International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304, ISSN(Online): 2455-9563
Vol.12, No.03, pp 85-90, 2019

Sedimentation Rate and Arthritis Inflammatory Score in Adjuvant Arthritis Model

Fitri Handajani*, Sulistiana Prabowo

Hang Tuah University, Indonesia

Abstract : Backgroud : Adjuvant arthritis induction using *Complete freund's adjuvant* (CFA) in Wistar rats resulted in increased *reactive oxygen species* (ROS) production, NF-κB activation and increased joint inflammation and destruction. Tuna (*Thunnus obesus*) contains omega-3 omega-6 fatty acids that play role in inhibition of NF-κB activation. We hypothesised that tuna extract is able to reduce arthritis severity. This study aimed to determine the effect of tuna extract in erytrocyte sedimentation rate (ESR) and arthritis inflammatory score in adjuvant arthritis model.
Method : Three groups of male Wistar rats (*Rattus norvegicus*) (8-12weeks, n=8/group) ie. Non treated, adjuvant arthritis by induction with Complete Freund's Adjuvant (CFA) and adjuvant arthritis + tuna extract (12 g/kg BW per oral/day) at day 22 post CFA induction for 7 days. At day 29 ESR was measured in all groups by Wintrobe method and arthritis inflammatory score was measured clinically.
Results : The results of Mann Whitney test showed there was significant difference (p=0.005) of ESR between non treated group and adjuvant arthritis groups (1.4 $\pm$ 0.5 vs 2.4 $\pm$ 0.5). There was significant reduction (p=0.044) of ESR in adjuvant arthritis group received tuna extract vs. without (1.9 $\pm$ 0.4 vs. 2.4$\pm$ 0,5). There was significant difference (p=0.001) of arthritis inflammatory score between non-treated group and adjuvant arthritis groups (1 vs 3.5). There was significant reduction (p=0,010) of arthritis inflammatory score in adjuvant arthritis received tuna extract vs. without (2.6 vs. 3.5).
Conclusion : ESR and arthritis inflammatory score in adjuvant arthritis model were significantly reduced by tuna extract treatment.
Key words : tuna, ESR, inflammation, adjuvant arthritis model.

Background

Rheumatoid arthritis is an autoimmune disease of heterogeneous syndrome associated with chronic inflammation of unknown cause. The syndrome is in the form of local joint and systemic disorders including abnormalities in the cardiovascular, pulmonary and skeletal disorders. The main area affected by rheumatoid arthritis are the synovial membrane, bursae and tendon sheaths. [1,2]

DOI: http://dx.doi.org/10.20902/IJPTR.2019.120310

Epidemiological studies show that in industrialized countries, rheumatoid arthritis affects 0.5-1% in adults, with 5-50 per 100,000 new cases each year. [3]

Rheumatoid arthritis' patients presented with increase ROS and an inflammantory process. Increase ROS that cannot be controlled by endogenous antioxidants cause oxidative stress and inflict oxidative damage resulted in increased severity of the arthritis.[1,2]

Erythrocyte sedimentation rate (ESR) is commonly used as the indicator of acute inflammation despite the fact that ESR is only an indicator for non-specific inflammation, it can be used to evaluate therapy response and often used as one of prognosis indicator in rheumatoid arthritis' patients.[4]

As a maritime country, Indonesia has plenty of biological resources from the seas. These products has enormous potential for the application of biotechnology, medicines, and cosmetics. Fish have a lot of nutrient such as proteins, minerals, vitamins, fats and it was the largest producer of omega 3, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA).[5,6,7]

Tuna contains many vitamins, minerals, arachidonic acid and omega-3 fatty acids. Omega-3 and omega-6 fatty acids play a role for reducing cellular inflammatory mediators, pro-inflammatory cytokines and enzymes that degrade cartilages, through inhibition of activation in NF-κB transcription factors. In this study, omega-3 and omega-6 that are contained in tuna are expected to reduce the inflammation, oxidative stress and the joint damage in rheumatoid arthritis' patients.[8,9,10,11]

According to a research conducted by Tadeschi *et al.* (2017) it is stated that the increase fish consumption is related to reduction of disease activity in rheumatoid arthritis' patients.[11] In this study, we used adjuvant arthritis as a model for rheumatoid arthritis to understand the mechanism and the development of new therapies for rheumatoid arthritis' patients.[12]

The aim of this study was to determine the effect of tuna extract on erythrocyte sedimentation rate and arthritis inflammatory score in adjuvant arthritis.

