
The relationship between NFκβ-p65 and PGE2 gene expression in osteoarthritis synoviocyte cells after mesenchymal stem cell Wharton Jelly Treatment

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Submitted: 26-02-2020

Reviewed: 21-04-2020

Accepted: 27-07-2020

ABSTRACT

The genes involved in cartilage degradation in the pathogenesis of osteoarthritis (OA) are the Nuclear Factor Kappa Beta (NFκβ) gene, which plays a vital role in modulating cartilage damage involving activation of pro-inflammatory cytokines, including prostaglandin E2 (PGE2). This pure experimental research aimed at analyzing the relationship between the expression of NFκβ genes after administration of mesenchymal stem cells in osteoarthritis synoviocytes in vitro from Wharton's Jelly (WJ-MSCs). The samples were isolated from synovial tissues of patients with osteoarthritis who underwent Total Knee Replacement (TKR) surgery and then divided into six treatment groups, each with four replicates. Groups I and II were the synoviocyte-control classes of 24h and 48h (synoviocytes, incubated for 24 and 48 hours respectively). Groups III and IV were control groups of 24h and 48h WJ-MSCs (mesenchymal stem cells derived from the Wharton's Jelly, incubated for 24 and 48 hours respectively). Finally, Groups V and VI were the 24-hour and 48-hour co-cultural treatment groups (synoviocyte co-culture and WJ-MSCs, Incubated, for 24 and 48 hours respectively). Using qPCR, PGE2 gene and NFκβ gene expressions were observed in each population. Tests showed 0.61 and 0.61, respectively, after the administration of WJ-MSCs, the expressions of the NFκβ-p65 and PGE2 genes in the 24h classes. In the 48h group, meanwhile, both numbers fell to 0.15 and 0.47, respectively. These indicate a strong correlation between the NFκβ-p65 gene and PGE2 gene expressions in OA synovial cells after the administration of MSC-WJ ($r = 0.903$).

Keywords: NFκβ gene, PGE2 gene, osteoarthritis, correlation

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INTRODUCTION

Up to now, clinical progress in understanding molecular and cellular pathways Osteoarthritis (OA) has illustrated strategies to prevent (Chung et al., 2018) the harmful effects of up-regulated cytokines or other inflammatory mediators, and their related signals. At the molecular level, degenerative joint disease ends up in the imbalance between catabolic and anabolic activities, wherever the first injury response happens in joint animal tissue (Kim and Yoo, 2018). Inflammatory reaction and animal tissue degradation expressions of the many genes, like lymphokine (IL)-1 and neoplasm death issue (TNF)- α , ar primarily regulated by NF κ B. NF κ B stimulates the TNF- α and IL-1 β cytokines that contribute to the inflammatory method in degenerative joint disease. The transcription of the PGE2 sequence conjointly includes NF κ B. NF κ B may be a sequence that plays a key role in degenerative joint disease pathological process (Saito, 2017).

The fast development of stem cell sciences in the world of research and medicines has broadened the potential of stem cells. Stem cells have famed to achieve success in delivering life-saving therapies to heal those affected by diseases that not result in ancient or surgical procedures of expedient. that embody chronic disorders and abnormalities, e.g. trauma and malignancy, whose prevalence is inclined to extend dramatically (Halim et al., 2010).

This experimental research was carried out in vitro using the OA patients synoviocyte tissue. It is part of a study of mesenchymal stem cells in vitro and in vivo on a greater scale. The innovation lies not only in the use of gene markers NFKB and PGE2, but also in the attempt to establish associations between the functions of both genes in OA pathogenesis, which are obtained as part of the study objectives, Is observed following in vitro administration of Mesenchymal stem cell Wharton Jelly to osteoarthritis synoviocyte cells.

