The Effect of Mesenchymal Stem Cells on IL-1β and IL-6 in Synoviocytes-Derived Osteoarthritis Grade IV

Rizki Rahmadian[1,4,5], Menkher Manjas[1,4,5], Roza Silvia[2,4,5], Hirowati Ali[3,4,5]
rizki_md@yahoo.com[1,4,5], menkherm@yahoo.com[1,4,5], roza.silvia_md@yahoo.com[2,4,5], hirowati_ali@yahoo.com[3,4,5]

Department of Orthopedic Surgery, Faculty of Medicine, Universitas Andalas M.Djamil Hospital[1]
Department of Histology, Faculty of Medicine, Andalas University[2] Department of Biochemistry, Faculty of Medicine, Andalas University[3]
Graduate School of Biomedical Sciences, Andalas University[4]
Andalas Cancer and Stem Cell Research Center, Andalas University[5]

Abstract. Osteoarthritis is a degenerative joint disease involving activated synovial tissue due to inflammation. Cytokines seem to enhance the matrix components of lead to destruction of cartilage. Mesenchymal stem cells (MSCs) are widely used for cell-based nowadays approaches, thus activated cytokines, IL-1β andIL-6 as a result of synovial inflammation that are left to be investigated.This is an in vitro study of IV-IL and IL-6 on IV synoviocytes-derived osteoarthritis treated with MSCs. Synovium was harvested from 3 patients with grade IV OA who were diagnosed by orthopedic surgeon and TKR underwent. Cells were isolated using explants culture method and cultured in DMEM with 10% fetal bovine serum, 1% penicillin streptomycin, and amphotericin B. Cells were incubated at 370C and 5% CO2. After third passages, cells were co-cultured with MSCs. Expression of IL-1β and IL-6 are observed using realtime polymerase chain reaction (PCR) after 24 hours and 48 hours. The expression of IL-1β is marked up after 24 hours of co-cultured with MSCs (57.72 ± 18.37) compared to control and shows a tendency to downgrade after 48 hours of event, statictically not significant. However, IL-6 showed a higher expression in synoviocytes after 24 hours of co-cultured with MSCs, and was significantly down regulated after 48 hours. Both IL-1 β and IL-6 showed higher expressions on 24 hours co-culturing synoviocytes with MSCs. Not IL-1β, expression of IL-6 is significantly decreased after 48 hours MSC treatment, implying that MSV grade IV syndicarthritis-derived osteoarthritis.

Keywords: IL-6, IL-1β, Mesenchymal Stem Cells, Osteoarthritis

1 Introduction

Osteoarthritis is one of the most common and frequent causes of disability in the world and has a considerable impact on the health of the community [1]. About 30-50% of adults over the age of 65 experience OA [2]. With regard to gender, it is known that women have a higher level of disability and pain due to OA than men. The prevalence of knee OA in France in women is around 6.6%, while in men 4.7% [3]. The case of OA is increasing along with the increase in the elderly population and the number of injuries due to excessive exercise or trauma due to accidents in the young population so that joint damage occurs.

Efforts to maintain joints were carried out by clinical experts to prevent TKR's actions because the TKR had to carry out major operations that required time, a lot of energy and cost

ICOMHER 2018, November 13-14, Padang, West Sumatera, Indonesia

DOI 10.4108/eai.13-11-2018.2283792

and were expensive. Attempts to maintain joints are carried out in several ways, namely, injection of hyaluronic acid (HA), platelet-rich, plasma, and cell-based therapy. Cell-based therapy has 2 types, namely chondrocyte cell-based and mesenchymal stem cell-based. Chondrocyte cell-based therapy, which is a cell derived from cartilage tissue consists of 2 types including Autologus Chondrocytes Implantation (ACI) and Matrix-Induced Autologus Chondrocytes Implantation (MACI).

