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RESEARCH ARTICLE

Correlation between Oxidative Stress with Cartilage Thickness and Catalase Activity in Osteoarthritis Model Rat after MSC-WJ Treatment

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Abstract

Objective: *This study was conducted to investigate the involvement of oxidative damage in rat model of osteoarthritis treated with Mesenchymal Stem Cell Wharton Jelly (MSC-WJ) and to elucidate whether oxidative stress indicators correlate with cartilage damage and catalase activity.* ***Methods****: This research is an experimental research with Posttest-Only Control Group Design. The sample consisted of 30 OA rat divided into 5 groups: group I was the OA rat control group, group II was the OA rat group after 4 weeks, group III was the OA rat group after 8 weeks, group IV was the OA rat group after 4 weeks of treatment with MSC-WJ and group V is the OA rat group after 8 weeks treated with MSC-WJ. Malondialdehyde (MDA) were measured by Ohkawa method, Cartilage Thickness were measured by using the Betaview program, as well as the 3.1MP Beta camera Sony exmor CMOS Sensor and catalse (CAT) activity were measured by modification of Shinha.* ***Results****: The results showed that the analysis of the Correlation between MDA with Cartilage Thickness (r = – 0.665) and CAT activity (r = – 0.666) in osteoarthritis Model Rat after Mesenchymal Stem Cell Wharton's Jelly Treatment.* ***Conclusion****: This study demonstrated a strong negative correlation between oxidative stress and knee cartilage thickness and catalase activity of MSC-WJ treated OA rats.*

Keywords: *Osteoarthritis; Mesenchymal Stem Cell Wharton's Jelly; MDA; Cartilage Thickness; Catalase*

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INTRODUCTION

Osteoarthritis (OA) is a complex disorder that affects many different joints, being a major cause of disability in the general population. It is characterized by morphological, biochemical, molecular and biomechanical changes of the cells and the extracellular matrix (ECM) that cause softening, fibrillation, ulceration, loss of articular cartilage, synovial inflammation, subchondral bone sclerosis, osteophyte formation and subchondral cysts. OA is a multifactorial and polygenic disease and its pathogenesis is influenced by several genetic and environmental factors associated with the activation of molecular pathways that contribute to the development of articular injuries.[1]

Swelling of the joints is one of the clinical features of OA that is associated with inflammation and reflect their synovitis due to thickening of the synovium or effusion.[2] Inflammation is triggered by external mediators such as cytokines and proteases, and internal cellular mechanisms that lead to increased production of inflammatory mediators and the lack of elimination of oxidized proteins. This protein in turn increases the concentration of reactive oxygen species (ROS) in cells, which further increases the oxidative damage that triggers inflammation.[3] Oxidative stress can promote cellular aging, and in particular chondrocyte aging.[4] Oxidative stress can also cause cell apoptosis and release of cellular content into the extracellular environment. Overall, degradation products and cellular content containing oxidized molecules can form a vicious circle, which is formed by newly formed ROS and further degradation products.[5]

Synovial inflammation plays an important role in the pathogenesis of OA and oxidative stress is present in the OA cartilage membrane. Under basal conditions, synovial cells express both iNOS and NADPH oxidase.[6] Inflammatory reactions in the synovium are controlled by several biochemical factors, including prostanoids, cytokines (IL-1α, IL-1β, TNF-α, PGE2), proteases and ROS produced by synoviocytes and chondrocytes. ROS contribute to inflammation-associated tissue degradation.[7]

Joint cartilage secretes various antioxidants as a cellular defense against the harmful effects of ROS. ROS are free radicals that contain oxygen molecules such as hydroxyl radical (•OH), hydrogen peroxide (H_2O_2), superoxide anion ($O_2^{\bullet -}$), nitric oxide (NO) and hypochlorite anion (OCl^-).[8] An understanding of the molecular pathways and their interactions with different joint tissues needs to develop new approaches for the prevention and treatment of OA. Recent studies have concluded that the development of OA is significantly associated with oxidative stress and reactive oxygen species (ROS).[9,10]

ROS are produced at low levels in articular chondrocytes, mainly by NADPH oxidase, and they act as integral actors of intracellular signaling mechanisms that contribute to the maintenance of cartilage homeostasis as they modulate chondrocyte apoptosis, gene expression, ECM synthesis and degradation as well as cytokine production.[11-14] ROS production and oxidative stress were found to be increased in patients with OA.[15-17] OA cartilage has significantly more ROS-induced DNA damage than normal cartilage and this damage is mediated by interleukin-1 (IL-1).18 Evidence for the

implication of ROS in cartilage degradation comes from the presence of lipid peroxidation products.[14,19-22] In contrast, antioxidant enzymes, such as superoxide dismutase (SOD), CAT, GPX and PON1 were decreased in OA patients. This confirms the role of oxidative stress in the pathogenesis of OA.[16,17,23-27]

