RESEARCH ARTICLE

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Histopathological Effects of Omega-3 in Reducing Cartilage Destruction Progression in Mice with Knee Joint Osteoarthritis

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Abstract

Primary and secondary osteoarthritis relates to inflammatory processes and inflammatory mediators and is destructive to the articular cartilage. Omega-3 is known to be an alternative treatment for rheumatoid arthritis due to the anti-inflammatory effect. This study is an experimental study with simple random sampling using 36 Wistar mice, which were divided into an intervention group and a control group, to understand the effect of omega-3 in slowing progress cartilage destruction in knee joint with osteoarthritis. This study is performed at the Clinical Pharmacology Laboratory, Faculty of Medicine, Universitas Padjadjaran, and the Anatomical Pathology Laboratory, Faculty of Medicine, Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital, in November 2018. Osteoarthritis was induced using intraarticular 1 mg monosodium iodoacetate injection to the mouse knee joint. The intervention group received oral omega-3 every day while the control group did not. Samples from the knee joint were extracted to evaluate the cartilage destruction histopathologically. Results were then analyzed using the Mann-Whitney test and a significant difference of the osteoarthritis grades was identified between the intervention group and the control group on day 7 (p=0.003), day 14 (p=0.003), and day 21 (p=0.003). In addition, a significant difference in the osteoarthritis grading changes was also found between the study group and the control group on day 7 and day 21 (p=0.004). Hence, omega-3 has the ability to slow down the histopathological cartilage destruction progress in mice with knee joint osteoarthritis.

Key words: Cartilage destruction, osteoarthritis, omega-3

Efek Pemberian Omega-3 terhadap Perlambatan Progresivitas Destruksi Kartilago Sendi Lutut Tikus yang Mengalami Osteoartritis Secara Histopatologis

Abstrak

Osteoartritis primer dan sekunder berhubungan dengan proses inflamasi dan mediator inflamasi dan merusak tulang rawan artikular. Omega-3 dikenal sebagai pengobatan alternatif untuk rheumatoid arthritis karena efek anti-inflamasi. Penelitian ini adalah penelitian eksperimental dengan pengambilan sampel acak sederhana menggunakan 36 tikus *Wistar*, yang dibagi menjadi kelompok intervensi dan kelompok kontrol, untuk memahami efek omega-3 dalam memperlambat perkembangan kerusakan tulang rawan pada sendi lutut dengan osteoarthritis. Penelitian ini dilakukan di Laboratorium Farmakologi Klinik, Fakultas Kedokteran, Universitas Padjadjaran, dan Laboratorium Patologi Anatomi, Fakultas Kedokteran Universitas Padjadjaran/Dr. Rumah Sakit Umum Hasan Sadikin Bandung, pada November 2018. Seluruh hewan uji dilakukan induksi osteoartritis dengan monosodium iodoasetat sebanyak 1 mg yang disuntikkan ke dalam sendi lutut. Kelompok perlakuan diberikan omega-3 1 kali per hari per oral, sedangkan kelompok kontrol tidak diberikan omega-3. Sampel jaringan sendi lutut diambil dan dilakukan penilaian destruksi kartilago secara histopatologis. Hasil kemudian dianalisis dengan menggunakan uji Mann-Whitney dan perbedaan yang signifikan dari nilai osteoartritis diidentifikasi antara kelompok intervensi dan kelompok kontrol pada hari ke-7 (p=0,003), hari ke-14 (p=0,003), dan hari ke-21 (p=0,003). Selain itu, perbedaan yang signifikan dalam perubahan penilaian osteoarthritis juga ditemukan antara kelompok studi dan kelompok kontrol pada hari ke-7 dan hari ke-21 (p=0,004). Simpulan, omega-3 memiliki kemampuan untuk memperlambat progresivitas destruksi pada sendi lutut tikus yang mengalami osteoartritis secara histopatologis.

