai peroksidasi lipid. Histologi sendi diperiksa dengan mikroskop berikut hematoxylin-eosin pewarnaan. Hasil penelitian menunjukkan bahwa pengobatan dengan ALA pada 30 mg dan 60 mg secara signifikan menurun diameter bersama dibandingkan dengan kelompok CFA (p = 0,003; p = 0,001 masing-masing) dan waktu latency tikus pada stimulasi thermal juga secara signifikan meningkat dibandingkan kelompok CFA (p = 0,015; p = 0,026 masing-masing). Pengukuran MDA dalam kelompok CFA dan kelompok ALA tidak memiliki perbedaan yang signifikan. Pewarnaan histologis menunjukkan bahwa pemulihan selaput sinovial dari sendi dalam kelompok ALA tidak berpengaruh. Hasil penelitian menunjukkan bahwa ALA memiliki efek untuk menekan perkembangan peradangan pada RA tetapi tidak melalui jalur stres oksidatif. ***(FMI 2016;52:98-103)***

Kata kunci: *arthritis rheumatoid, adjuvant lengkap Freund, alpha lipoic acid, antioksidan*

ABSTRACT

Rheumatoid arthritis (RA) is an autoimmune diseases which is characterized by chronic inflammation of the synovial tissue in joints. This research was designed to investigate the effect of alpha lipoic acid as antioxidant on rats with complete freund's adjuvant (CFA)-induced RA by intra articular injection of complete freund's adjuvant (CFA). ALA was administered orally once a day for 7 days at 30, 60 and 120 mg doses a week after CFA injection. The severity of arthritis was evaluated by joint diameter and latency time on thermal stimulation. Joint diameter and latency time on thermal stimulation will measured on day 0, 3, 5, 7, 10, 12 and 14. Measurement of malondialdehyde (MDA) level in plasma was performed using thiobarbituric acid (TBA) method to assess lipid peroxidation. Histology of joint was examined by microscope following hematoxylin-eosin staining. The result showed that treatment with ALA at 30 mg and 60 mg significantly decreased the joint diameter compared to CFA group (p=0.003; p=0.001 respectively) and rat's latency time on thermal stimulation was also significantly increased compared to CFA group (p=0.015; p=0.026 respectively). Measurement of MDA in CFA group and ALA group had no significant difference. Histological staining indicated that the recovery of the synovial membranes of joint in ALA group had no effect. Results indicated that ALA has the effect to suppress the development of inflammation in RA but not through oxidative stress pathway. ***(FMI 2016;52:98-103)***

Keywords: *Rheumatoid Arthritis, Complete freund's adjuvant, alpha lipoic acid, antioxidant*

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INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disorder that is characterized by chronic inflammatory of joints and effecting primarily synovial membrane. The cardinal features of RA include pain, swelling, morning stiffness (commonly more than an hour), warmth, redness. Additional features present include malaise, tiredness and night pain (Miossec & Tebib 2006, Dubey & Adebajo 2008, Orhan et al 2013). Prevalance of RA is 0.5-1% from population of world and increases at age 40 until 70 years (Saxena et al 2014, Wahl & Schuna 2014).

Chronic inflammatory in RA produce cytokines such as tumor necrosis factor (TNF)-α and interleukin (IL)-1

that leading to synovial fluid (Bhowmick et al 2008, Dubey et al 2008, Emery 2011). These cytokines bind to their receptors and activate the NFκB pathway. Activation of this pathways could produce matrix metalloproteinase (MMP) enzym. MMP is a destructive enzyme to joint and could be the trigger of RA (Malemud 2009, Waldburger & Firestein 2009). IL-1 and TNF-α could also increase influx of neutrophils into synovial fluid. Neutrophils produces reactive oxygen species (ROS) by NADPH oxidase (Tsuji et al 2006, Wright et al 2010). ROS is a small molecule which has an unpaired electron that could attact polyunsaturated fatty acids (PUFA) and cause lipid peroxidation. Malondialdehyde (MDA) is a product of lipid peroxide-tion (Stadtman & Baerlett 2002, Afonsu et al 2007, Ishibashi 2013). Even though the etiology of RA is not fully known yet, ROS had been suspected to play a role in RA. Report by Jaswal et al (2003) increasing levels of MDA have been found in RA patients and it was affected by the increasing of the ROS level.