Lipid peroxidation derived from chondrocytes is a major source of oxidative stress where ROS can cause cartilage collagen degradation, structural destabilization of the ECM, and cartilage aging through attack of polyunsaturated fatty acids on membrane lipids.[28-30] Lipid peroxidation produces various hydroperoxide and aldehyde products, such as malondialdehyde (MDA) and 4-hydroxynonenal (HNE) which are highly reactive to cellular components and ECM. Excessive binding of these reactive aldehydes to ECM and cellular proteins can alter cellular function, membrane permeability, electrolyte balance, thereby causing matrix protein degradation.[31] Elevated levels of MDA and HNE have been reported in plasma and synovial fluid of OA patients and these are the most commonly applied parameters for lipid peroxidation indicators. 29,32-34 MDA mediates the oxidation of cartilage collagen while HNE causes ROS emission that activates the MAPK pathway.[35]

Hydrogen peroxide (H_2O_2) induces chondrocyte cell death in a dose-dependent manner.[36] H_2O_2 produced by the hypoxanthine-xanthine oxidase (XOD) system causes inhibition of articular chondrocyte growth.[37] H_2O_2 produced in excess amounts can be a mediator of cellular damage. Excessive hydrogen peroxide production can occur both exogenously and endogenously. Exogenously, hydrogen peroxide can be formed during the acute inflammatory phase of osteoarthritis and rheumatoid arthritis involving neutrophils and macrophages. Endogenously, the production of hydrogen peroxide by chondrocytes occurs via NADPH oxidase. Excessive exposure to hydrogen peroxide on chondrocytes can cause various effects, namely cell apoptosis, production of lipid peroxidation, inhibition of synthesis and degradation of proteoglycans.[38] Catalase is an antioxidant that plays an important role in neutralizing hydrogen peroxide.

This study aims to determine the correlation of MDA with cartilage thickness and catalase in OA rats after treatment with Mesenchymal Stem Cell Wharton Jelly (MSC-WJ).

METHODS

Animals and Experimental Design

Male white rats (*Rattus novergicus*) weighing between 250 - 300 grams. The experimental animals were placed in clean cages, disinfected and free of pathogens and given standard food in the form of pellets and drinking ad libitum. The experimental animals were adapted for 1 week before treatment.

Osteoarthritis Induction

Osteoarthritis was induced by single injection of intraarticular iodoacetate into the knee joints of anesthetized mice by means of intraperitoneal injection of 0.1 ml 10% xylazine and 0.2 ml 10% ketamine.[39-41] Prepare an monosodium iodoacetate (MIA) solution with a concentration of 20 mg / ml (sterile 0.9% NaCl solvent). After anesthetizing the rat's left leg was bent 90° at the knee. The patellar ligament is felt under the kneecap and is injected into this area. Each mouse received 0.05 mL intraarticular injection into the left knee

using a gas tight glass syringe with 27 0.5-inch needle sizes.[39,42] Twenty four rats (three weeks after induction of MIA, Janusz *et al*. (2001) were divided into 4 groups. The OA rat group after 4 weeks, the OA rat group after 8 weeks, the OA rat group was treated with MSC-WJ for 4 weeks and the OA rat group was treated with MSC-WJ for 8 weeks. The treated group of OA rat was given 50 µL of MSC-WJ at a dose of 1 x 10^6 cells to the left knee, the control group and the untreated group were given complete medium after anesthesia.

Blood Collection and Knee Joints

Blood and Knee Joints were collected from the healthy rat group, the OA rat group after 4 weeks, the OA rat group after 8 weeks, the OA rat group was treated with MSC-WJ for 4 weeks and the OA rat group was treated with MSC-WJ for 8 weeks.

Histological Analysis

The rat knee joint was fixed with 10% formalin buffered phosphate, then decalcified in 8% HCl for 1 week. Furthermore, it is processed into paraffin blocks and cut with a microtome with a thickness of 4 µm. The results of the cutting in the form of a ribbon are placed on the surface of warm water at a temperature of 45^0C with the aim of eliminating folds in the ribbon due to cutting. Each ribbon (strip) was stained with Haematoxilin and Eosin.