Kata kunci: Destruksi kartilago, osteoartritis, omega-3

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Introduction

Osteoarthritis is a chronic disorder of the synovial joints which is characterized by progressive softening and disintegration of articular cartilage that is accompanied by a new growth of cartilage and bone at the joint margin; cyst formation and sclerosis at subchondral bone; synovitis; and capsular fibrosis. Osteoarthritis is believed to be exclusively a degenerative disease of the cartilage. However, the latest evidence has proven that osteoarthritis is a multifactorial entity, involving multiple causative factors like trauma, mechanical forces, inflammation, biochemical reactions, and metabolic derangements.[1,2]

Osteoarthritis is a common musculoskeletal disorder that causes disability in the world, with 30% of the world's population in the 65 years old and above group have osteoarthritis. Most of the cases involve knee joints (40.7%), followed by the hip joints (24.7%).[3]

Patients suffering from primary or secondary osteoarthritis will experience inflammation (synovitis) in their joints. With this inflammation, the synovial macrophages will release cytokines, such as interleukin-1β (IL-1β). IL-1β has a destructive effect on the articular cartilage, which will trigger more inflammation in the joint.[2,4,5]

There is currently is no medication available to completely heal osteoarthritis. The treatment of osteoarthritis is focused on reducing the progression of the disorder or arthroplasty if other methods of treatment have failed. The options to reduce osteoarthritis progression are lifestyle modification to reduce body weight; quadriceps and hamstring strengthening; and chondro-protector supplement consumption, such as glucosamine, chondroitin sulfate, and hyaluronic acid.[6,7]

Omega-3 fatty acid is an essential fatty acid that is found mostly from coldwater fish like salmon. There are three types of omega-3 fatty acids: *α-linoleic acid* (ALA), *eicosapentaenoic acid* (EPA), and *docosahexaenoic acid* (DHA). The omega-3 fatty acid is most commonly known as a supplement for preventing atherosclerosis but is recently recognized to also works as an anti-inflammatory agent through the inhibition of arachidonic acid production by competitively inhibit the binding of arachidonic acid to cyclooxygenase and inhibit the production of interleukin-1β.[8,9]

This study aimed to understand the effect of omega-3 in reducing the progression of cartilage destruction in knee joints with osteoarthritis.

Methods

This study was an animal experimental study using simple random sampling technic. The subjects were 150-250 gram 36 adult Wistar strain male mice injected with monosodium iodoacetate intraarticularly at knee joint. Exclusion was made by excluding mice with infection and behavioral change. This study was conducted at the Pharmacology Laboratory of the Faculty of Medicine, Universitas Padjadjaran, from November 2018 to October 2018 and the approval for the study was obtained from the Department of Orthopaedic and Traumatology and Research Ethics Committee of Dr. Hasan Sadikin General Hospital (Research Ethics Committee reference number: LB.04.01/A05/EC/286/X/2018).

After an adaptation period of seven days at the laboratory, all mice were injected with monosodium iodoacetate 1 mg intraarticularly at the knee joint. The mice were then divided into 2 groups with 18 mice in each group. Group I, which was the study group, was given omega-3 fatty acid 310 mg/kg BW once a day using a feeding probe while Group II as a control group did not receive omega-3 fatty acid. On day 7, day 14, and day 21, six mice were taken from each group. The mice were terminated and the articular cartilage of the knee joint was harvested. Samples were then stained with Safranin O and assessed for the articular cartilage destruction using the histopathological approach at the Anatomical Pathology Laboratory of the Faculty of Medicine, Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital. The rate of cartilage destruction was then graded using the Osteoarthritis Research Society International (OARSI) classification for osteoarthritis cartilage histopathology. Analysis was then performed on the data using the Mann-Whitney test due to the ordinal nature of the data. SPSS 18 for Windows was used for this analysis.

Results

A milder stage of osteoarthritis was observed in the study group with OARSI classification means of 1.17 on day 7, 1.5 on day 14, and 1.67 on day 21. The control group showed a more severe stage of osteoarthritis with the OARSI classification means of 3 on day 7, 4.5 on day 14, and 5.5 on day 21.

Differences in osteoarthritis grades between the study and control group was tested using

Table 1 OARSI Osteoarthritis Cartilage Histopathology Classification[10]