An antioxidant could be involved in lowering the MDA level. Although endogenous antioxidant is a radical scavenger for ROS, when the number of endogenous antioxidants are not as many as radical scavengers exogenous antioxidant is required. ALA is one of the antioxidants having potential benefit in chronic disease. ALA is dithiol compound synthesized enzymatically in the mitochondrion from octanoic acid. It acts as a co-factor for mitochondrial α-ketoacid dehydrogenases in energy metabolism. Now, it's marketed widely and used as a therapy for preventing and could be anti-inflammatory for diabetic polyneuropathy (Shay et al 2009, Golbidi 2011). To evaluate the potency of ALA as antioxidant therapy in RA, a rat model of RA was made by intra articular injection of CFA into the knee joint. Indicators of inflammation were evaluated by latency time on thermal stimulation that showed hyperalgesia and joint diameter to infer articular swelling. This study also measured MDA level as an indicator of oxidative stress and observed the changes of synovial membrane in the RA model by histophatology evaluation hematoxylin-eosin staining.

MATERIALS AND METHODS

Materials

α-lipoic acid (ALA) were obtained from PT. Dexa Medika (Palembang, Indonesia), complete freund's adjuvant (CFA) (Sigma Aldrich), saline, 2-thiobarbituric acid (TBA) (Sigma-Aldrich). Reagents for MDA: trichloroacetic acid (TCA), HCl, NaOH (Merck), TEP (TCI) and other reagents, such as propylene glycol, formaldehyde, and aquadest were obtained from PT. Bratachem (Surabaya, Indonesia).

In vivo study

8-12 weeks old male rats were used for the experiments. Rats were housed in individual cages and maintained under 12-hours light/dark cycle with food and water ad libitum. The animal were habituated to the apparatus before the onset of the experiments. The experimental procedures followed the guidelines for the care and use of laboratory animals and the study was approved by the Animal Ethics Committee, Faculty of Veterinary, Airlangga University, Indonesia.

Rats were allocated randomly and divided into five groups, i.e saline (n=6), CFA (n=6), CFA-treated (n=18) groups. As a rat model of RA, the rats were intra-articularly injected with 25 μL of CFA and saline as a control. In the CFA-treated group, rats received 30, 60 and 120 mg/kg ALA orally. ALA was administered orally once a day for 7 days after a week of CFA administration. Rats were sacrificed on day 14. Blood was collected by venipuncture into EDTA. After centrifugation (3000 rpm for 25 minutes) plasma was immediately kept at -20°C. The joint were separated and kept in 10% neutral buffered formalin for 24 hours at room temperature.

Measurements were performed on day 0 (before any treatment) then on day 3, 5, 7, 10, 12 and 14. Joint diameter was measured to infer joint swelling as an indicator of inflammation. Joint diameter was measured with manual caliper. For latency time on thermal stimulation, rats were placed on the hot plate with the temperature adjusted to 51,5°C. The latency to the first paw licking, escape behavior (jump), rearing, tapping were taken as an index of nociceptive treshold.

Plasma MDA level was determined as thiobarbituric acid-reactive substances (TBARS) using spectrophotometer λ 529 nm. Briefly, 0.5 mL plasma was placed in 10 mL volumetric flash. Then, 0.5 mL TCA, 1.2 mL NaTBA 1%, and HCl 0.1 N ad 10 mL were added. The tubes were mixed and incubated in a waterbath at 900 C for 30 minutes followed by rapid cooling and then centrifuged at 3000 rpm for 15 min. The absorbance was measured photometrically at 529 nm in the supernatant and the concentrations were expressed as μmol/L.

Following sacrification, ankle joints were collected into 10% formalin solution for at least 24 hours before placement in decalcification solution containing EDTA 7,4 for 1 month, softening of the tissue was controlled by punching regularly. The tissues were embedded in parafin wax and sections were cut at 5 μm thickness and

stained with hematoxylin-eosin by routine procedures. Then, changes of histomorphological in the joints from CFA group compared to naive group.