The thickness of the cartilage is assessed using the Betaview program, as well as the Beta 3.1MP Sony exmor CMOS Sensor at 100 x microscopic magnification by measuring the thickness of cartilage from the joint surface to the bone and cartilage boundaries perpendicularly. Measurements were carried out at 10 different points with a distance of approximately 200µm from each measurement point.

Flow cytometry Analysis

Mesenchymal stem cells used for the test were passage 3. MSC-WJ passage 1 originated from the human umbilical cord (UC-MSC) obtained from the UPT of Stem Cell Medicine Technology RSCM FKUI. Based on flow cytometry analysis, this MSC-WJ has expression of CD73-APC cell surface markers 99.8%, CD105-PerCP-Cys5.5 95% and CD90-FITC 99.9%. Then MSC-WJ passage 1 is silenced (developed) and MSC passage 3 is obtained.

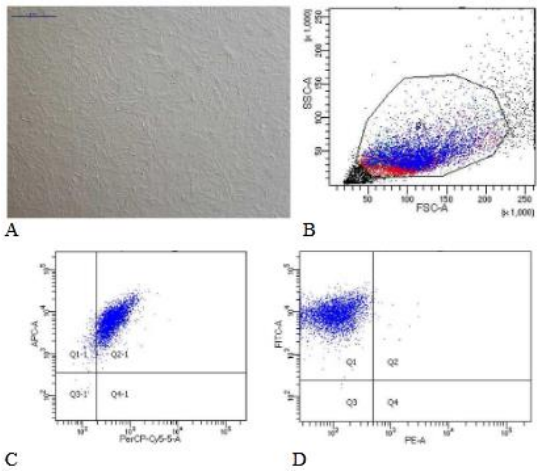


Figure 1: Data on Characteristics of Mesenchymal Stem Cells Wharton Jelly. (A) Cells MSC-WJ reach confluence. Scale bar: 500 µM. Photographs of cells taken using a Nikon Ti-S microscope; (B) Data flow cytometry. Forward scatter (FCS) plot & side scatter (SSC) plot. Population gated events (P1): 20,000; (C) Cell surface markers expression: CD73-APC 99.8% and CD105-PerCP-Cy5.5 95%; (D) Cell surface markers expression: CD90-FITC 99.9% and Lin (-) -PE 0.4%

Determination of MDA concentration

MDA concentration was measured fluorometrically as 2-thiobarbituric acid-reactive substance (TBARS) in serum by the

Ohkawa method. SF sample was mixed with 8.1% sodium dodecyl sulfate, 20% acetic acid and 0.8% 2-thiobarbituric acid. The method was modified by adding sodiumsulphate (100 mmol/l) and 3,5-diisobutylo-4-hydroxytoluen(2.5mmol/l). After vortexing, SF sample was incubated for 1 h in 95^0C and butanole - pyridine 15:1 (v/v) was added. The mixture was shaken 10 min and then centrifuged. Butanole - pyridine layer was measured fluorometrically at 552 nm (515 nm excitation). TBARS value is expressed as malonyldialdehyde equivalent. Tetra ethoxypropane was used as the standard. Data are shown as micromole MDA/l SF (mmol/l).[43]

Assay of Catalase Activity

This was carried out using the method described by Sinha method. 0.2 ml of sample (serum, and liver, heart, and kidney homogenates' supernatant) was mixed with 0.8 ml distilled H_2O to give 1 in 5 dilution of the sample. The assay mixture contained 2 ml of solution (800 µmol) and 2.5 ml of phosphate buffer in a 10 ml flat bottom flask. Properly diluted enzyme preparation (0.5 ml) was rapidly mixed with the reaction mixture by a gentle swirling motion. The reaction was run at room temperature. A 1 ml portion of the reaction mixture was withdrawn and blown into 1 ml dichromate/acetic acid reagent at 60 seconds intervals. The hydrogen peroxide content of the withdrawn sample was determined.[44]

Research Ethics

This research has received ethical consideration and approval from the Research Ethics Committee Team of the Faculty of Medicine, Andalas University with registration number 349/ KEP/FK/2019.

Statistical Analysis

The data are expressed as means ± standard deviations and testing of correlations by use of *Pearson's test*. The p value <0.05 was said to be statistically significant.

RESULT AND DISCUSSION

Study of 24 male osteoarthritis rats induced by monosodium iodoacetate (MIA) for 3 weeks. The OA rats were divided into 4 groups, two groups of untreated OA rats (OA rats 4 weeks and 8 weeks) and the other 2 groups treated with MSC-WJ for 4 weeks and 8 weeks.