Method

This study was an experimental research (experiment design) that used the post test only control group design method conducted at the Laboratory of Biochemistry, Faculty of Medicine, Hang Tuah University, Surabaya, Indonesia. We tested 24 Wistar strain male rats (*Rattus norvegicus*) which were divided into 3 groups (8 each): 1) groups of untreated rats, 2) groups of rats with adjuvant arthritis which were induced with Complete Freund's Adjuvant (CFA) by intradermal injection of 0.1 ml CFA at the base of the rat's tail, and 14 days later 0.1 ml CFA booster was given intradermally to the right and left feet. After 7 days, there will be some symptoms of adjuvant arthritis in the form of swelling, redness, and pain in the joints of the feet, 3) treatment groups, which are rats with adjuvant arthritis who were given tuna fish extract orally for 7 consecutive days from the first day of the emergence of symptoms of adjuvant arthritis with the dose of 12 grams / kg of body weight, starting on the 22nd day. At the end of the study the erythrocyte sedimentation rate (ESR) and arthritis inflammatory score of all group of rats were measured (in day 29).

Induction of Adjuvant Arthritis Using Complete Freund's Adjuvant (CFA)

The animals were injected intra dermally with 0.1 ml Complete Freund's Adjuvant (CFA) at the base of the tail. After 14 days 0.1 ml CFA booster was given at the right and left feet. After 7 days, the symptoms of adjuvant arthritis occured in the form of swelling, redness and pain in the feet joints. This arthritis model called Adjuvant Arthritis (AA) and has been widely used as an animal model of rheumatoid arthritis (AR).[13]

The Procedure to Make Tuna Extract (*Thunnus obesus*)

Ethanol extract from tuna fish (*Thunnus obesus*) was made in the following ways:

Tuna (*Thunnus obesus*) were cleaned and cut into small pieces, dried until the water content was 20-30%. Each 116 grams Tuna was extracted using maceration technique with 1.5 liters of 85% ethanol. The extract was concentrated using filtered rotary vacuum evaporator at 40^{o}C for approximately 2 hours. The extract

then washed three times with 100 ml chloroform and the upper layer (non-lipid fraction), then the ethanol fraction was removed and dried using N_2 and a tuna extract was formed.

Procedure for Administering Tuna Extract

The rats were weighed, then the pediatric nasogastric tube were inserted through the mouth until it reached the rat's stomach. The tuna extract was given at a dose of 12 g / kg body weight through a syringe and inserted into the nasogastric tube to reach the stomach of the rats.[14]

Procedure to Measure Erythrocyte Sedimentation Rate (ESR)

Blood samples with EDTA anticoagulants were homogenized before the collation. After that the sample were put in the Wintrobe tube using the Pasteur pipette until the 0 mark, and the tube is placed upright. Wait for 1 hour and see how many mm of erythrocyte descend.