MATERIALS AND METHOD

Tools and Materials

The research materials consisted of synovial tissues from Prof. Dr. M.Djamil Hospital in Padang City, a total tissue isolation kit for RNA (PureLink Invitrogen USA ®), kit iScript Reverse Transcriptase cDNA synthesis (BioRad), NF τ B-p65 primary gene (Macrogen ®), MMP-13 primary gene (Macrogen ®), HPRT1 primary gene (Macrogen ®), collagenase, deoxyribonuclease I in Dulbecco's Modified Eagle Medium (DMEM), Fetal-13 primary gene (Macrogen ®), Collagenase, Deoxyribonuclease I in Dulbecco Modified Eagle Medium (DMEM), Fetal Bovine Serum (FBS) from The Life Technologies ®, RT-PCR (ThermoFisher Scientific ®), NanoDrop 2000 Spechtrofotometer (ThermoFisher Scientific ®), and ThermoFisher Scientific ® centrifuge. The Andalas University of Cancer and Stem Cell Deoxyribonuclease I in Dulbecco Modified Eagle Medium (DMEM), Fetal Bovine Serum (FBS) from The Life Technologies ®, RT-PCR (ThermoFisher Scientific ®), NanoDrop 2000 Spechtrofotometer (ThermoFisher Scientific ®), and ThermoFisher Scientific ® centrifuge. The Andalas University of Cancer and Stem Cell Study Center works for the Indonesian Institute of Medical Education and Research (IMERI), Faculty of Medicine, University of Indonesia. have procured mesenchymal stem cells from Wharton Jelly (MSC-WJ), i.e. have procured mesenchymal stem cells from Wharton Jelly (MSC-WJ)

Method

Samples were collected from a total of ten patients aged 50-70 years, male and female. 10⁵ cells were used for each group. (Ibrahim et al.,2015).

Co-culture MSC-WJ and synoviocyte cells

Synoviocyte cells were grown up to 50–60 per cent, then cultivated with MSC-WJ and synoviocyte cells incubated in a CO2 incubator for 24 and 48 hours (Rosengren et al., 2007).

Primer designs

The base sequences of the primers of the NF κ B gene are according to a reference article, namely primer forward 5'-CGCATCCAGACCAACAA-3' and primer reverse 5'-AGATGGGATGAGAAAGGACAGG-3'. The MMP-13 gene for the primer forward was 5'-CACTTTATGCTTCCTGATGACG-3' and for the reverse primer was 5'-TCCTCGGAGACTGGTAATGG-3' (Yamada et al., 2010). As a control in the Real-Time PCR examination, the HPRT1 gene was used with the primer forward 5'-CCTGGCGTCGTGATTAGTGAT-3' and the reverse primer: 5'-CCCATCTCCTTCATCACATCTC-3' (Watanabe et al., 2017).

RNA isolation

A total of five hundred mikroliter cells that had been treated with stem cells and triazole were homogenized and placed in an exceedingly 1.5 ml PCR tube. Afterward, they were more with one hundred μ L of chloroform, subjected to a homogenous incubation for five minutes, and centrifuged at 12,000 g for quarter-hour at 4°C. the highest layer (supernatant) was removed to a new tube, more with 250 μ L of isopropanol, and shaken (in back and forth motion), then allowed to square for ten minutes at 4 °C. This mixture was then combined with five hundred five hundred of seventy five p.c grain alcohol, shaken, and centrifuged for five minutes at 4 °C with speed of 7.500 g. The highest layer containing grain alcohol was stripped, dried for quarter-hour, then applied RNase-free water with twenty five hundred and diluted per the NanoDrop polymer concentration victimisation the BioRad manual package operational procedures, USA.

PCR gradient amplification

The SYBR Green amplification kit was used to replicate every gene. The PCR system was set as follows: predenaturation for 30 seconds at 95°C, denaturation for 5 seconds, gradient annealing during five seconds at 55 °C (consisting of 50 cycles), and incremental melting curve from 65 to 95 °C, with an rise of 0.5 °C per five seconds.

cDNA synthesis

The total synthesis of desoxyribonucleic acid consisted of five μ g total ribonucleic acid, 1x RT buffer, twenty pmol oligodT, 4 mM dNTP, 10 mM DTT, forty U SuperScript TMII RTase catalyst, and twenty μ L DEPC-H₂O, severally. At 52°C for fifty minutes the entire desoxyribonucleic acid was synthesized with the operative procedures of the BioRad, USA manual kit.