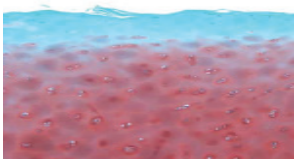
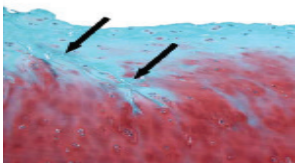
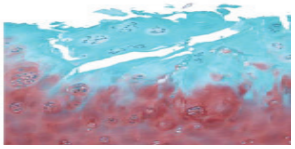
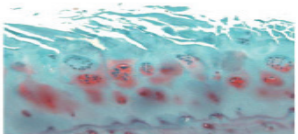
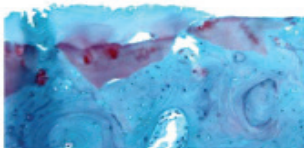
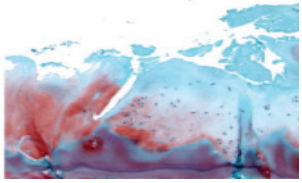
Grade	Key Feature	Description	Histopathological Finding
0	Normal	Matrix: normal Cells: intact with appropriate orientation	
1	Surface intact	Matrix: edema and/or superficial abrasion Cells: death with proliferation and hypertrophy	
2	Surface discontinuity	Matrix: discontinuity at superficial zone	
3	Vertical fissures	Matrix: vertical fissures into midzone	
4	Erosion	Cartilage matrix loss	
5	Denudation	Surface: sclerotic bone	
6	Deformation	Bone remodeling	

Table 2 Osteoarthritis Grade

Day	Study Group				Control Group			
	n	Minimum	Maximum	Mean	n	Minimum	Maximum	Mean
Day 7	6	1	2	1.17	6	2	4	3
Day 14	6	1	2	1.5	6	4	5	4.5
Day 21	6	1	2	1.67	6	4	6	5.5

Table 3 Comparison of Osteoarthritis Grades between Study and Control Groups

Day	Group	N	Mean	Standard deviation	P-value
7	Study	6	1.17	0.408	0.003
	Control	6	3.00	0.632	
14	Study	6	1.50	0.548	0.003
	Control	6	4.50	0.548	
21	Study	6	1.67	0.516	0.003
	Control	6	5.50	0.837	

