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EFFECTS OF ESTROGEN ON KYNURENINE PATHWAY AND NF-kB IN TNF-α INDUCED NEUROINFLAMMATION

TNF-α ARACILI NÖROİNFLAMASYONDA ÖSTROJENİN KİNÜRENİN YOLU VE NF-kB ÜZERİNE ETKİLERİ

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Abstract

Neuroinflammation involves glia activation, releasing of inflammatory mediators such as cytokines and chemokines, and formation of reactive oxygen and nitrogen species. It plays a central role in many neurodegenerative diases processes such as Alzheimer's disease, Parkinson's disease, dementia. Estrogen deprivation, commonly associated with aging, loss of learning and memory skills in postmenopausal women and Alzheimer's disease. In this study, we studied effects of 17-ßestradiol on kynunerine pathway and NK-kB gene expression in neuroinflammation. According to our results, estrogen increased expression of kynunerinase gene and decreased IDO-1 gene expression after TNF-alpha incubation in differentiated SH-SY5Y cells. However, it did not change NF-kB gene expression.

Keywords: estrogen; neuroinflammation; TNF-α; Alzheimer's disease; kynureninase; NF-kB; IDO-1

Özet

Nöroinflamasyon, glia aktivasyonunu, sitokinler ve kemokinler gibi inflamatuar mediatörlerin salınmasını ve reaktif oksijen ve nitrojen türlerinin oluşumunu içerir. Alzheimer hastalığı, Parkinson hastalığı, demans gibi birçok nörodejeneratif hastalık sürecinde merkezi bir rol oynar. Genellikle yaşlanmayla ilişkili olan östrojen yoksunluğu, özellikle menopoz sonrası kadınlarda öğrenme kaybı ve hafıza becerileriyle ilişkilidir ve Alzheimer hastalıgı ile ilişkilidir. Bu çalışmada, 17-b östradiolun, kynunerine yolağı ve NF-kB gen ifadesi uzerine etkisini arastirdik. Elde ettiğimiz sonuçlara göre östrojen, farklılaşmış SH-SY5Y hücrelerinde TNF-alfainkübasyonu sonrası kinüreninaz geninin ekspresyon düzeyini arttırırken, IDO-1 gen ekspresyon düzeyini azalttı. Bununla birlikte, NF- kB gen ekspresyon seviyesini değiştirmedi.

Anahtar Kelimeler: *östrojen; nöroinflamasyon; TNF-α; alzheimer hastalığı; kinüreninaz; NF-kB; IDO-1*

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1. Introduction

The neuroinflammation; is a response that includes all the cells, including the central nervous system, neurons, macroglia and microglia, and can be a negative factor in acute and chronic brain disorders in connection with the brain. Cellular and molecular immunological components such as cytokines, reactive oxygen and nitrogen species and glial cells; Activation of microglia and astrocytes causes neuroinflammation. Neuroinflammation plays an important role in many neurodegenerative diseases such as multiple sclerosis (MS), Alzheimer's disease (AD), ALS, Parkinson's disease and autism (Carson, Doose, Melchior, Schmid, & Ploix, 2006). In order to activate complex neuroinflammatory pathways and microglia; factors such as genetic, environmental, age and past experiences have significant effects(Hof & Mobbs, 2009).

There are many potential mechanisms that estrogen can influence the symptoms of Alzheimer's risk and phenotype (Rosini, Simoni, Caporaso, & Minarini, 2016). The degeneration of cholinergic neurons and accumulation of amyloid β plaques cause the disease to progress gradually (Chen et al., 2016) (Shamim & Laskowski, 2017).