This analysis used Livak-Schmittgen's (2001) relative quantification method, or quantitative analysis using the Threshold delta or Plaffl method.

$$\begin{aligned}\Delta C_{T \text{ experimental}} &= C_{T \text{ target in experiments}} - C_{T \text{ housekeeping in experiments}} \\ \Delta C_{T \text{ control}} &= C_{T \text{ target of control}} - C_{T \text{ housekeeping of control}} \\ \Delta\Delta C_{T} &= \Delta C_{T \text{ experimental}} - \Delta C_{T \text{ control}}\end{aligned}$$

The levels of expressions were determined using the equation Comparison of rates of gene expression = $2^{-\Delta\Delta C_{T}}$ where the gene HPRT1 as a control gene (housekeeping).

Sample size

The sample size was determined using the Federer formula (1977). The experiments used six groups, each consisting of 4 repetitions. The division of groups is as follows:

- Group I : 24h synoviocytes-control group, i.e., Synovial OA cells incubated during 24 hours
- Group II : 48h synoviocytes-control group, i.e., OA synovial cells incubated during 48 hours
- Group III : 24h WJ-MSCs control group, i.e., a control group consisting of mesenchymal stem cells sourced from the Wharton's Jelly and incubated during 24 hours
- Group IV : 48h WJ-MSCs control group, i.e., a control group consisting of WJ-MSCs incubated during 48 hours
- Group V : 24h co-culture treatment group, i.e., co-culture of OA synovial cells and WJ-MSCs that were incubated during 24 hours
- Group VI : 48h co-culture treatment group, i.e., co-culture of OA synovial cells and WJ-MSCs that were incubated during 48 hours

Ethical approval

The research permit is in the process of submission to the Research Ethics Committee of the Medical Faculty, Andalas University. In the event of approval, it will receive a registration number: 550/KEP/FK/2017.

Statistical analysis

The research data and experiment results, including the NFK β gene and PGE-2 gene expressions and the Pearson's correlation coefficients, are presented in tables and graphs. These data were processed in the statistical SPSS 22 program.

RESULTS AND DISCUSSION

Expression of NFK β and PGE2 genes

Prior to the analysis, a preliminary test of data normality and homogeneity was performed. Among both classes, the Shapiro-Wilk normality check yielded important values of 0.05, indicating the data were normally distributed.

The results of the descriptive analysis, i.e., skewness ratio of <2 in all groups, support this conclusion. Also, the Levene's test revealed that the homogeneity of variance was $0.16 \geq 0.05$, indicating that the data used in the NFK β and PGE2 gene research have the same variant (homogeneous). After administration of WJ-MSCs, the Pearson test was performed to know the expressions of the NFK β gene and the PGE2 gene correlation.

Table 1. Analysis of NF κ Bp65 gene relative expression levels using the Livak-Schmittgen method (2001)

Groups (n=4)	C _T average NFK β ($\bar{x} \pm SD$)	C _T average HPRT1 ($\bar{x} \pm SD$)	$\Delta C_T =$ NFK β - HPRT1	$\Delta \Delta C_T =$ ΔC_T treatment - ΔC_T control	$2^{-\Delta \Delta C_T}$
24 h synoviocytes-control	35.72 \pm 0.32	33.29 \pm 0.15	2.43	0.00	1
24h WJ-MSCs control	35.64 \pm 0.24	32.50 \pm 0.28	3.14	0.71	0.67
24h co-culture treatment	36.35 \pm 0.35	33.34 \pm 0.32	3.01	0.58	0.61
48h synoviocytes-control	35.94 \pm 0.20	33.95 \pm 0.19	1.99	0.00	1
48h WJ-MSCs control	38.56 \pm 0.38	33.90 \pm 0.33	4.66	2.67	0.16
48h co-culture treatment	37.09 \pm 0,14	32.41 \pm 0.28	4.68	2.69	0.15

Table 2. Analysis of PGE₂ gene relative expression levels using the Livak-Schmittgen method (2001)