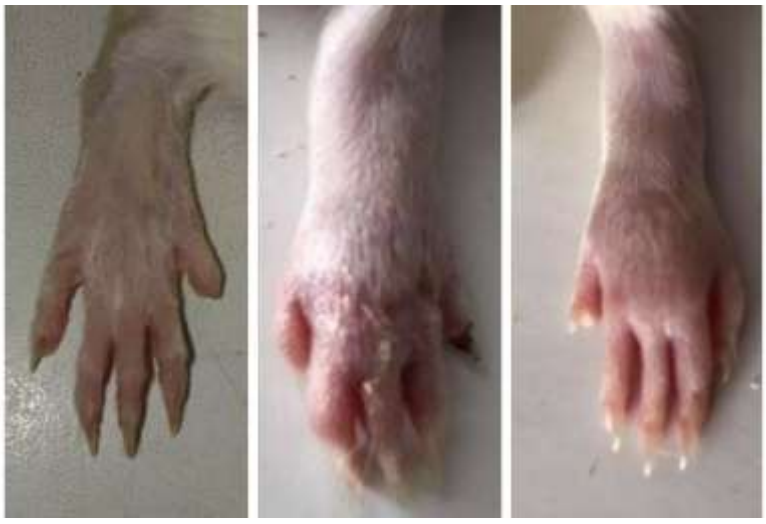
Procedure to Measure Arthritis Inflammatory Score

The arthritis inflammatory score in this study was assessed based on a modification of a method introduced by Brand *et al*., 2007:

Arthritis inflammatory score	
1	There is no redness and swelling
2	Mild redness and swelling limited in the tarsal or ankle joint
3	Mild redness and swelling extended from the ankle to the tarsal
4	Moderate redness and swelling extended from the ankle to the metatarsal
5	Severe redness and swelling covering the ankles, feet, and fingers or ankylosis from the limbs

Result

The results showed that adjuvant arthritis showed increase inflammatory score (Figure 1B) compared to rat's feet that did not received any treatment (Figure 1A). There was reduction in the severity of arthritis inflammation in the rat's feet with adjuvant arthritis that were given tuna extract (Figure 1C) compared to the rat's feet with adjuvant arthritis that did not receive tuna extract (Figure 1B). Images of untreated rat, rat with adjuvant arthritis and rat with adjuvant arthritis receiving tuna fish extract can be seen in Figure 1.



A B C

Figure 1. A. Rat's foot with no treatment. B. Rat's foot with adjuvant arthritis. C. Rat's foot with adjuvant arthritis that were given tuna extract for 7 days.

The Mann Whitney test results showed that there were significant differences (p = 0.005) of ESR between the untreated group compare to adjuvant arthritis group (1.4 ± 0.5 vs 2.4 ± 0.5). There were significant differences (p = 0.044) of ESR between adjuvant arthritis group compared to adjuvant arthritis received tuna extract (2.4 ± 0.5 vs 1.9 ± 0.4).

The results showed that adjuvant arthritis has significant increase in ESR, and tuna extract were significantly decrease the ESR increase.

Mean and standard deviation (SD) of ESR in groups of rats that were not treated, groups of rats with adjuvant arthritis and groups of rats with adjuvant arthritis received tuna extract were shown in Table 1.

Table 1. Mean and standard deviation of Erythrocyte Sedimentation Rate (ESR)

Group	Mean ± Sd (Mm/Hour)
1	1.4 ± 0.5
2	2.4 ± 0.5
3	1.9 ± 0.4

Note:
Group 1 : Non treatment group
Group 2 : Adjuvant arthritis group
Group 3 : Adjuvant arthritis group + 7 days tuna extract

The Mann Whitney test results showed that there were significant difference (p = 0.001) of the arthritis inflammatory score between the untreated group compared to the adjuvant arthritis group (1 vs 3.5). There were significant differences (p = 0.010) of the arthritis inflammatory score between adjuvant arthritis group compared to adjuvant arthritis received tuna extract (3.5 vs. 2.6).

The results indicates that adjuvant arthritis showed significant increase in arthritis inflammatory score, and the addition of tuna extract administration significantly decrease the arthritis inflammatory score.

The mean of arthritis inflammatory score in untreated group, the adjuvant arthritis group and adjuvant arthritis received tuna extract can be seen in Table 2.