However, with weaknesses such as the availability of limited healthy joint cartilage, difficulties in isolation, chondrocytes expansion, and differentiation of chondrocytes isolated in culture make attention in changing cell-based OA therapy to the use of mesenchymal stem cells (MSC) which can be a source of cells potential for cartilage repair [4]. Mesenchymal stem cells have been considered as a promising alternative source of cells for cartilage repair. Stem cells or stem cells are stem cells that can form new cells. Mesenchymal stem cells are wrong one type of multipotent adult stem cell (ASC). Based on the description above, where the use of stem cells can be used as an alternative for OA therapy, the authors are interested in conducting in vitro studies of mesenchymal stem cells against cells isolated directly from the tissues and synovial fluid of OA patients, wherein the synovial tissue and fluid containing pro and anti-inflammatory factors, so that the co-culture of OA cells with mesenchymal stem cells, will reduce these pro-inflammatory factors, which indicate the presence of the cells being carried out by stem cells-OA cell.

2 Method

2.1 Explant Culture

The method used is the explant planting method. The DMEM medium and complete medium are warmed using waterbath. After that, make sure the biosafety cabinet is ready for use. Prepare six well plates containing 3 ml of washing medium for each well. The tissue that has been obtained is washed up to six times. Separate fat from the tissue to be planted. Then the minch tissue becomes a small size using tweezers and sterile scissors. After that, planting on a plate that had previously been filled with a complete medium. Incubation in a CO2 incubator. Check every day until the cells differentiate and splitting is done until the cell count is sufficient for treatment. OA primary cells were cultured until 70-80% confluent, land were cultured together with stem cells. Cells were observed after 24 hours and 48 hours.

2.2 RNA Isolation, cDNA synthesis and Realtime PCR

RNA isolation using Trizol (Invitrogen). Every 1 µg of RNA will be synthesized into cDNA using a Reverse Transcriptase Script (BioRad). Realtime PCR is performed using specific primers for IL-1β and IL-6.

2.3 Data Analysis

Data were analyzed using Oneway ANNOVA to observe the expression of IL-1β and IL-6 in time dependent manner. Data considered significant with $p<0.05$.

3 Result

3.1 Characteristics of Sinoviocytes

Sinoviocytes cells have morphology in the from of fibroblast that are cultured in a plate containing a complete medium as a source of nutrition. Sinoviocytes cells morphology can be seen on the figure 3.1.

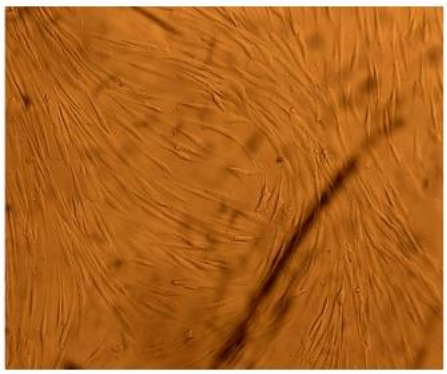

Fig. 3.1. Synoviocytes morphology

3.2 Effect of Mesenchymal Stem Cell on IL-6 Gene Expression in Osteoarthritis Sinoviocytes

The result of examination IL-6 gene expression in osteoarthritis sinoviocytes using the real time pCR method showed that the after 24 hours after incubation an increase occurred, but a significant decrease occured in the treatment 48 hours after incubation.

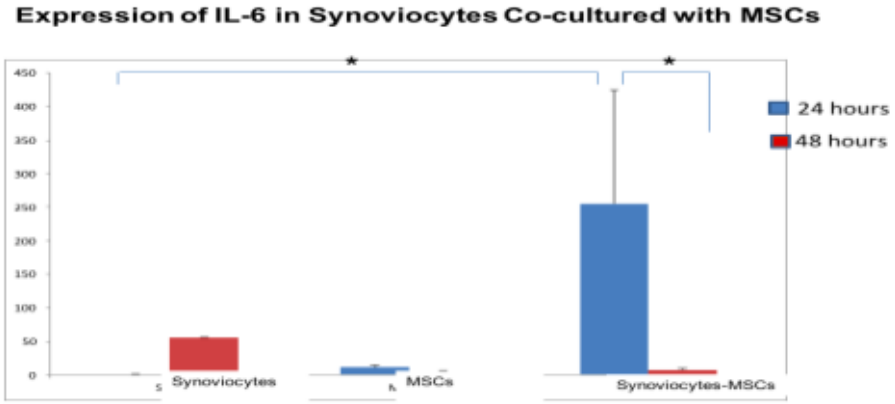

Fig. 3.2. Graph of the effect of mesenchymal stem cell on IL-6 gene expression in osteoarthritis synoviocytes

The result of IL-6 gene expression on realtime optimization PCR can be seen on graph amplication curve and melting peak graph showed on figure 3.3.

