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Chronic Noise Exposure Activates Apoptosis Signaling Pathway in the Rat Auditory Cortex

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Abstract

Background: Today, hearing disorders are one of the most common problems in an industrial society. High-intensity sounds can induce apoptosis and metabolic changes in the auditory neural pathway and auditory cortex.

Aim: In this study, we investigated the effect of chronic noise exposure on Bax, Bcl-2 and Caspase-3 genes expression in the auditory cortex of rats.

Methods: Chronic exposure to 110 dB white noise was applied in male rats for 6 hours for 5 consecutive days. Superoxide dismutase (SOD) activity was measured in the serum of rats prior to and following noise exposure. Bax, Bcl-2 and Caspase-3 genes expression in the auditory cortex was determined by Real time PCR assay.

Results: Our results showed that the serum SOD level was significantly decreased in rats exposed to noise. In addition, the gene expression of Caspase-3 and Bax/Bcl-2 ratio was markedly increased in the auditory cortex of rats were subjected to chronic noise exposure compared to control rats.

Conclusion: Chronic noise exposure can activate apoptosis signaling pathway in the auditory cortex of rat.

Conflicts of Interest: The Authors declare no conflicts of interest.
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Introduction

Hearing loss is one of the most important causes of disability worldwide. Over the past few years, it has been found that hearing loss caused by noise pollution is the most common cause of chronic disability. Noise-induced hearing loss is a common occupational disease, and some factors such as genetic disorders and personal habits can alter the level of vulnerability to noise pollution (1, 2). High-intensity sounds can result in the loss of or cellular changes in various components of the adult auditory system (3). Numerous studies have shown that hearing loss is associated with the production of oxidative stress factors in the cochlea and auditory cortex which are produced in the mitochondria of mammalian cells (4-6). Reactive oxygen species (ROS) as signaling molecules are involved in the regulation of the apoptotic process (7).

Programmed cell death or apoptosis depends precisely on the interaction of certain gene products that activate or inhibit the cellular suicide process (8). The two major gene families, Caspases and Bcl-2, are involved in the apoptosis pathway and have two groups of

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pro-apoptotic and anti-apoptotic molecules. The average ratio of these proteins determines the fate of a cell (9),(10).

High-intensity sounds can induce apoptosis in the nerve tissue in the lower auditory pathway (3, 11). But other auditory structures such as the auditory cortex have also shown metabolic changes after exposure to noise(12). Therefore, this study investigated the effect of noise pollution on the activity of signaling molecules involved in the regulation of the apoptosis process in the auditory cortex.

Methods

Experimental animals

Twelve male Wistar rats, aged 2–3 months and weighing 200–250 g, were obtained from the Experimental Animal Center of Shahid Beheshti Medical University and kept at a constant temperature (21 ± 1°C), with 12/12-hour light/dark cycle. All experimental procedures were approved by the Ethical Committee of Shahid Beheshti University of Medical Sciences based on National Institutes of Health Principles of Laboratory Animal Care (NIH publication no. 85-23, revised1985). Rats were randomly assigned into two groups: control and noise exposure (n = 6/ each group). This study was approved by the Research Ethics Committee of Shahid Beheshti University of Medical Sciences (approval number: IR.SBMU.RETECH.REC.1396.1172) based on National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1985).

Noise exposure

Rats were exposed to 110 dB sound pressure level (SPL) which lasted 6 hours for 5 consecutive days. SPL was recorded using a sound level meter (TES-1358 Sound Analyse, Taiwan). The exposures were performed in a glass chamber (13).

SOD activity assay

Before and after the noise exposure paradigm, blood samples were obtained from the lateral tail vein of all rats, in an anesthetic condition. Blood samples were centrifuged at 4°C. The serum SOD activity was measured using a SOD Assay Kit (Cayman Chemical Company, Ann Arbor, MI, USA) according to the manufacturer's guidelines.

Tissue Preparation

Rats were decapitated after the end of the noise exposure protocol. Then the brains were immediately removed. The auditory cortex was dissected in a cold Petri dish, frozen in liquid nitrogen, and stored at -80°C until used for the real-time PCR.

RNA isolation

RNA was extracted from auditory cortex tissue using TRIZOL reagent according to the manufacturer’s protocol. The RNA quantity was measured with a spectrophotometer; first-strand cDNA synthesis by using 1 g RNA with 2 M oligo dT primer and 200 U MMLV in a total volume of 20 µL. The mixed suspension was incubated at 42°C for 60 minutes, followed by a final step incubation at 72°C for10 minutes.

Real-time PCR

Aliquots (5 µL) of cDNA were subjected to PCR assay using following primer pairs: Bax (F: 5'-CGGCGAATTGGAGATGAACTGG-3', R: 5'-CTAGCA AAGTAGAAGAGGGCAACC-3'), Bcl-2 (F: 5'-TGTGGATGACTGACTACCTGAACC-3', R: 5'-CAGCCAGGAGAAAT CAAACAGAGG-3') and Caspase-3 (F: 5'-GTGGAACTGA CGATGATA TGGC-3', R: 5'-CGCAAAGTGACTGGATGAACC-3') (14).

β-Actin was used as control using the following primer: F (5 TGTCCACCTTCCAGCAGATGT 3) and R (5AGCTCAGTAACAGTCCGC CTAGA 3). SYBR Green (TaKaRa) PCR assays for each

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sample were done in duplicate in 96-well optical plates.

Statistical analysis

All data are presented as the mean ± SEM. The analysis was performed using Student's t-test with GraphPad Prism version 5.04 Windows (Graph Pad Software, San Diego, CA, USA). p-value <0.05 was considered statistically significant.