Oxidative stress is thought to be one of the main causes of Alzheimer's disease. ROS induces neuroinflammation by stimulating gene transcription by the release of cytokines such as pro-inflammatory TNF-α (Akbar et al., 2016)(Morales et al., 2014). The levels of TNF-α in healthy people's brain are low and their physiological role is uncertain. An increase in TNF-α levels is observed in chronic inflammation and inflammation plays a leading role, especially in the early stages of the disease. Pro-inflammatory mediators and nuclear transcription factor (NF-B) are directly or indirectly involved in the production of a large number of pro-inflammatory cytokines such as TNF-α. NF-κB is a key control role in inflammation and is an important target for anti-inflammatory therapeutic interventions (Ivanenkov, Balakin, & Lavrovsky, 2011). In the TNF-α mediated inflammation model, it was observed that ER activation and 17-β estradiol inhibited nuclear translocation of NF-kB, (Ghisletti, Meda, Maggi, & Vegeto, 2005).

In addition to this, pro-inflammatory cytokines can directly induce the production of oxidative species by affecting neuronal functions (Yamada, Akimoto, Kagawa, Guillemin, & Takikawa, 2009). It has been shown that estrogen inhibits the inflammatory response in the brain, and therefore estrogen depletion leads to an inflammatory state and causes IDO-1 activation (Jayawickrama, Nematollahi, Sun, Gorrell, & Church, 2017).

2. Materials and Methods

2.1. Cell Culture

The SH-SY5Y (ATCC) neuroblastoma cells were grown on a complete medium of Dulbecco's Modified Eagled Medium (DMEM) medium supplemented with 10% fetal bovine serum, 100 U / ml Penicillin / Streptomycin and L-Glutamine on a recommended 37°C medium containing 5% .

2.2. Differentiation of SH-SY5Y Cells

SH-SY5Y was cultured in an incubator containing 37 ° C 5% CO2 in DMEM medium containing human neuroblastoma cell line, 10% FBS, 2mM glutamine, 100U / ml 100U / ml Penicillin / Streptomycin and L-Glutamine, 0.1% non essential amino acid. The cell medium was changed every 3 days to pass 90% density. All applications were glued when the cells reached a density of 75%. 24 hours after the cells were seeded, differentiation was initiated by reducing the FBS ratio in the culture medium to 1% and by adding 10 [mu] M retinoic acid on the 4th and 7th days. To evaluate the morphological differentiation in the cells, they were analyzed under inverted microscope at days 4 and 7 (Kalkan, Durasi, Sezerman & Atasever-Arslan, 2016).

2.3. TNF-α Implementation to SH-SY5Y Cell Line

Cells treated with SH-SY5Y RA were supplemented with 10 μM TNF-α in cell media on day 8 of RA-treated cells and incubated for 24 hours. The estrogen was dissolved in ethanol to prepare a 10 -7 M solution. The solution was added to the cells incubated with TNF-α for 24 hours and incubated with TNF-α for another 6 hours.

It was studied in 3 groups:

1.Differentiated-RA (SH-SY5Y TNF (-))

2.Differentiated-RA (SH-SY5Y TNF (+))

3.Differentiated-RA (SH-SY5Y TNF (+), estrogen (+))

2.4. RNA Isolation

After pipetting 1 ml of tri-reagent was added for 10 million cells, it was then allowed to stand at room temperature for 5 minutes, then 200 μl of chloroform was added. After 15 sec vortexing, it was left in the room for 2-3 minutes. Subsequently, centrifugation was carried out for 15 min at +4 ° C. and the upper phase was placed in a new tube and 500 μl of isopropanol was added. Vortexed and allowed to stand in the room for 5-10 minutes. Supernatant was centrifuged for 10 minutes at +4 degrees. 750 μl 75% EtOH was added. Supernatant was centrifuged for 5 min at +4 degrees. The alcohol was evaporated by standing on ice for 10 minutes. 50 μl RNAase free water was added to dissolve the RNA pellet. 5 ependorhea divided to -80 degrees. Each tube was prepared to be disposable and thus the degradation of the RNA was prevented.

