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Original Article:
The Effect of Vitamin E on the Expression of Mitochondrial Apoptotic Pathway genes in the Cerebellar Tissue of the Parkinson's Rat Model

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Abstract

Introduction: Parkinson's disease is one of the debilitating diseases which is more common amongst the elderly; oxidative stress has been found to be one of its major causes. In this study, therefore, the effect of vitamin E on the expression of Bcl-2 family genes was studied.

Materials and Methods: A total of 24 adult rats were purchased from the Pasteur Institute of Iran. The rats were later divided into 4 groups: control group, control group receiving VitE, PD group, PD group receiving VitE. After RNA extraction, cDNA synthesis, and primer design of Bax and Bcl-2 genes, their expressions were studied in different rat groups by real-time polymerase chain reaction (RT-PCR) technique.

Results: The mitochondrial apoptotic gene expressions were affected by vitamin E administration. Vitamin E decreased the expression of Bax gene and overexpressed Bcl-2 gene.

Conclusion: Vitamin E plays an important role in reducing the effects of oxidative stress and, consequently, apoptosis in the cerebellar tissue cells of rats with Parkinson's disease.

Keywords: Rat, Bax, Bcl-2, Parkinson's disease

Introduction

With an increase in life expectancy, the prevalence of neurodegenerative diseases has also been on the rise. Aging-associated diseases are becoming an epidemic in all industrialized countries. It is estimated that Parkinson's disease will quadruple by 2040 [1]. Neurodegenerative diseases have a variety of symptoms that affect different parts of the brain, and there are several reasons not yet fully understood. Altered mitochondrial function due to oxidative stress, abnormal accumulation of proteins and proteasomes, altered iron metabolism, and other factors occur in these diseases. All of these factors lead to impaired pathways and the onset of cell death. The removal of oxidized proteins in the cell done by proteasomes produces Reactive Oxygen Species (ROS) by altering the redox state. ROS-producing factors can damage mitochondria and neurons [2].

The Central Nervous System (CNS) is very sensitive to oxidative stress; the main reason for which is the high consumption of oxygen by the brain consuming 20% of the total oxygen in the body [3]. One of the factors studied in Parkinson's disease is the inadequacy of the brain's antioxidant system, which indicates that brain tissue has lower antioxidant activity than other tissues in the body. For example, brain tissue has 10% antioxidant activity of liver tissue [4]. Thus, the production of ROS, oxidative stress, and low levels of antioxidant activity in the brain can damage the nervous system [5]. Loss of redox balance in organisms due to antioxidant deficiency and increased ROS will play an important role in the development of neurodegenerative diseases including Parkinson's [6]. Domination of oxidative stress in neurons can cause the oxidation of several molecules which then paralyzes the defense systems within the tissues [7]. Vitamin E has antioxidant capacity that neutralizes oxidative stress against free radicals and is considered as a natural antioxidant [8]. It is a fat-soluble vitamin and an important antioxidant in preventing lipid peroxidation. It is also responsible for protecting membrane against free radicals produced by lipid peroxidation [9].

Oxidative stress can lead to damage of mitochondrial membranes by ROS production, cytochrome C separation from mitochondrial membranes, and apoptosis induction [10]. The release of cytochrome C is controlled by Bcl-2 family proteins located in the inner mitochondrial membrane [11]. Bcl-2 family proteins have been shown to play an important role in the isolation of cytochrome C from mitochondrial membranes. Anti-apoptotic proteins of the Bcl-2 family proteins include Bcl-2 and Bcl-XL, which prevent the cytochrome C separation from the inner mitochondrial membrane. In contrast, the pro-apoptotic proteins of the Bcl-2 family are Bax, Bcl-2, and Bid proteins, which separate cytochrome C from the inner mitochondrial membrane leading to its accumulation in the cytosol [12].

Therefore, the aim of the present study was to investigate the effect of vitamin E on changes in the expression of genes involved in mitochondrial apoptosis Bax and Bcl-2 in rat model of Parkinson's disease.

Materials and Methods

Materials

D-α-tocopheryl acid succinate (Sigma Aldrich, German) was administrated to the rats at dose of 24 I.U./kg, intramuscular [13].