Statistical snalysis

Values were expressed as means ± standard deviation. One way ANOVA was performed to test the significance of the difference between the naive and ALA groups. When F values were found significant ($P<0.05$), Tukey's procedure for multiple range tests was then conducted.

RESULTS

Joint diameter and hot plate test

Signs of RA model were detected after CFA intra articular injection, it showed an increase in joint diameter and a decrease of latency time on thermal stimulation compared to naive group. Figure 1 and 2 showed a week after CFA induction, ALA administration orally once a day for 7 days improves the condition of RA which is characterized by a decrease in joint diameter and an increase of latency time on thermal stimulation. ALA at doses 30 and 60 mg/kgBW significantly decreased the ratio of joint diameter ($p=0.003$; $p=0.001$ respectively) compared to CFA group (fig.1). Latency time of ALA group on thermal stimulation was increased compared to CFA group (fig.2). The difference were observed on day 10 and 12 at dose 30 mg/kgBW ($p=0.039$; $p=0.015$, respectively) and on day 12 ALA 60 mg/kgBW compared to CFA group ($p=0.026$).

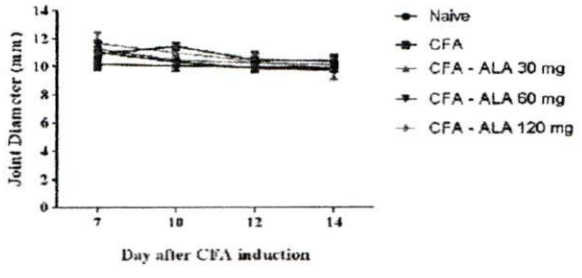

Fig. 1 Joint swelling assessed by measuring the joint diameter. CFA-induced rats showed an increased in joint diameter after injection. ALA administration orally once a day for 7 days at 30, 60 or 120 mg/kgBW doses one week after CFA injection could decreased joint diameter.

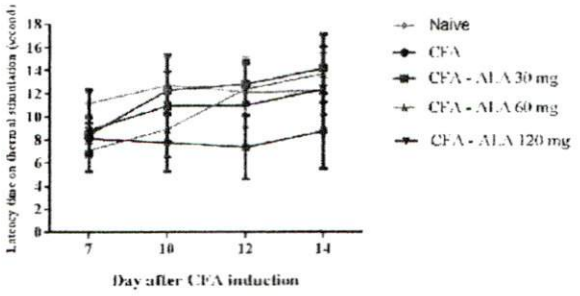

Fig. 2. Latency time on thermal stimulation as a sign of hyperalgesia. CFA-induced rats showed decreased in latency time on thermal stimulation after injection. ALA administration orally once a day for 7 days at 30, 60 or 120 mg/kgBW doses one week after CFA injection could increased latency time on thermal stimulation.

Plasma MDA level

Lipid peroxidation was identified by MDA level. None of the groups receiving CFA injection and ALA administration (30, 60 and 120 mg/kgBW) showed significant alterations in MDA level compared to the CFA group ($p=0.989$; $p=0.999$; $p=1.000$, respectively).

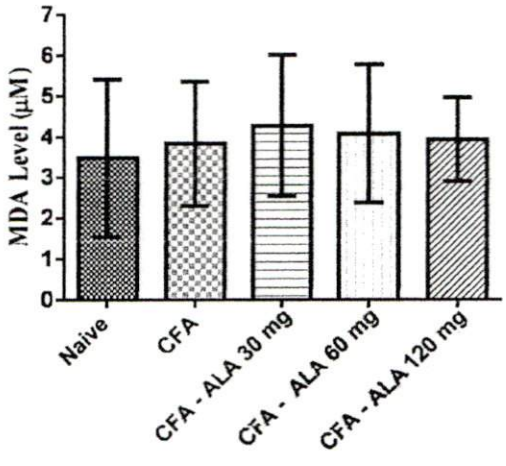

Fig. 3. Plasma MDA level in rats after CFA intra articular injection and ALA administration orally once a day for 7 days at 30, 60 or 120 mg/kgBW doses a week after CFA injection. Plasma MDA level was determined by TBARS method using spectrophotometer UV-Vis λ 529 nm.