Results

Effects of noise exposure on serum SOD level

SOD test was used to determine protection against the oxidative stress condition. The decreased SOD activity was associated with enhanced levels of oxidative damage. SOD level was reduced in the noise exposure groups (n = 6) (p<0.001) compared to control group (Figure. 1).

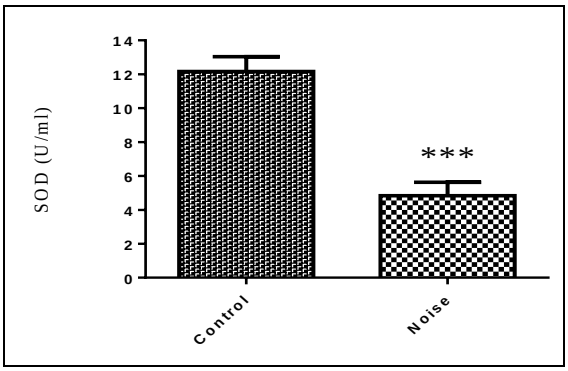

Figure1. Superoxide dismutase activity in the serum of rat. The effects of noise exposure on the serum SOD activity (mean±SEM). ***p < 0.001 compared to the control group (Student's t test).

Gene expression analysis

Expression of Bax, Bcl-2 and Caspase-3 genes expression was evaluated in noise and control groups. According to t- test analysis, Caspase-3 (n = 3, p < 0.01) and Bax/Bcl-2 (n = 3, p < 0.01) ratio in the auditory cortex of rats exposed to noise was more than in the control group (n = 3), (Figure. 2).

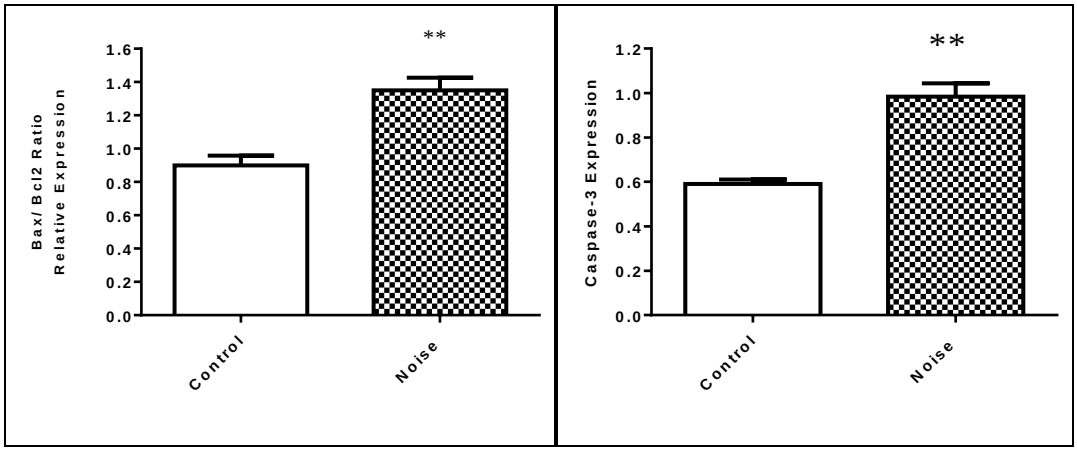

Figure 2: Effects of noise exposure on gene expression of Bax, Bcl-2 and caspase-3 in the auditory cortex of the rats. Bax to Bcl-2 gene expression ratio (Left) and caspase-3 gene expression increased (Right) in noise group compared to control group. ** p < 0.01 compared to the control group (Student's t test). The mRNA levels were measured and data were normalized to β-Actin. The expression of genes was expressed as folds compared to the control.

Discussion

In this study, we examined whether acoustic trauma can activate apoptosis signaling pathway in the rat auditory cortex. For this purpose, rats were subjected to chronic noise exposure. Then, serum SOD activity and auditory cortical genes expression of Caspase-3, Bax and Bcl-2 were assayed. Apoptosis can occur through two major pathways of apoptotic cell death including an extrinsic (death receptor-dependent) or an intrinsic (mitochondria-dependent) pathway (15).

Protein members of the Bcl-2 family control the mitochondrial pathway of apoptosis (16).

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This apoptotic pathway controls cell death by consisting of anti-apoptotic and pro-apoptotic protein of BCL-2 family. The anti-apoptotic members of the intrinsic pathway such as Bcl-2 prevent apoptosis. In contrast, protein such as BAX acts as a pre-apoptotic agent that results in the release of the cytochrome C from mitochondrial intermembrane space into the cytosol. Then cytosolic cytochrome c could induce caspase-3 via caspase-9 activation (17, 18). Studies have shown that metabolic damage due to oxidative stress plays a major role in hearing loss (19). Noise stress results in stimulation of the central nervous system and apoptosis of the cochlear tissue (20-22). Some studies have discussed the effect of the oxidative stress and oxygen free radicals on hearing loss (23). Yamane and colleagues have reported increased levels of superoxide anions in the cochlea after exposure to 120 -125 dB noise for 3 hrs in guinea pigs (24). Ising and colleagues, have shown that acoustic trauma can increase oxygen free radicals (25). In noise-induced hair cell apoptosis, activation of both caspases 9 and 8 has been observed after exposure to noise which indicates the involvement of both the membrane and mitochondrial pathways (26). Hu and colleagues, have also shown a different expression of apoptosis-related genes in the cochlea of rats exposed to noise (27).

Conclusion

In summary, this study revealed that chronic noise exposure can activate signaling pathways involved in stimulating the apoptotic process in the auditory cortex which play an important role in processing complex sound parameters.

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Not declared.

Conflicts of Interest

The authors declare no conflicts of interest.

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Ethics:

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