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Trehalose Increase Proliferation and Decrease Apoptosis in Hair Cells Induced by Hydrogen Peroxides

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Abstract

Background: Hydrogen peroxide (H_2O_2) a key reactive oxygen species, which is made through redox procedure, can induce oxidative damage to several biological macromolecules and play critical role in neurodegenerative diseases.

Aim: The current study examined the neuroprotective effect of trehalose on hair cells death induced by H_2O_2.

Methods: Hair cells were co-treated by trehalose and H_2O_2. The effects of trehalose on BAX/BCL2 expression ratio and cell viability were assessed by Real time PCR and MTT assay, respectively.

Results: The results of Real time PCR and MTT assay indicated that H_2O_2 induce cell death, and trehalose has neuroprotective effect and decreases cell death.

Conclusion: Our data showed that trehalose has the protective effect on hair cells death induced by H_2O_2.

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

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Introduction

Hair cells are auditory sensory receptors that are located in organ of Corti in the cochlea of the inner ear. These cells are divided into outer and inner hair cells based on their location and the type of activity. Outer hair cells mostly play the role in amplification of sounds, while inner hair cells play a role in perceiving sounds. Hair cells are responsible for converting sound into electrical impulses that are transmitted to the brain (1, 2).

Sensory and auditory nerve damage in the inner ear is known as sensorineural or nerve-related hearing loss (3). Hair cells in lower vertebrates can spontaneously repair, while damage to mammalian cochlear hair cells is not returnable. Therefore, the use of biological approaches to repair mammalian cochlear hair cells offers more extensive treatment for hearing loss (4, 5). Trehalose (also called mycosis) is a simple carbohydrate consisting of two-glucoses. It has 45% of the sweetness of sucrose (6, 7).

Trehalose and sucrose are sugars that are not able to cross cell membranes. Trehalose is a food additive that improves the quality of dried and processed foods by making them tastier. Trehalose is present in some fungi. Its hydrolysis produces two molecules of glucose (8). The presence of these sugars in the diluent causes a hypertonic environment which results cell shrinking. Recent research has shown that trehalose can inhibit the accumulation of

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proteins such as amyloid beta in Huntington and Prion (9). In this study, this sugar used as a medicine against these diseases and we evaluated the protective effects of trehalose on H_2O_2- induced cytotoxicity in cultivated hair cells.

Methods

Hair cells extraction and culturing

Animals (Wistar rats, weighing 200 g to 250 g, Iran) were purchased from Shahid Beheshti University of medical sciences.

This study was approved by the etic committee of Shahid Beheshti University of medical sciences. The hair cells were extracted from cochlea (and cultured in DMEM/F12 (Stem Cell Technology Company, Tehran, Iran), and supplemented with BFGF, EGF, 100 U/ml penicillin, 100 mg/mL streptomycin and 2 mM/mL L-glutamine and incubated in a humidified incubator with 5% CO_2 at 37°C (1).

Treatment of hair cells by trehalose and H_2O_2

The studied groups included group A: hair cells, B: hair cells and H_2O_2, group C: hair cells treated with trehalose and H_2O_2.

In order to examine the protective effect of trehalose against H_2O_2-treated hair cells, after 24 h pre-conditioning with 4 M trehalose, the cells were rinsed twice with PBS and then incubated with a basal medium containing 150 µM H_2O_2 for 20 minutes. It should be noted that dilutions of trehalose and H_2O_2 were freshly prepared and used in DMEM/F12 without FBS (6).

MTT assay

MTT assay was used to measure cellular metabolic activity as an indicator of cell viability, proliferation and cytotoxicity. In first step, hair cells were cultivated in 96-well plate, and were then treated with trehalose at 37 °C in a CO2 incubator. After incubation, MTT solution (100 µL) was applied to each well and the plate was incubated in a CO2 incubator for 4 h at 37° C.

The supernatants were then aspirated and the formazan crystals were solubilized by adding DMSO (100 µL, Carl Roth GmbH & Co., Karlsruhe, Germany) per well for 30 min at 37° C in the CO2 incubator. After this, dissolved formzan crystals absorbance was quantified by using the plate reader at 570 nm.

Real Time-PCR

Step 1: To conclude the abundance of copies of genes with bax, bcl2, whole RNA was collected from removal hair cells at 1 week using RNA Extraction kit (Invitrogen, USA) rendering to the manufacture procedure. Step 2: cDNA synthesis, the reaction product used directly for Real time PCR reaction or stored at -20 ° C. Step 3: Pico drop device was used to check the quantity of cDNA. Step 4: Real Time PCR reaction. Primers' sequences used in the Real Time PCR are presented in table 1.

Table 1: Primers' sequences used in the Real Time PCR.