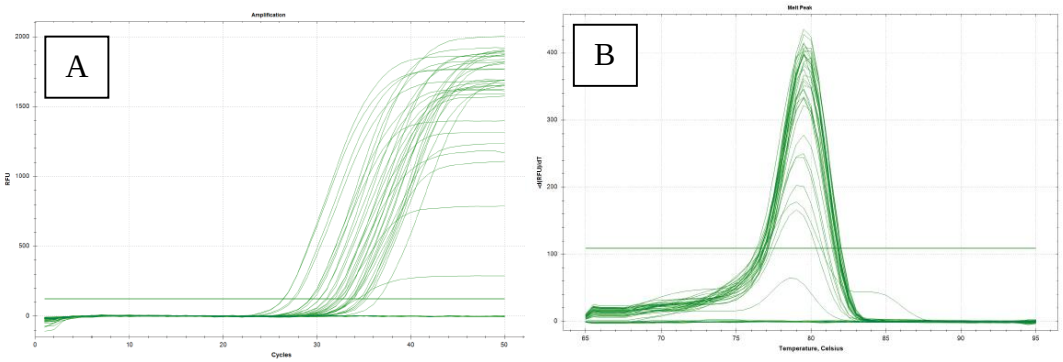

Fig. 3.3. Graph of the results of realtime optimization of primary PCR primary IL-6 gene. (A) Graph of amplification curve results in realtime PCR and (B) Melting peak graph from realtime PCR results

3.3 Effect of Mesenchymal Stem Cell on IL-1β Gene Expression in Osteoarthritis Synoviocytes

The result of examination IL-1β gene expression in osteoarthritis sinoviocytes using the real time pCR method showed that the after 24 hours after incubation an increase occurred, but a significant decrease occured in the treatment 48 hours after incubation.

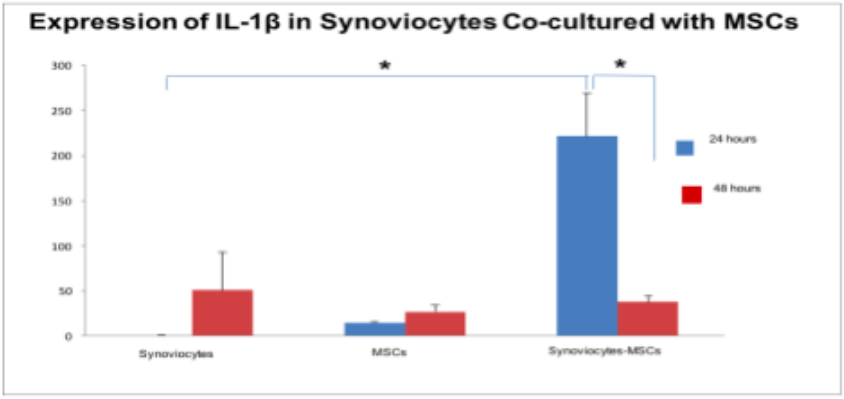

Fig. 3.4. Graph of the effect of mesenchymal stem cell on IL-1β gene expression in osteoarthritis synoviocytes

The result of IL-1β gene expression on realtime optimization PCR can be seen on graph amplication curve and melting peak graph showed on figure 3.5.