Groups	C _T average PGE2 ($\bar{x} \pm SD$)	C _T average HPRT1 ($\bar{x} \pm SD$)	$\Delta C_T =$ PGE2- HPRT1	$\Delta\Delta C_T =$ ΔC_T treatment - ΔC_T control	$2^{-\Delta\Delta C_T}$
24h synoviocytes-control	35.05±0.22	33.29±0.19	1.76	0.00	1
24h WJ-MSCs control	34.72±0.16	32.50±0.28	2.22	0.46	0.73
24h co-culture treatment	35.82±0.23	33.34±0.09	2.48	0.72	0.61
48h synoviocytes-control	34.87±0.33	33.95±0.14	0.92	0.00	1
48h WJ-MSCs control	35.52±0.25	33.90±0.32	1.62	0.70	0.62
48h co-culture treatment	34.44±0.16	32.41±0.27	2.03	1.11	0.47

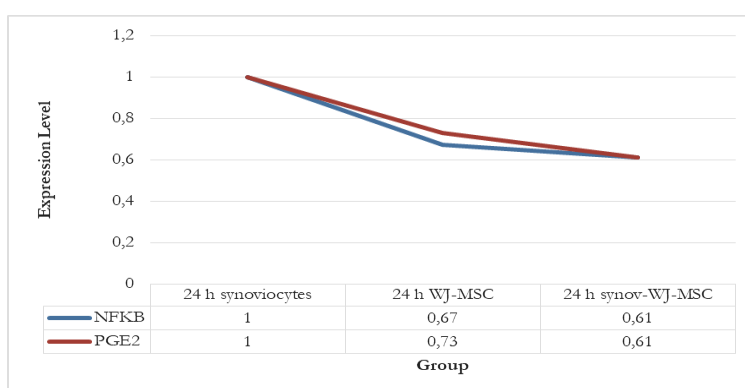
Tables 1 and table 2 show that the 48h synoviocytes-control and 48h co-culture groups produced the lowest NFκβ-p65 gene expression levels; the latter even yielded the lowest PGE2 gene expression level. Meanwhile, the NFκβ-p65 and PGE2 gene expressions in the 24h co-culture treatment group (co-culture of OA synoviocytes and WJ-MSCs) were higher than its 48h counterpart, but lower than the 24h WJ-MSCs control group.

Correlation between NFκβ gene expression and PGE2 gene expression

Table 3. Correlation between the expressions of NFκβ gene and PGE2 gene (Pearson's correlation test)

Variables	NFκβ-p65	PGE2	r ²	p
NFκβ-p65	1	0.903	0.909	0.01
PGE2	0.903	1		

Table 3 provides a strong correlation, which is apparent from the analogous rates and patterns, between the populations of NF β genes and PGE 2 genes. Based on the correlation coefficients of the Pearson, it was observed that NFκβ gene expression is closely correlated with PGE2 gene expression, with r= 0.903 (in the range of 0.70-0.99) and p=0.01. Likewise, Figure. 1 And Figure.2 indicate that the NFκβ gene is expressed in lower the PGE2 gene expression.

**Figure 1. The correlation of NFκβp65 and PGE2 gene expressions during 24 hours of incubation**

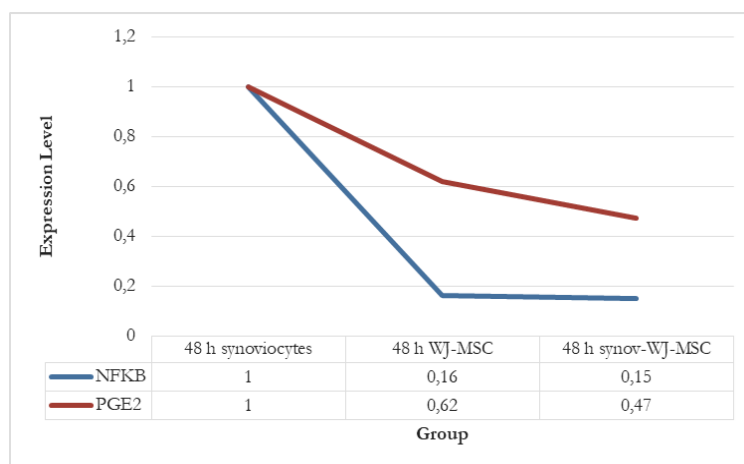


Figure 2. The correlation of NFK β 65 and PGE2 gene expressions during 48 hours of incubation