Table 2. Mean of arthritis inflammatory score

Group	Mean	Minimum	Maximum
1	1	1	1
2	3.5	3	4
3	2.6	2	3

Note:
Group 1 : Non treatment group
Group 2 : Adjuvant arthritis group
Group 3 : Adjuvant arthritis group + 7 days tuna extract

Discussion

The erythrocyte sedimentation rate (ESR) is a test that indicated acute inflammatory that is not specific thus cannot be used to determine the clinical diagnosis. It is however, still often used due to its affordability and as a measure for treatment response. ESR are affected by plasma proteins including globulin, fibrinogen and albumin, and especially fibrinogen, so that a slight increased of fibrinogen will affect ESR. Plasma proteins affected ESR so that in acute inflammation the rise of ESR occurs more slowly, and ESR will remain high for several days or weeks even though the inflammatory process has been resolved.[4]

In this study it was shown that in adjuvant arthritis group induced by CFA, ESR was significantly increased compared to the untreated group. It showed that the acute inflammatory process in adjuvant arthritis result in the increase of erythrocyte sedimentation rate.

In this study, tuna extract was significantly able to reduce erythrocyte sedimentation rate. It was shown that the inflammation process of adjuvant arthritis that received tuna extract was significantly reduced.

The inflammatory process can be identified if there are some symptoms such as redness, burning, pain, swelling / edema and disturbed function. The redness occur because of the secretion of pro-inflammatory cytokines which result in peripheral vasodilation. The edema occur due to the release of cytokines which results in disruption of membrane permeability and may cause the intravascular extravasation to extravascularly. In adjuvant arthritis model the severity of inflammatory symptoms can be evaluated by the size and the extent of the swelling and redness in the feet joints and even the damage can be measured by X-ray examination or histopathology. In this study we only use the indicator of redness and swelling severity through modification of the scoring system from Brand *et al*., 2007. [15]

The expression inhibition of NF-κB transcription factors by omega-3 and omega-6 found in tuna can result in a reduction of the pro-inflammatory cells number in the inflamed joints and also a reduction in pro-inflammatory cytokines. As a result, inflammatory symptoms such as heat, pain, redness, swelling and dysfunction in the joints will decrease. This situation results in a reduction of swelling and redness in the mice feet.[16,17,18,19,20,21]

Furthermore, the inhibition of NF-κB transcription in this study can result in the reduction of the pro-inflammatory cell number that would reduce the release of proteolytic enzymes, named the metalloproteinase-1 (MMP-1), MMP-3 and MMP-9. This results in the reduction of protein degradation in joint cartilage and decrease inflammatory process. Omega-3 is also able to inhibit collagen degradation, reduce osteoclast activation so it can inhibit joint cartilage damage which result in the reduction of joint pain and functional disturbances that occur due to inflammation.[9,16,17,19,20,21] All of the factors mentioned before may play a role in reduction of the inflammation severity of arthritis in adjuvant arthritis.

In summary, this study showed that there was an increased of the arthritis inflammatory score in adjuvant arthritis group compared to the untreated group. The increased of inflammatory symptoms in the form of joint redness and swelling was significantly reduced by administration of tuna extract. This study also showed that administration of tuna extract significantly reduce the severity of joint inflammation in adjuvant arthritis. This is likely to be associated with the reduction of the inflammatory process due to active inhibition of the NF-κB transcription factor expression by omega-3 and omega-6 fatty acids found in tuna extract.

Conclusion

Adjuvant arthritis model showed significantly increase of the ESR and arthritis inflammatory score. Tuna extract significantly decreased ESR and inflammatory score in adjuvant arthritis. This might be due to the omega-3 and omega-6 fatty acids in tuna which inhibit the inflammatory process through inhibition of NF-kB activation.