Animals

To perform this experimental study, 24 adult male rats were purchased from the Pasteur Institute of Iran, Tehran. The animals were kept under standard conditions of 12 hours of light and 12 hours of darkness 25±2C and a relative humidity of 50%±10%. All animals were fed the same proportions of corn, wheat, barley, and pellets under the same nutritional conditions, and free access to water was available to all.

The rats were randomly divided into 4 groups.

1) Control group

2) Control group receiving 24 I.U./kg, i.m. D-a-tocopheryl acid succinate

3) Parkinson's disease group

4) Parkinson's disease group receiving 24 I.U./kg, i.m. D-a-tocopheryl acid succinate

Induction of Parkinson's disease

3% sodium pentobarbital (45 mg/kg i.p.) was used to anaesthetize rats. Then, unilateral lesions of the left medial forebrain bundle was performed, followed by stereotaxic injection of 6-hydroxydopamine (6-OHDA). For the preparation of 6-OHDA, it was first dissolved in sterile 0.01% ascorbate saline (4 µg/µL) and was injected unilaterally (0.5 µL/min) at the coordinates described by the atlas of Paxinos and Watson [14].

RNA extraction and cDNA synthesis

RNA extraction kit (Denazist, Iran) was used to extract RNA from cerebellar tissue. The manufacturer's instructions were applied for RNA extraction. Agarose gel and nanodrop were used to determine the quality and quantity of extracted RNA, respectively.

cDNA synthesis was performed using the CDNA Synthesis Kit (Easy cDNA Synthesis Kit, DenaZist, Iran) based on the manufacturer's instructions. Quantitative measurement of DNA was performed using a nanodrop device.

Primers

The primers for Bax (F-3'-AGG GTG GCT GGG AAG GC-5',R- 3'-TGA GCG AGG CGG TGA GG-5') and Bcl-2(F-3'-ATC GCT CTG TGG ATG ACT GAG TAC-5', R-3'- AGA GAC AGC CAG GAG AAA TCA AAC-5') genes were designed by Gene runner, Allel ID,

and Primer express softwares. The β-Actin gene (F-3'-CAC CAT TGG CAA TGA GCG GTTC-5', R-3'- AGG TCT TTG CGG ATG TCC ACGT-5') sequence was applied as internal control.

Real time PCR

Real time PCR (ABI 7300) was done using master mix and specific gene primers. Required components for Real time PCR are listed in Table 1. To amplify Bax and Bcl-2 cDNAs, RT-PCR time and temperature were programmed as the following. First, the cDNA denaturation was performed at 95°C for 30 s. Then, in the extension phase, 40 cycles of 95°C for 5 s and 60°C for 31 mins were applied. Finally, 95°C, 60°C and 95°C were programmed for 15 s, 30 s and 15s, respectively.

Statistical analysis

To evaluate the significant differences in the mean expressions of Bcl-2 and Bax genes (mean±SD) in different groups, the GraphPad prism software version 8, a one-way Analysis of Variance (ANOVA) and the Tukey post hoc test at the probability level of $P<0.05$ were used.

Rusults

Bax gene expression

The results of the present study showed overexpression of Bax gene in rats with Parkinson's disease. The administration of 24 I.U./kg, i.m. D-a-tocopheryl acid succinate down-regulated Bax gene in cerebral tissue (Figure 1).

Bcl-2 gene expression

Bcl-2 gene expression down-regulated in rats with Parkinson's disease compared with the control rats. However, administration of vitamin E to rats up-regulated the expression of this gene in healthy rats and rats with Parkinson's disease (Figure 2).

Discussion

In the present study, among the rats receiving vitamin E, down-regulation of Bax gene and overexpression of Bcl-2 gene were observed in cerebellar tissue: this in turn indicates that vitamin E prevents apoptosis.

The Bcl-2 family maintains the integrity of the mitochondrial outer membrane and is composed of anti-apoptotic and pro-apoptotic members. This family regulates the release of cytochrome C into the cytosol, resulting in the activation of caspase-3 [12].