During the inflammatory process of OA pathogenesis, a widespread wide variety of pro-inflammatory mediators, e.g., nitric oxides (NO) and PGE2, are produced by using the inducible Nitric Oxide Synthase (iNOS) and cyclooxygenase-2 (COX-2). COX-2 is induced via pro-inflammatory stimulation, then COX-2 is authorised as an isoform accountable for the production of prostaglandins in various inflammatory models. Regulation of the PGE2 gene is very excessive in diseases such as rheumatoid arthritis, osteoarthritis and carcinoma.

High COX2 expression and PGE2 release play a major role in the initiation and development of cancer inflammation (Zhang et al., 2019). PGE2 intervenes the flagging pathway to expand NF κ B actuation, in which COX-2 can advance NF κ B phosphorylation. The subsequent cellular reaction culminates in the activation of various transcription factors, with a prominent role being played by NF κ B. This transcription factor contributes to several strong pro-inflammatory mediators, including cytokines and chemokines that can inflict damage to local tissue. PGE2 genes involved in cartilage damage caused by OA are also dependent on the activity of NF κ B (Lawson et al., 2013).

The results confirmed a advantageous NF κ β relationship between NF κ β gene and PGE2 gene expressions, in which low NF κ β gene expression stages coincided with low PGE2 gene expression levels. This nice correlation happens in synoviocytes within the synovium in the pathophysiology of OA. Various research have demonstrated that the expression of countless pro-inflammatory cytokine genes, including Tumor Necrosis Factor-alpha (TNF- α), Interleukin (IL)-1 β , IL-6, and PGE-2, is associated with NF κ β activation. NF κ β is one of the most important transcription elements in regulating a variety of cellular genes concerned in the inflammatory response of the acute immune section and the method of cellphone survival. According to (Megias et al., 2009), NF κ β is the primary regulator of the expression of pro-inflammatory genes.

The positive correlation is consistent with what various prior pupils have found. For instance, (Prosperi et al., 2012) are proposing that NF κ β flagging, which is a middle man or woman of provocative procedures, is additionally working as a controller and integrator with vitality digestion. It was explained that the NF κ β -p38 MAPK pathway is an essential component of cell transduction signals, especially in the regulation of genes involved in the inflammatory process, such as COX-2 and PGE-2 gene expressions (Tornatore et al., 2012). Macrophages are brought on by the involvement of micro organism, viruses, or external apprehensive stimulations. Lipopolysaccharide (LPS), an endotoxin formed by gram-negative bacteria, can set off macrophages that in impact trigger signalling, such as the NF- κ B pathway and the Protein Kinase pathway of mitogen activation.

This signalling system facilitates the secretion of pro-inflammatory cytokines, such as the tumor necrosis factor (TNF) α , interleukin (IL) 1β , and IL-6, and stimulates the release of inflammatory response elements such as NO and PGE2 (Luan et al., 2014). (Sun et al., 2009) additionally suggested that MTT-III-dep activates the development of PGE-2 and COX-2 expression via NF κ B in murine macrophages.

NF κ B inhibition can inhibit the expression of inflammatory elements and pro-inflammatory cytokines. Thus, therapeutic strategies that also avoid inflammatory ailments want to be developed by using studying compounds that inhibit iNOS and COX-2, control the expression of NO and PGE2, and inhibit the secretion of pro-inflammatory cytokines such as TNF- α , IL- 1β and IL-6 (Chung et al., 2018).