Effect of ALA on sinoviocyte proliferation

Sinoviocyte proliferation in synovial membrane was observed by hematoxylin-eosin staining in all groups. Effect of ALA on sinoviocytes proliferation in rat's synovial membrane after CFA intra articular injection and ALA administration orally once a day for 7 days at 30, 60 or 120 mg/kgBW doses one week after CFA injection in fig.4 showed increased number of sinoviocyte in CFA group (panel B) compared to naive group (panel A). Panel C, D and E showed sinoviocyte in rats receiving ALA 30, 60 or 120 mg/kgBW. In RA, proliferation sinoviocyte not only increased the number of syioviocytes but also adhered to the cartilage and aggravates the RA. The calculation of sinoviocyte in synovial lining was started from the intimal synovial layer. When these synoviocytes proliferates into the cartilage, the counting was started from the sinoviocyte cell's lining on the cartilage. Based on the number of sinoviocyte, ALA group at 30mg/kgBW had less number of sinoviocyte compared to CFA group.

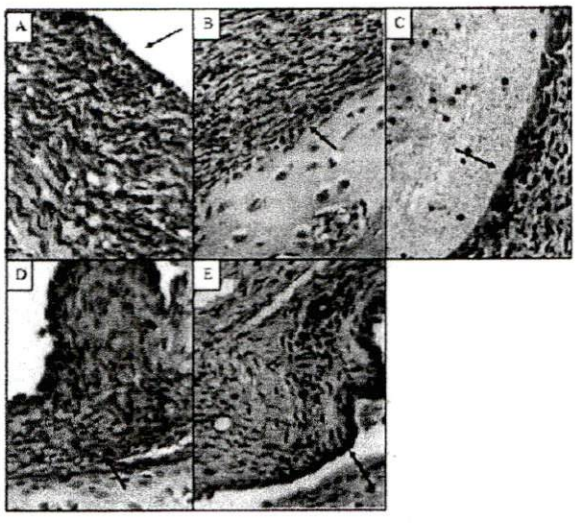

Fig. 4. The black arrows show sinoviocyte. (HE) staining for synovial membrane (magnification 1000x). (A) Naive group, (B) CFA group, (C) CFA-ALA30mg/kgBW,(D)CFA-ALA 60mg/kg BW,(E)CFA-ALA120mg/kgBW

DISCUSSION

The RA model should not only induce relevant nociceptive behavior but also mimic as closely as possible the structural articular changes observed in the human RA disease. CFA intra articular injection leads to the development of RA as seen in human (Zhang & Ren 2011, Snekhalatha et al 2012). Chen et al (2012) reported that CFA successfully induced RA in 100% (10 of 10) of the injected rats. The result of latency time on thermal stimulation and joint diameter evaluation revealed that intra articular injection of CFA induced hyperalgesia and articular swelling in rats. These are the results of CFA contains Mycrobacterium butyricum and it could be the most potent initiator to activate the immune system. CFA induces secretion of cytokines so TNF-α and IL-1 are released. IL-1 and TNF-α have an important role on the pathogensis of RA (Choy 2001, Kinne et al 2007). These cytokines bind to their receptors and activate NFκB pathway and produce matrix metalloproteinase (MMP). MMP is a destructive enzyme to joints and could be the trigger of RA (Malemud 2009, Waldburger & Firestein 2009). In ALA group, the joints diameter was decreased and latency time on thermal stimulation was increased. It is because ALA could inhibit NFκB activation through inhibition of IKK2. IKK2 is essential for IκBα degradation and subsequent NFκB activation because IκBα degradation is a critical step for NFκB activation in response to TNF-α stimulation, the process of degradation mediates the migration of NFκB from cytoplasma into the nucleus (Ying et al 2011).