References

1. Aya, Muhammad Alhawagari, Amanda H-S, Hideki Kitaura, Osagami Kinigawa, Deborah VN. NF-κB-inducing kinase controls lymphocyte and osteoclast activities in inflammatory arthtritis. *Journal of clinical investigation*. 2005;115(7).
2. Mikirova N, A Rogers, J Casciari. P Taylor. Effect of High Dose Intravenous Ascorbic Acid On The Level of Inflamtion In Patients With Rheumathoid Arthritis. *Modern Research In Inflammation*. 2012;1(2):26-32.
3. Rich R. Robert, Thomas A. Fleisher, Benjamin D. Schwartz, William T. Shearer, Warren Strober. Text book of clinnical immunology principles and practice. *Elsevier*. 2008;3:767-786.
4. Aji, Sumariyono, Kusumawidjaja, Abdullah. Korelasi Antara Kadar Matriks Metalloproteinase 9, Laju Endap Darah, Faktor Reumatoid, dan Lama Sakit dengan Gambaran Radiologis pada Pasien Artritis Reumatoid. *Jurnal Penyakit Dalam Indonesia*. 2015;2(2).
5. Sukarsa. Studi aktivitas asam lemak omega 3 ikan laut pada mencit sebagai hewan model percobaan. *Buletin Teknologi Hasil Perikanan*. 2004;7(1).

6. Healy, Wallace, Miles, Calder, Newshole. Effect of low-to-moderate amount of dietary fish oil on neutrofil lipid composition ang function. *Lipids*. 2000;35(7).
7. Peer Trainer. Tuna. *Nutritian Facts*. 2009.
8. Cleland, James, proudman. The role of Fish oils in treatment of Rheumatoid Arthtritis. *Drugs*. 2003;63(9):845-853.
9. Seves, Temme, Brossen, Szipj, Hoekstra, Hollander. Sustainability aspects and nutritional composition of fish : evaluation of wild and cultivated fish species consumed in the Netherlands. *Climatic Change*. 2016;135:597-610
10. Simopoulos. Omega-3 Fatty acid in inflamation and autoimmune diseases. *Journal of the American collage of nutrition*. 2002; 21.
11. Tadesci SK, Banthon, Giles, Tzu-Chieh, Yoshida, Solomon. The relationship between fish consumption and disease activity in rheumatoid arthritis. *Arhtritis care & research*. Juni 2017:1-20.
12. Snekhalatha U, Anburajan M, Venkatraman B, Menaka M. "Evaluation of complete Freund's adjuvant-induced arthritis in a wistar rat model". *Zeitschrift für Rheumatologie, DOI 10.1007/s00393-012*. 2012:1083-1088.
13. Prabowo S. Pengaruh stresor dingin terhadap proses keradangan pada arthritis adjuvan: penelitian experimental pada arthritis adjuvan (Hewan model untuk arthritis rematoid). *Disertasi program pasca sarjana Universitas Airlangga*. 2004.
14. Riswanto. Laju endap darah. *Laboratorium Kesehatan*. http://labkesehatan.blogspot.com/2009/12/laju-endap-darah-led.html. 2009.
15. Brand D, Latham K, Rosloniec. "Colagen-induced arthritis". *Nature Protocol*. 2007;2(5):1269-1275
16. Buddhachat, Siengdee , Chomdej, Soontornvipart, Nganvongpanit. Effects of different omega-3 sources, fish oil, krill oil, and green-lipped mussel against cytokine-mediated canine cartilage degradation. *In Vitro Cell.Dev.Biol.—Animal*. 2017;53:448–457
17. Calder. 2017. *Omega-3 fatty acids and inflamatory proces: from molecules to man*. *biochemical society transactions* 45 (5), 1105-1115
18. Rani, Kumar, Rao and Shameem. 2016. *Seasonal variation of proximate composition of tuna fishes from Visakhapatnam fishing harbor, East coast of India*. *International Journal of Fisheries and Aquatic Studie*, 4(6): 308-313
19. McInnes Iain BFRCP and Schett GMD, 2011. "Mechanisms of disease the pathogenesis of rheumatoid arthritis". *The New England Journal of Medicine*, vol. 365, pp. 2205-19
20. Mirshafiey A and Mohsenzadegan M, 2008. "The role of reactive oxygen species in immunopathogenesis of rheumatoid arthritis". *Iran Journal Allergy Asthma Immunology*, December ; vol. 7, no. 4, pp. 195- 202
21. Schett George. 2008. Review*:* "Immune cells and mediators of inflammatory arthritis". *Autoimmunity*; 41(3): 224–229.

*****