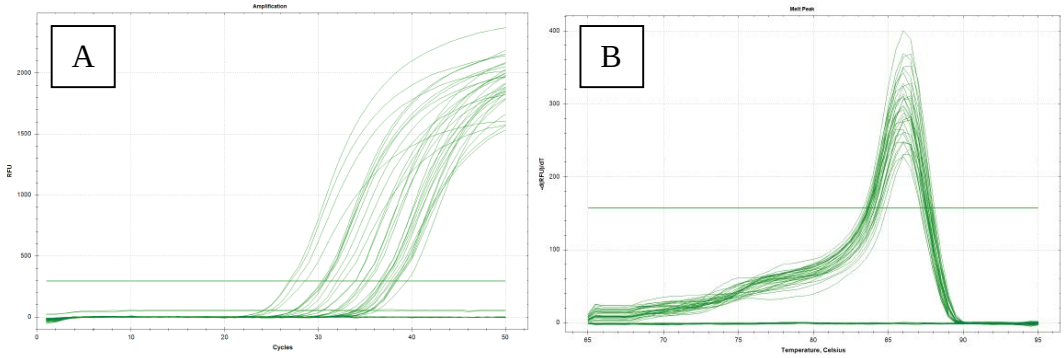

Fig. 3.5. Graph of the results of realtime optimization of primary PCR primary IL-1β gene. (A) Graph of amplification curve results in realtime PCR and (B) Melting peak graph from realtime PCR results

4 Discussion

The result of examination IL-1β gene expression and IL-6 gene expression in osteoarthritis sinoviocytes showed that the after 24 hours after incubation an increase occurred, but a significant decrease occured in the treatment 48 hours after incubation. Fan et al (2012), although interleukin- IL-1β (IL-1β) is one of the most important inflammatory mediators, growing evidence indicates that IL-1β signaling elicits the immunosuppressive properties of MSCs [5]. On the research Tang et al (2015), the result of the present study demonstrated that MSCs suppressed the inflammatory response and extracellular matrix degradation in IL-1β in a osteoarthritis rat model [6]. Other than that, Li et al (2013) both RT-PCR showed that myogenic diferentiation of MSCs was associated with significant downregulation of IL-6 expression [7].

Production of IL-6 in disease-affected joint tissue is a response from IL-1β and TNF-α [8]. IL-1β is mostly produced by synovial macrophages and chondrocytes in the OA joint [9]. The synovial intimal cells, termed synoviocytes, are believed to be responsible for the production of synovial fluid components, for absorption from the joint cavity, and for blood/synovial fluid exchanges. Two types of synoviocytes, macrophagic cells (type A cells) and fibriblast-like cells (type B cells) have been identified. Type A synoviocytes are non-fixed cells that can phagocytose actively cell debris and wastes in the joint cavity, and possess an antigen-presenting ability. These type A cells, derived from blood-borne mononuclear cells, can be considered resident macrophages (tissue macrophages) like hepatic Kupffer cells. Type B synoviocytes are characterized by the rich existence of rough endoplasmic reticulum, and dendritic processes which from a regular network in the luminal surface of the synovial membrane [10].

4.1 Interleukin-6

Interleukin-6 (IL-6) is one of several pro-inflammatory cytokines that exists at a high level in the synovial fluid of individuals with a clinical diagnosis of rheumatoid arthritis (RA) and osteoarthritis (OA) [11]. One cytokine that has been shown to play a role in cartilage destruction is interleukin 6 (IL-6). IL-6 increases in synovial fluid (SF) from OA compared to healthy donors [12].

IL-6 is a 26 kDa pleiotropic cytokine. Originally known as B cell stimulating factor stimulates cell growth B. Similarly, also known as hepatocyte stimulating factor for activating hepatocyte to produce acute phase reactants such as C-reactive protein and amyloid A. IL-6 is produced by various cell types including lymfocytes, monocytes, fibroblasts, synovial, and endothelial cells [13].

In OA, IL-6 can stimulate chondrocyte cells and synovial cells to produce prostaglandin, collagenase and metalloproteinase which plays an important role in inducing cartilage degradation. In healthy patients and patients suffering from OA, IL-6 is able to be secreted by chondrocytes and be an important role in cartilage proliferation and metabolism [14]. According to Qu et al (2015) in his research It was found that increased expression of IL-6 in bone OA patients, caused by IL-6 secretion from monocytes facilitated by inflammatory factors including IL-1β and prostaglandin E as a consequence of the inflammatory response in synovial tissue in OA [15].

Cytokines IL-6 contribute to several diseases, including osteoarthritis [16]. Production of IL-6 in disease-affected joint tissue is a response from IL-1β and TNF-α. Increased IL-6 concentration was seen in synovial fluid and serum and was connected with the intensity of lesions seen in X-Ray images. The effect of the presence of IL-6 on cartilage joints is not much different from other cytokines, namely decreasing collagen type II production and increasing enzyme production from MMP. The effect can be increased under injuri conditions. IL-6 can cause changes in the subchondral bone layer, so its involvement is also highly considered as the main thing in OA [8].