In the development of inflammation in RA, cytokines also increase the influx of neutrophils. Neutrophil produces ROS and the role of increased oxidative stress in the development of chronic disease is a subject of great interest. Previous reports also indicate the role of oxidative stress in the pathogenesis of RA (Mirshafiey & Mohsenzadegan 2008). Unexpectedly, the present study the value of MDA level as an indicator of lipid peroxidation showed no significant difference in CFA group compared to naive group. ALA which is known as potent antioxidant, did not significantly change plasma level of MDA. A report by Sahin et al (2006) also in line with this finding stated there was also no significant different between MDA level among the different experimental group. This strongly suggested that the mechanism of ALA may affect both oxidative stress and inflammatory process simulataneously through the inhibition of NFκB (Shay et al 2009, Mirtaher et al 2009). Cytokines such as TNFα and IL are not only upregulated by NFκB but also act as activators of NFκB leading to perpetuation of pro-inflammatory condition (Malemud 2009, Waldburger & Firestein 2009). Furthermore, ROS also known as considerable cause for oxidative stress in RA plays an essential role as both upstream and downstream pathways of NFκB. These intricate interactions form a positive feedback loop in which oxidative stress and inflammation amplify each other mutually (Henrotin et al 2003). Hence, ALA could interrupt these interaction

via inhibiting NFκB. Contradictory to this present study there was no correlation between oxidative stress and RA. ALA could inhibit NFκB activation through anti-oxidant-independent mechanism.

This present study, histopathology evaluation by hema-toxylin eosin staining indicated that there was an increased number of sinoviocyte in the synovial membrane of the CFA group. The earliest changes occur in the synovial membrane, leading to sinoviocyte proliferation. Sinoviocyte proliferation occurs in the surface of the membrane. When the condition continues, this leads to the thickening of synovial membrane (Capitanescu et al 2011). Sinoviocytes play critical roles in normal joint homeostatis. It secretes high-levels of long-chain, polymeric hyaluronan into joint cavity and controls the volume of the synovial fluid, which has both lubricating and immunomodulatory properties. But, the increasing number of sinoviocyte can stimulate inflammation in the joint of RA (Mor et al 2005). In ALA 30 mg/kgBW group, number of synoviocytes observed was not as many as the observation in CFA group or the other ALA groups. But, sinoviocytes were still higher than the number of sinoviocyte on the naive group because when inflammation occured the intra articular pressure rises sufficiently to inhibit blood flow into the synovial membrane whereas the inflammatory cells can still infiltrate into the joint cavity (Haywood & Walsh 2001).

CONCLUSION

ALA has a potential effect for suppressing the develop-ment of inflammation in RA but not through oxidative stress pathway.

REFERENCES

Afonsu, V., Champy, R., Mitrovic, D., Collin, P., and Lomri, A. (2007). Reactive Oxygen Species and Superoxide Dismutases: Role in Joint Diseases, Joint Bone Spine. 74, 324-329.

Bhowmick, K., Chakraborti, G., Gudi., N. S., Moideen, A. VK., and Sheety, H. V. (2008). Free Radical and Antioxidant Status in Rheumatoid Arthritis. Indian Journal of Rheumatology. 3, 8-12.

Capitanescu, B., Simionescu, C., Margaritesu, C., Stepan, A., Ciurea, R. (2011). Clinical and Morphological Aspects of Sinovitis Early Rheumatoid Arthritis, Current Health Sciences Journal. 37, 17-20.

Chen, L., Bao, B., Wang, N., Xie, J., Wu, W. (2012). Oral Administration of Shark Type II Collagen Suppresss Complete Freund's Adjuvant-Induced Rheumatoid Arthritis in Rats. Pharmaceuticals. 5, 339-352.

Dubey, S., and Adebajo, A. O. (2008). Historical and Current Perspectives on Management of Osteorthritis and Rheumatoid Arthritis. In: Reid, D. M., Miller, C. G. (Eds.). Clinical Trials in Rheumatoid Arthritis and Osteoarthritis. London, Springe, pp. 12-13.

Emery, P. (2011). Pocket Reference to Early Rheumatoid Arthritis. UK, Springer Healthcare, pp. 1 - 4.

Golbidi, S., Badran, M., and Laher, I. (2011). Diabetes and Alpha Lipoic Acid. Frontiers in Pharmacology. 2, 1-15.

Haywood, L., and Walsh, D. (2001). Vasculature of The Normal and Arthritis Synovial Joint, Histology and Histopathology Cellular and Molecular Biology. 16, 277-284.

Henrotin, Y. E., Bruckner, P. Pujol, J. P. L. (2003). The Role of Reactive Oxygen Species in Homeostasis and Degradation of Cartilage, Osteoarthritis and Cartilage. 11, 747-755.