In line with the results obtained in this study, many reports have shown that up-regulation of Bax gene can induce apoptosis in cells, both in vitro and in vivo [15]. It has also been reported that under oxidative conditions, overexpression of Bax and down-regulation of Bcl-2 can lead to the induction of apoptosis in the rat's hippocampus [16]. Korsmeyer et al. [17] reported that Bcl-2 does not appear to have an effect on the production of ROS,

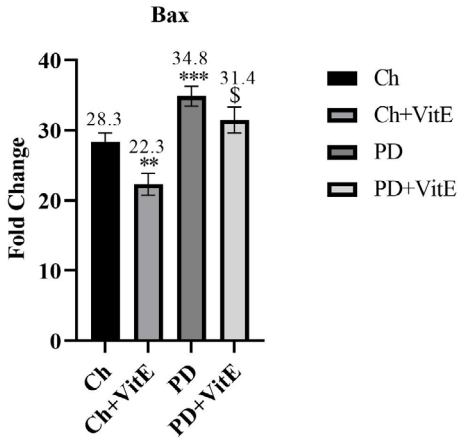

Figure 1. The effect of VitE on Bax gene expression in control and Parkinson's rats

** and *** showed significant differences comparing control rats at probability level of $P<0.01$ and $P<0.001$, respectively. $ represents significant differences comparing PD rats at probability level of $P<0.05$.

Ch: healthy control; Ch+VitE: healthy control receiving VitE; PD: rats with Parkinson's disease; PD+ VitE: rats with Parkinson's disease receiving VitE.

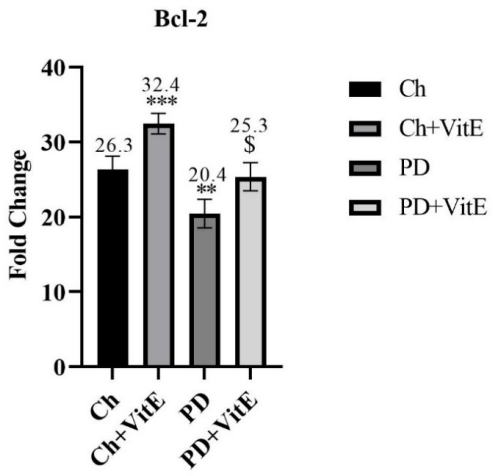

Figure 2. The effect of VitE on Blc-2 gene expression in control and Parkinson's disease rats

but that this gene protects cells from oxidative damage. Hildeman et al. also showed that overexpression of bcl-2 could protect cells from ROS-induced apoptosis [18]. However, the Bcl-2 cells protection mechanism against oxidative stress remains unknown. The researchers believe that Bcl-2 itself does not exert antioxidant activity, but it may have indirect activity in increasing levels or antioxidant activity inside the cell. Thus, the overexpression of Bcl-2 allows cells to better counteract the effects of ROS by increasing antioxidant enzymes [18]. The bcl-2 family proteins maintain the integrity of the mitochondrial outer membrane and are composed of anti-apoptotic and pro-apoptotic members. This family regulates the release of cytochrome C into the cytosol, resulting in the activation of caspase-3 [19].

Today, the therapeutic and protective effects of vitamin E on the central nervous system neurons in neurodegenerative diseases such as Parkinson's disease have been discussed [20]. Co-administration of vitamin E with vitamin C has been found to reduce the progression of the disease in the early stages of Parkinson's disease [21]. In addition, rapid improvement of cerebral ischemia due to intravenous injection of vitamin E has been reported [22]. The use of free radical scavengers such as vitamin E has also been shown to be effective in early Parkinson's disease [23]. In the present study, the reduction in apoptosis observed due to the administration of vitamin E could be attributed to the antioxidant effect of vitamin E and the inactivation of free radicals. Therefore, vitamin E can be considered in the treatment of Parkinson's disease.

Conclusion

In general, it can be concluded that vitamin E has therapeutic effects in Parkinson's disease and these effects can be attributed to the reduction of apoptosis as a result of reduced oxidative stress in the hippocampal tissue. Therefore, treatment of Parkinson's with vitamin E warrants further investigation in future studies.

Table 1. Required components for Real time PCR

Materials	Concentration
SYBR premix EX Taq™ 11 [2X]	25
PCR Forward primer [10 µM]	2
PCR Reverse primer [10 µM]	2
Rox Reference Dye or Dye 11 [50 X]	1
RT reaction solution [cDNA solution]	4
dH_2o [Sterilized distilled water]	16
Total	50

Ethical Considerations

Compliance with ethical guidelines

The current research was approved by ethical committee of Islamic Azad Tehran medical university Cod Number: IR.IAU.PS.REC.1399.208.

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Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflict of interest.

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