4.2 Interleukin 1β

Complex relationship analysis shows that the processes that occur in a joint are not just a set which (apparently) only includes catabolic effects. In addition, anabolic anti-inflammatory processes also occur continuously. This phenomenon is driven by various mediators, in which the key role is associated with reactions in cytokine tissue. The group of inflammatory cytokines is the most important group of compounds that participate in the pathogenesis of OA. The most numerous and accurate participation of cytokines has been documented in the literature. Cytokines accountable to the greatest extent to the loss of tissue metabolic homeostasis by promoting joint forming and destructive catabolic processes. The key role they play in the pathogenesis of OA is the result of the effect these compounds have on most of these cells is in the joints and impacts through intracellular pathway signal transduction in the production of cytokines and inflammatory compounds and other enzymes. Among the many representatives of this group, the most important is associated with IL-1 β , TNF α , IL-6 [8].

Interlukin-1β (IL-1β) is considered as one of the most important and most influential cytokines in its involvement in the OA pathogenesis. IL-1β is mostly produced by synovial macrophages and chondrocytes in the OA joint [9]. These cytokines can induce inflammatory reactions and catabolic influences independently [8]. The role of IL-1β in pathogenesis is to suppress type II collagen production and proteoglycan and stimulate synovial cells to release matrix metalloproteinase (MMP) [9]. Cytokine IL-1β also releases proteoglycan from the extracellular matrix towards synovial fluid, in addition IL-1β can inhibit the synthesis of collagen type II, IX, and XI, stimulate abnormal proteoglycan production, and reduce the expression of natural MMPs inhibitors called tissue inhibitors of metalloproteinase (TIMP) [17]. There is also its role in stimulating the production of ROS, such as peroxide and hydroxylate radicals which can directly damage joint cartilage [8].

The articular cartilage of a whole human adult is central to articulating joint function. This is very dependent on the integrity of the extracellular matrix, given the high loading force during movement especially in the joint holding the load. Unlike the first impression of static tissue that is more or less, it shows the articular cartilage, although in adult organisms the network rotation is slow. Thus, one of the most important questions in the study of osteoarthritis is to understand the balance of catabolic and anabolic factors in articular cartilage because this is the key to understanding the biology of cartilage maintenance and degeneration. There are many anabolic and catabolic pathways mixed in the articular cartilage. The balance between anabolism and catabolism is titrated at various levels, ranging from mediator-synthesis cells that express catabolic or anabolic factors. Also, at the effector cell level (ie chondrocytes) the expression of anabolic and catabolic genes competes for the balance of matrix homeostasis, namely the synthesis of matrix components and the expression and activation of matrix decomposing proteases. Also, there are several intracellular cross-speech layers between the anabolic and catabolic signaling pathways. Perhaps the most important lesson from this overview is the idea that anabolic-catabolic equilibrium such as the amount and not so much anabolism is clean or catabolism is limited. Thus, it may not be the goal of treating osteoarthritis to encourage anabolism or to break down catabolism, but anabolic-catabolic balance total activities require proper titration and balance [18].

5 Conclusion

The expression of IL-1β is marked up after 24 hours of co-cultured with MSCs (57.72 ± 18.37) compared to control and shows a tendency to downgrade after 48 hours of event, statictically not significant. However, IL-6 showed a higher expression in synoviocytes after 24 hours of co-cultured with MSCs, and was significantly down regulated after 48 hours. Both IL-1 β and IL-6 showed higher expressions on 24 hours co-culturing synoviocytes with MSCs. Not IL-1β, expression of IL-6 is significantly decreased after 48 hours MSC treatment, implying that MSV grade IV syndicarthritis-derived osteoarthritis.

References

[1] Egloff, C., Hugle, T., and Valderrabano, V. 2012. Biomechanics and pathomechanisms of osteoarthritis, Swiss Medical Weekly. The European Journal of Medicine Science, 142:w13583, 1-14.