Histophatology

The results of the histopathological examination of the knee joint tissue showed changes in cartilage thickness (Figure 2).

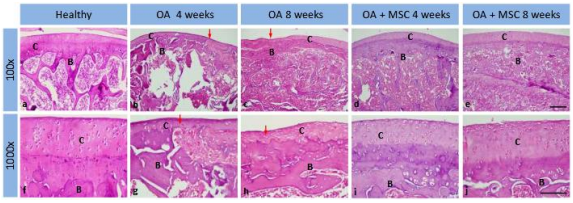


Figure 2. Comparison of histology of genu joint tissue between experimental and treatment groups. Healthy (a, f), osteoarthritis 4 weeks (b, g), osteoarthritis 8 weeks (c, h), osteoarthritis MSC-WJ treatment 4 weeks (d, i) and osteoarthritis treated by MSC-WJ 8 weeks (e, j). The joint tissue of osteoarthritis animals showed a decrease in joint cartilage thickness with a thin cartilage area (arrow). On MSC-WJ administration, there was an increase in joint cartilage thickness compared to positive controls (OA 4 weeks and OA 8 weeks). Hematoxylin eosin. 200 µm scale.

Biomarker Stres oksidatif

Table 1. MDA levels, knee cartilage thickness and catalase activity

Variable	Mean ± SD				
	Healthy	OA 4 weeks	OA 8 weeks	OA + MSC-WJ 4 weeks	OA + MSC-WJ 8 weeks
MDA	1.63 ± 0.61	2.78 ± 0.50	3.67 ± 0.72	2.03 ± 0.61	1.78 ± 0.42
Joint cartilage thickness	246.33 ± 62.13	124.48 ± 37.24	98.95 ± 17.61	172.85 ± 53.82	195.60 ± 17.40
CAT	10.30 ± 0.69	7.61 ± 0.45	6.70 ± 0.47	8.80 ± 1.09	9.62 ± 0.53

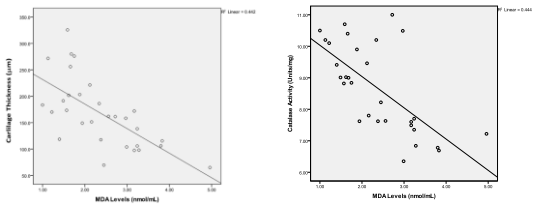
As can be seen in Table 1, MDA concentrations increased, whereas cartilage thickness and catalase activity decreased after MIA induced (4 and 8 weeks) compared to healthy rats.

As can be seen in Table 1, MDA concentrations increased, whereas cartilage thickness and catalase activity decreased after MIA induced (4 and 8 weeks) compared to healthy rats. However, there was a decrease in MDA levels, an increase in cartilage thickness and catalase activity after treatment with MSC-WJ (4 and 8 weeks).

Table 2. Correlation test result between MDA with Joint cartilage thickness and Catalase

Variable	Joint cartilage thickness			Catalase		
	n	Coefficient correlation (r)	p value	n	Coefficient correlation (r)	p value
MDA	30	- 0.665	0.0001	30	- 0.666	0.0001

The results showed a strong relationship between MDA as an indicator of lipid peroxidation with knee cartilage thickness (r = - 0.665) and catalase activity (r = - 0.666) which had a negative pattern. There was a significant relationship between MDA with knee cartilage thickness (p=0.0001) and catalase activity (p=0.0001) (Table 2). MDA contributed 44.2% to the thickness of knee cartilage (R2 Linear = -0.442) and contributed 44.4% to serum catalase activity (R2 Linear = -0.444) (Figure 3).



a b

Figure 3. Significant correlation between MDA levels with cartilage thickness (a) and catalase activity (b) was found both in vivo studies.

Relationship between MDA and knee cartilage thickness

The results showed a negative relationship between serum MDA levels and knee cartilage thickness, where decreasing MDA levels in MSC-WJ-treated rat serum caused an increase in knee cartilage thickness (Figure 2 and table 2).