2.5. cDNA preparation from RNA

The SensiFAST cDNA Synthesis Kit will be used to synthesize cDNA (complementer Deoxyribose Nucleic Acid). According to the kit protocol; To prepare the master mix, add 1 μg RNA, 4 μl 5x TransAmp buffer, 1 μl Reverse transcriptase enzyme and DNase / RNase free water to complete 20 μl. The program was programmed to heat 25 ° C for 10 minutes, 42 ° C for 15 minutes, 48 ° C for 15 minutes, 85 ° C for 5 minutes and finally 4 ° C incubation.

2.6. Real Time PCR

qPCR method was used to investigate changes in the expressions of NF-Kb, IDO-1, and kynureninase genes in the neuroinflammation models. RT-PCR The Roche LightCycler® FastStart DNA Master was performed with SYBR Green I.

3. Results and Discussion

The estrogen is required for the synthesis of the acetyl transferase enzyme and has been studied in postmenopausal women by elevating the acetyltransferase acetyl activity on the 17β-estradiol cognitive processes to reduce the likelihood of Alzheimer's disease progression and improve cognitive performance by estrogen replacement therapy. (Janicki and Schupf, 2010).

In this study, we established the cellular model of neuroinflammation induced with TNF-α pro-inflammatory cytokine, which triggers neuroinflammation and mediates the mechanism of action of 17-β estradiol in inflammatory response. Understanding the mechanism of action of 17-β estradiol is very important because of the limited study of how the molecular mechanism of action is affected in neuroinflammation models of 17-β estradiol.

First, we induced neuroinflammation in SH-SY5Y neuroblastoma cells differentiated with RA and incubated with TNF-α for 24 hours, and then we incubated a group of cells that were incubated with TNF-α for another 6 hours with 17-β estradiol. According to our results, estrogen increased expression level of kynureninase genes and decreased IDO-1 gene expression levels after TNF-α. incubation in differentiated SH-SY5Y cells. However, it did not change NF-kB gene expression level (Figure 1).

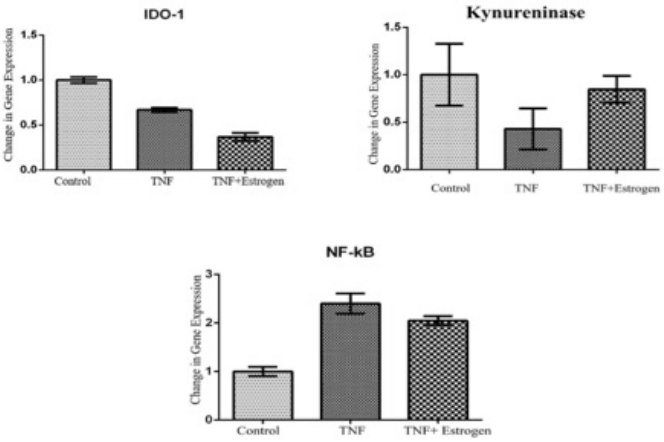

Figure 1. Expression levels of IDO-1, kynunerinase and NF-kB genes in SH-SY5Y cells differentiated-RA (SH-SY5Y TNF (+), estrogen (+)).

NF-kB is an important target for anti-inflammatory therapeutic interventions. In the inflammatory studies performed in recent years; 17-β estradiol is involved in the results obtained by inhibiting intracellular transport of NF-kB in inflammatory signal transduction pathways and inhibiting gene transcription induced by inflammatory agents (Tam, Mercado, Hoffmann, & Niwa, 2012) In our study, it was found that estrogen did not change NF-kB gene expression level in TNF-α induced neuroinflammation.

The expression of IDO-1, which is involved in the kynurenine pathway, is regulated by inflammatory cytokines, interferons, and TNF-α (Kincses, Toldi, & Vécsei, 2010). Also kynureninase is very important for kynurenine metabolism. Our results showed that estrogen has different effects on IDO-1 and kynureninase. While it increases kynureninase gene expression level, also it induces a decrease in IDO-1 gene expression. But its these effects do not change much against effects of TNF-α on the cells.

According to our results, 17-β estrodiol can have a protective effect on neuroinflammation. However, further investigations of estrogenic effects on neuroinflammation studies may improve therapeutic approaches to estrogen.

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