[2] Loeser, R F. 2010. Aging and Osteoarthritis The Role of Chondrocyte Senescence and Aging Changes in the Cartilage Matrix. National Institute of Helath Public Access. 17(8), 971-979.

[3] Sellam, J and Berenbaum, F. 2013. Is osteoarthritis a metabolic disease ?, Joint Bone Spine. Elseviier, 80, 568-573.

[4] Demoor, M., Ollitrault, D., Gomez-Leduc, T., Bouyoucef, M., Hervieu, M., et al. 2014, Cartilage tissue engineering molecular control of chondrocyte differentiation for proper cartilage, Biochimica et Biophysica Acta, 1840, Elsevier, 2414-2440.

[5] Fan, H., Zhaou, G., Liu, L., Liu, F., Gong, W., Liu, X., Yang, L., Wang, J., and Hou, Y. 20102. Pre-treatment with IL-1β enhances the efficacy of MSC transplantation in DSS-induced colitis. Cellular & Moleculer Immunology, 9, 473-481.

[6] Tang, J., Cui, W., Song, F., Zhai, C, Hu, H., Zuo, Q., and Fan, W. 2015. Effects of mesenchymal stem cells on interleukin-1β -treated chondrocytes and cartilage in a rat osteoarthritis model. Molecular Medicine Report, 12: 1753-1760.
[7] Li, P., Li, S.H., Wu, J., Zang, W.F., Dhingra, S., Sun, L., Weisel, R.D., and Li, R.K. 2013. Interleukin-6 downregulation with mesenchymal stem cell differentiation results in loss of immunoprivilege. J. Mol. Med. Vol 17, No 9, pp. 1136-1145.
[8] Wojdasiewicz, P., Poniatowski, L A., and Szukiewicz, D. 2014. The Role of Inflammatory and Anti-Inflammatory Cytokines in the pathogenesis of Osteoarthritis. Hindawi Publishing Corporation. Mediators of Inflammation. Review Article ID 561459, 1-19.
[9] Monemdjou, R., Fahmi, H., and Kapoor, M. 2010. Synovium in the pathophysiology of osteoarthritis. Therapy. 7(6), 661-668. 2.
[10] Iwanaga, T., Shkichi, M., Kitamura, H., Yanase, H., and Inoue, K.N. 2000. Morphology and functional roles of synoviocytes in the joint. Review. Arch. Histol. Cytol, Vol.63, No.1. p. 17-31 3.
[11] Akeson, G and Malemud, C.J. 2017. A Role for Soluble IL-6 Receptor in Osteoarthritis. J Funct Morphol Kinesiol, 2(3). 4.
[12] Tsuchida, A.I., Beekhuizeen, M., Bot, A.G.J., Bekkers, J.E.J., Dhert, W.J.A., Creemers, L.B., dan Saris, D.B.F. 2012. The Role of IL-6 in Osteoarthritis and Cartilage Regeneration. ORS Annual Meeting. 5.
[13] Choy, E. 2003. Interleukin 6 receptor as a target for the treatment of rheumatoid arthritis. Ann Rheum Dis;62(Suppl II):ii68–ii69. 6.
[14] Grassel S, Beckmann J, Rath B, Vogel M, et al. (2010). Expression profile of matrix metalloproteinase-2 and -9 and their endogenous tissue inhibitors in osteonecrotic femoral heads. Int. J. Mol. Med. 26: 127-133. 7.
[15] Qu, X.Q., Wang, W.J., Tang, S.S., Liu, Y dan Wang, J.L. 2015. Correlation between interleukin-6 expression in articular cartilage bone and osteoarthritis. Genetics and Molecular Research 14 (4): 14189-14195. 8.
[16] Dittrich, A., Hessenkemper, W., and Schaper, F.. 2015. Systems biology of IL-6, IL-12 family cytokines, Cytokine & growth factor Review, 26, Elsevier. 595-602. 9.
[17] Carmona, J U., Prades, M. 2009. Pathophysiology of Osteoarthritis. Continuing Education for Veterinarians. 28-40.
[18] Aigner, T., Soeder, S and Haag, J. 2006. IL-1ß and BMPS-interactive players of cartilage matrix degradation and regeneration. European cells and Materials Vol. 12, (pages 49-56) DOI: 10.22203/eCM.v012a06.