MDA is an indicator of the occurrence of lipid peroxidation carried out by reactive oxygen species (ROS). Where the production of ROS is increased in cases of OA.[15-17] Evidence for the implication of ROS in cartilage degradation comes from the presence of lipid peroxidation products,[14,19,45] which is seen by the increased production of MDA in animal models of OA.[46] The binding of MDA to the ECM and cellular proteins will cause matrix degradation.31 Osteoarthritis sufferers experience an increase in MDA in their synovial fluid and blood plasma.[47] Grigolo et al. (2003) found that chondrocytes from osteoarthritis knee, which are activated in

vitro generate ROS and significantly accelerate lipid peroxidation.[48] According to Hiran et al. (1997) that articular chondrocytes can release ROS using nicotinamide adeninedinucleotide phosphate (NADPH) oxidase.[49] NADPH oxidase expressed by chondrocytes is the main enzyme responsible for ROS generation in synovial fluid, which contributes to increased oxidative stress within joints and mediates the progressive cartilage degradation that accompanies OA.[50,51]

Exposure of articular chondrocytes to H_2O_2 inhibits proteoglycan synthesis,[52,53] apoptotic cell death,[54] lipid peroxidation[28] and proteoglycan (PG) degradation.[55] The ineffectiveness of scavengers against hydroxyl radicals in disease models suggests that H_2O_2 itself is a major mediator of cartilage degeneration.[36]

ROS can cause damage to all components of the ECM. Free radicals attack directly on proteoglycan and collagen molecules and prevent the formation of collagen fibrils.[37,56,57] In the presence of oxygen, •OH degrades collagen and changes its amino acid composition. Incubation of acid-soluble type I collagen with the superoxide radical anion, which is generated by the XOD system, degrades collagen and prevents the formation of collagen fibrils.[58] Most in vitro studies indicate that ROS play a role in cartilage degradation, as reflected by their effect on matrix components and chondrocyte properties.[14]

This study showed that treatment with MSC-WJ could increase cartilage thickness in animal models that experienced thinning during OA propagation. The results of our previous study also showed the occurrence of cartilage repair in OA model rat treated with MSC-WJ,[59] because MSCs can differentiate into chondrocytes that play a role in producing cartilage matrix.[60]

MSCs are multipotent stem cells that have the ability to self-review and intrinsically repair and regenerate the tissues in which they reside after damage or trauma. MSCs have the capacity to differentiate into various other connective tissues such as cartilage, bone, tendon, adipose tissue. [61]

Relationship between MDA and Catalase

The results showed a negative relationship between serum MDA levels and catalase, where decreasing MDA levels in the serum of MSC-WJ-treated rats led to increased catalase activity (Figure 2 and Table 2).

Disruption of the joint components through knee injury can lead to increased production of $O_2^{\bullet-}$, in part due to the release and oxidation of hemoglobin from erythrocytes by activation of NADPH oxidase.[62,63] $O_2^{\bullet-}$ formed can be converted into H_2O_2 or •OH. In vivo hydrogen peroxide is detoxified and metabolized by the antioxidant enzymes CAT and glutathione peroxidase (GPX).[46] H2O2 produced by the hypoxanthine-xanthine oxidase (XOD) system causes inhibition of articular chondrocyte growth.[37]

Under normal circumstances, ROS are eliminated by scavenging and detoxification reactions, which are catalyzed mainly by antioxidant enzymes: SOD, CAT and glutathione transforming enzymes, including GPX, glutathione reductase (GR) and glutathione-S-transferase (GST). The first line of defense against ROS is SOD, which removes O_2^- by catalyzing a dismutation reaction. CAT protects cells and tissues by directly decomposing hydrogen peroxide.[64] The

absence or dysfunction of some of these defense systems makes cells and tissues susceptible to oxidative damage.[24] Ivanova and Ivanova (2000) found that there was no SOD and low levels of CAT in the synovial fluid (SF) of osteoarthritic joints.[65]

It has been demonstrated that MSCs are resistant to oxidative stimuli in vitro associated with antioxidant enzymes which are constitutively expressed as SOD1, SOD2, CAT and GPx.[66] In addition to using constitutive antioxidants, MSCs are also capable of significant adaptations in response to redox stress.[67]

Treatment with MSCs showed a decrease in ROS levels [68.69] because MSCs have been shown to reduce H_2O_2 and $O_2^{\cdot-}$ levels in cases of inflammatory diseases.[70,71,72] So that the use of catalase will be reduced to decompose H_2O_2. In addition, MSCs also constitutively express antioxidant enzymes such as SOD1, SOD2, CAT, and GPx.[67] So that catalase activity can be increased in animal models of OA treated with MSC-WJ. Therefore, both CAT and GPx may play a role in MSC therapy in reducing oxidative stress by removing H_2O_2 which acts as an inducer of cell death in vitro.[73]

CONCLUSION

Based on the results of the research that has been done, it can be concluded that there is a strong correlation between MDA serum dan ketebalan tulang rawan dan aktivitas katalese lutut tikus OA yang diterapi MSC-WJ.

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