Gene	Primer type	Sequence	Annealing temperature	Product size (Base pairs)
bax	F	CCCGAGAGGTCTTTTTCCGAG	65	210bp
	R	CCAGCCCATGATGGTTCTGAT		
bcl2	F	TACAGGCTGGCTCAGGACTAT	65	230bp
	R	CGCAACATTTTGTAGCACTCTG		
GAPDH	F	CCACAACTC TTCCATTCTC	59	200bp
	R	CCAAGATTCACGGTAGATAC		

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Data analysis

All results are presented as mean ± SEM. Statistical analysis was performed by one-way analysis of variance (ANOVA), and p-values < 0.05 was measured as statistically significant.

Results

H_2O_2-induced cell death in different groups

Cytotoxicity assay was done to investigate protective effect of trehalose pretreated hair cells against H_2O_2. Treatment of cultivated hair cells with trehalose and H_2O_2 markedly decreased cell death compared to H_2O_2 only treated cells (Figure 1).

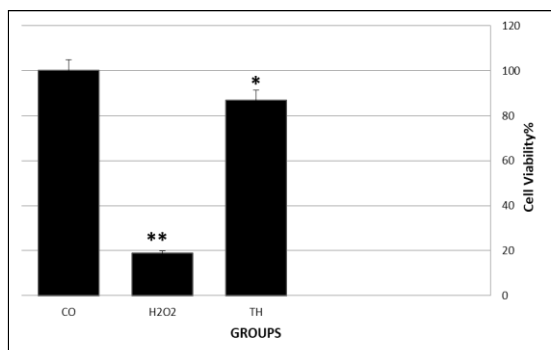

Figure 1. Results of the effect of trehalose on cell viability in the all groups. The results showed that trehalose reduced cell death in TH group in comparison to H_2O_2 group (*p<0.05) and significant difference between H_2O_2 and CO group (**p< 0.01) (CO: control, TH: Trehalose, H_2O_2).

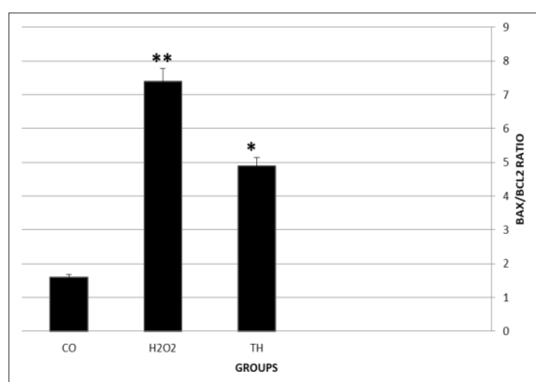

Figure 2. Real time PCR result showed that bax/bcl2 ration in experimental groups. Result showed that a significant difference between H2O2 group and CO group (**p<0.01) and significant difference between TH and H_2O_2 groups (* p< 0.05) (CO: control, TH: Trehalose, H_2O_2).

Trehalose reduce apoptosis rate

Results of Real time PCR assay showed that the BAX/BCL2 ratio in H_2O_2 group significantly difference in comparison with CO group and significant difference between TH compared to H_2O_2 group (Figure 2).

Discussion

According to the results of this study, it was found that some antioxidants such as trehalose can be used to protect neuronal cell species such as inner ear hair cells against oxidative stress caused by various neurological diseases. The results also showed that the rate of apoptosis and cell mortality in the group with trehalose was significantly different from the control group. Due to having neuroprotective effect, the viability of hair cells, and the expression of the bcl2 gene were significantly increased. These results were consistent with other studies (9). Based on our findings, trehalose has protective effects on hair cells induced by H_2O_2. However, a reason why neurodegenerative diseases are developing can be due to hypoxia, which causes vital damage to the neurons, neural tissue and also leads to nervous system disorder. Consequently, it is essential to protect neuronal cells from the damages in medical science (10, 11). Changes in neurons such as apoptosis in neurodegenerative diseases like Huntington, Parkinson, etc., can eventually result in paralysis and numbness in the wound area (15).

We put the hair cells in treatment with H_2O_2 then we investigated the viability test in hair cells using the MTT method. In other study, oxidative H_2O_2 produced stress in primary cerebral cortical neurons, by which the activity of the SOD enzyme in the cells decreases, leading to cellular death (16). Based on several studies, ROS potentially increases in numerous neurodegenerative diseases. An Increase in H_2O_2 followed by a ROS increase occurs when the produced ROS is greater than the

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antioxidants produced in the hair cell (12). The results of this study showed that trehalose reduces free radicals and hair cell mortality with protective effects. This increase causes oxidative stress in the hair cell that result in impairment in the function of mitochondrial which causes neuronal death (13, 14). The results of this study also showed that trehalose reduces apoptosis by improving BAX/BCL2 ratio. These two markers are markers affecting apoptosis and trehalose has inhibitory effects on their ratio. The results of this study were consistent with the studies of other researchers (6, 7). In general, trehalose can be used as a supplement in people prone to damage to the inner ear and hair cells as a preventative agent.

Conclusion

The results of this study showed that trehalose can be used as a protective agent against oxidative stress caused by inner ear diseases.

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Conflicts of Interest

The authors declare no conflicts of interest.

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