According (Signaling and Kopitar-jerala, 2015), expressions of iNOS and COX-2 in glial cells reportedly increase in inflammatory conditions, main to the extended synthesis of NO and PGE2. Such rules of NO and PGE2 production in the course of neuroinflammation performs a vital role in the law of inflammatory balance. NF κ B is a key transcription factor that increases the expression of pro-inflammatory enzymes and cytokines only if it has migrated to the nucleus. In un-activated cells, NF κ B is typically placed in the cytoplasm in association with I κ B α , which suppresses the activation signal and prevents translocation into the nucleus. When I κ B α is phosphorylated and degraded—a precursor of the activation of NF- κ B, NF κ B is remoted and translocated to the nucleus (Signaling and Kopitar-jerala, 2015).

The pathogenesis of osteoarthritis ought to be maintained in stability with the regulatory mechanism of complicated homeostasis; otherwise, mobile ailment or harm can occur. Activation of the important transcription thing NF κ B controls the expression of many genes that encode cytokines, chemokines, enzymes, and adhesion molecules involved in the synthesis of mediators from inflammatory reactions. In the cytosol, NF κ B is current in an inactive nation that types complexes with the I κ B protein, and its activation occurs via induction of phosphorylation, which mediates proteasomal degradation of I κ B. Suppression of NF κ B activation has been linked to anti-inflammatory activity. IL- 1β -induced NF κ B will inhibit COX-2 expression and PGE2 production. The effects of this study can be used as new therapeutic goals for OA via inhibiting pro-inflammatory cytokines which play an vital function in the cartilage degradation cycle.

There has been a developing variety of studies focusing on the physiology and pathophysiology of inflammation because many disorder morbidities are now realized as the product of inflammatory strategies involving seasoned and anti-inflammatory mediators (Rasheed et al., 2016).

(Guillot et al., 2019) found that inhibition of the NF κ B and PGE-2 pathways can purpose a minimize in tiers of pro-inflammatory cytokines like IL-6, IL-1 Beta, and VEGF in the synovial membrane of human knee joints suffering from OA; also, the NF κ B and PGE2 pathways are shown to be interrelated. The NF κ Bp65 gene, which is one of the NF κ B families, is worried in the expression of numerous genes which make a contribution to the inflammatory response in the course of the inflammatory process.

Pro-inflammatory cytokines and chemokines stimulate the NF κ B transcription process. Activation of NF κ B will trigger gene expression that reasons harm to articular joints, ensuing in osteoarthritis. In fact, NF κ B regulates the expression of more than one adaptive genes, such as Major Complex Histocompatibility (MHC), and genes that exceedingly modify apoptosis regulation. (A.Krum et al., 2010). Also, NF κ B is necessary to modulate immune response (Tornatore et al., 2012).

During the inflammatory process, the PGE2 gene is accountable for the inflammatory response to the pathogenesis of osteoarthritis. Its activation includes the function of NF κ B as a cytokine that regulates the occurrence of inflammatory responses. NF κ B activation can also activate gene expression that reasons damage to articular joints and, eventually, osteoarthritis (Biology et al., 2013).

Mesenchymal stem cells remoted from the Wharton's Jelly (WJ-MSCs) can reduce the expression level of the NFKB-p65 gene as a cytokine regulating the system of transcription of pro-inflammatory genes. Based on the interconnection between NFKB-p65 and PGE2 proven in this research, this technique is most in all likelihood to have an effect on the PGE2 gene expression, which in turn can improve the prevalence of injury to synovial tissue in OA. This effect seems due to the fact PGE2, a pro-inflammatory cytokine, plays a giant section in inflicting damages to the matrix of cartilage in joints. The results of this study provide a reference for the use of stem cells, specifically WJ-MSCs, as promising osteoarthritis therapy in the future.

CONCLUSION

There is a strong correlation between the expressions of NFKB gene and PGE2 gene in osteoarthritis synoviocytes after the administration of Mesenchymal stem cells isolated from Jelly Wharton's.

ACKNOWLEDGMENTS

We would like to thank the Andalas Cancer Treatment Center and Stem Cell (ACRC), Andalas University, and the Indonesian Medical Education and Research Institute (IMERI), Faculty of Medicine, University of Indonesia for providing the synovial tissues. Their appreciation also applies to the University of Ahmad Dahlan for financially funding the work and to Dr Rizki Rahmadian, Sp. OT (K), M. Kes to the assistance in obtaining synovial tissue samples.

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