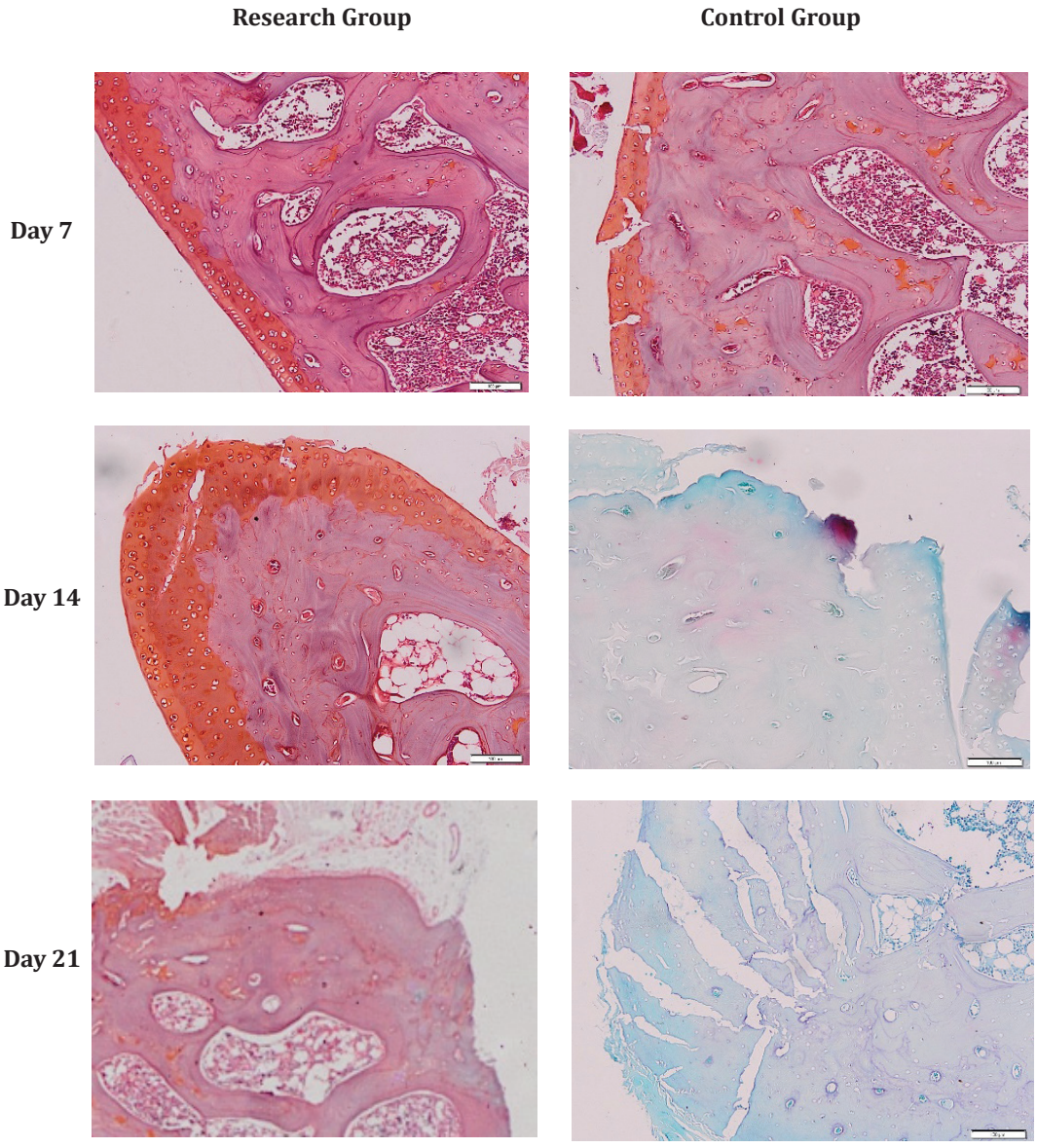


Figure Histopathological Findings

the Mann-Whitney test, resulting in a significant difference in osteoarthritis grade between the study and control groups on day 7 (p=0.003), day 14 (p=0.003), and day 21 (p=0.003).

The difference in osteoarthritis grading changes between the study and control groups was tested using the Mann-Whitney test, resulting in a significant difference in osteoarthritis grading change between the study group and the control group between day 7 and day 14 (p=0.026) and between day 7 and day 21 (p=0.004). Between day 14 and day 21, there was no significant difference between the study group and the control group in osteoarthritis grading change (p=0.240).

Discussion

This study was an experimental study using Wistar strain mice as the animal model. To induce osteoarthritis in the animal model, intraarticular injection of monosodium iodoacetate was given to the knee joint. Monosodium iodoacetate is known to induce osteoarthritis in animal models. After injected with monosodium iodoacetate, an inflammatory reaction in the joint is triggered, which will create lesions at the articular cartilage in the form of proteoglycan matrix destruction, subchondral sclerosis, subchondral cyst, osteophyte formation, and disturbance in joint function, which are similar to osteoarthritis in human.[11,12]

In this study, osteoarthritis was seen in the study group that received daily omega-3 intake, which was milder than the one observed in the control group, 7 days after the monosodium iodoacetate (p=0.003). On days 14 and 21, the study group also presented a milder stage of osteoarthritis compared to the control group (p=0.003 on both days).

Osteoarthritis is a dynamic process that involves the destruction and repair with inflammation plays a vital role in the process. The osteoarthritic joints will experience synovitis, where the synovium releases cytokines such as interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) that will induce the secretion of protease enzyme which will destroy the articular cartilage. The destruction of articular cartilage will, in turn, induce more inflammation and release more cytokines.[3,4]

Omega-3 is an essential fatty acid, meaning that it is not produced in the human body. After being digested in intestines, the omega-3 will be delivered to the synovium. In the synovium, the omega-3 will reduce inflammation by inhibiting the arachidonic acid production, competitively inhibiting the arachidonic acid binding with cyclooxygenase and the production of interleukin-1β. By inhibiting inflammation, protease enzyme will not be secreted and articular cartilage destruction will stop.[8,9]

Rajaei et al.[8] stated that a daily consumption of omega-3 of 3,000 mg per day (50 mg/kg BW) would effectively reduce inflammation in patients with rheumatoid arthritis. In this study, the FDA dose conversion formula was used to calculate the dose of omega-3 provided to the mice to see if the same dose would be applicable for osteoarthritis.

To calculate the progressivity of osteoarthritis, a comparison was made regarding the osteoarthritis grading changes between each group. Between day 7 and day 14, the control group had a higher osteoarthritis grading change (p=0.026). Meanwhile, between day 14 and day 21, both groups showed a similar osteoarthritis grading change (p=0.240). This might be caused by the fact that the monosodium acetate injection was only given at the beginning of the study, which may lead to a decreased concentration in the period between day 14 and day 21. Yet, the overall progression between day 7 and day 21 showed that the control group had a significantly higher osteoarthritis grading change when compared to the study group (p=0.003). In conclusion, omega-3 effectively reduces the progression of cartilage destruction in osteoarthritis.

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