Ishibashi, T. (2013). Molecular Hydrogen: New Antioxidant and Anti-inflammatory Therapy for Rheumatoid Arthritis and Related Diseases. Current Pharmaceutical Design, 19, 6375-6381.

Jaswal, S., Mehta, H. C., Sood, A. K., and Kaur, J. (2003). Antioxidant Status in Rheumatoid Arthritis and Role of Antioxidant Therapy. Clinica Chimica Acta. 338, 123-129.

Malemud, C. J. (2009). Intracellular Signaling Pathways in Rheumatoid Arthritis. Journal of Clinical and Cellular Immunology. 4, 1-15.

Miossec, P., and Tebib, J. (2006). Diagnosis of Rheumatoid Arthritis at an Early Stage. In: Bouysset, M., Tourne, Y., Tillmann, K. (Eds.). Foot and Ankle in Rheumatoid Arthritis. France, Springer, pp. 1-3.

Mirshafiey, A., and Mohsenzadegan, M. (2008). The Role of Reactive Oxygen Species in Immunopathogenesis of Rheumatoid Arthritis. Iranian Journal of Allergy, Asthma and Immunology. 4, 195-202.

Mirtaheri, E., Gargari, B. P., Kolahi, S., Dehghan, P., Asghari-Jafarabadi, M., Hajalilou, M., Novin, Z. S., Abbasi, M. M. (2015). Effects of Alpha Lipoic Acid Supplemetation of Inflammatory Biomarkers and Matrix Metalloproteinase-3 in Rheumatoid Arthritis Patiens, Journal of the American College of Nutrition. 0, 1-8.

Mor, A., Abramson, S., Pillinger, M. (2005). The Fibroblast Like Synovial Cell in Rheumatoid Arthritis: A Key Player in Inflammation and Joint Destruction, Clinical Immunology. 115, 118-128.

Orhan, C. E., Onal, A., Uyanikgil, Y., Ulker, S. (2013). Antihyperalgesic and Antiallodynic Effect of Sirolimus in Rat Model of Adjuvant Arthritis, European Journal of Pharmacology. 705, 35-41.

Saxena, R. (2014). Arthritis as a Disease of Aging and Changes in Antioxidant Status. In: Preedy, V. R (Ed.), Aging: Oxidative Stress and Dietary Antioxidants. London, Academic Press, pg. 52.

Shay, K. P., Moreau, R. F., Smith, E. J., Smith, A. R., and Hagen, T. M. (2009). Alpha-Lipoic acid as a Dietary Supplement: Molecular Mechanisms and Therapeutic Potential. Biochimica et Biophysica Acta. 1790, 1149-1160.

Snekhalatha, U., Anburajan, M., Venkatraman, B., Menaka, M. (2012). Evaluation of Complete Freund's Adjuvant-Induced Arthritis in a Wistar Rat Model (Comparison of Thermography and Histopathology). Zeitschrift für Rheumatologie. 4, 375-382.

Stadtman, E. R., and Berlett, B. S. (2002). Reactive Oxygen-Mediated Protein Oxidation in Aging and Disease. In: Gilbert, D. L., and Colton, C. A. (Eds.). Reactive Oxygen Species in Biology Systems: An Interdisciplinary Approach. New York, Kluwer Academic Publishers, pg. 660.

Wahl, K., and Schuna, A. A. (2014). Rheumatoid Arthritis. In: Dipiro, J. T., Talbert, R. L., Yee, G. C., Matzke, G. R., Wells, B. G., and Posey, L. M. Pharmacotherapy: A Pathophysiologic Approach. New York, Mc Graw Hill, p. 3201-3203.

Waldburger, J., and Firestein, G. S. (2009). Signaling Pathways in Rheumatoid Arthritis. In: Tak, P. (Ed.), New Therapeutic Targets in Rheumatoid Arthritis. Switzerland, Birkhauser Verlag, p. 167-168.

Zhang, R., and Ren, K. (2011). Animal Models of Inflammatory Pain. In: Ma, C., Zhang, J. (Ed.). Animal Models of Pain. London, Humana Press, pp. 23-30.

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