Neuroscience 286 (2015) 281–292

NADPH OXIDASE 2-DEPENDENT OXIDATIVE STRESS, MITOCHONDRIAL DAMAGE AND APOPTOSIS IN THE VENTRAL COCHLEAR NUCLEUS OF D-GALACTOSE-INDUCED AGING RATS

Z. DU, [a†] **Q. YANG,** [a†] **L. LIU,** [b] **S. LI,** [a] **J. ZHAO,** [a] **J. HU,** [a] **C. LIU,** [a] **D. QIAN** [a] **AND C. GAO** [a*]

[a] *Department of Otorhinolaryngology, Nanshan Affiliated Hospital of Guangdong Medical College, 89 Taoyuan Road, Nanshan District, Shenzhen 518052, China*

[b] *Guangxi Medical University, College of Pharmacy, Nanning 530021, China*

Abstract—Aging has been associated with oxidative stress and the accumulation of mitochondrial DNA (mtDNA) mutation. The previous study has established a mimetic rat model of aging using D-galactose (D-gal) and revealed that chronic injection of D-gal can increase NADPH oxidase (NOX)-dependent oxidative stress, mitochondrial damage and apoptosis in the peripheral auditory system. However, the effects of NOXs in the central auditory system (CAS) were still obscure. The current study was designed to investigate potential causative mechanisms of central presbycusis by using the D-gal-induced aging rats. We found that the levels of H_2O_2 and the expression of NADPH oxidase 2 (NOX2) and its corresponding subunits P22^{phox}, P47^{phox} and P67^{phox} were greatly increased in the ventral cochlear nucleus (VCN) of D-gal-treated rats as compared with controls. And, the levels of a typical biomarker of oxidative stress, 8-hydroxy-2-deoxyguanosine (8-OHdG), and the accumulation of mtDNA common deletion (CD) were also increased in the VCN of D-gal-treated rats as compared with controls. Moreover, the damage of mitochondrial ultrastructure, a decline in ATP levels, the loss of mitochondrial membrane potential (MMP), an increase in the amount of cytochrome c (cyt c) translocated to the cytoplasm and caspase-3 activation were observed in the VCN induced by D-gal. In addition, we also found that the terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate (dUTP) nick-end-labeling (TUNEL)-positive cells in the VCN were increased in D-gal-treated rats. Taken together, these findings suggest that NOX2-dependent oxidative stress may contribute to mitochondrial damage and activate a caspase-3-dependent apoptosis pathway in the CAS during aging. This study also provides new insights into the development of presbycusis.
© 2014 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: age-related hearing loss, central auditory system (CAS), NADPH oxidase 2 (NOX2), oxidative damage, mitochondrial DNA common deletion (mtDNA CD), apoptosis.

INTRODUCTION

Aging is the universal physiological phenomenon occurring in living organisms that is characterized by progressive degenerative changes in many organs. Age-related hearing loss, also known as presbycusis, is thought to result from age-related degeneration of the peripheral and central components of the auditory system (Frisina and Walton, 2006; Howarth and Shone, 2006). The cochlear nucleus (CN) is the first relay station in the central auditory pathway and consists of two major divisions, the dorsal cochlear nucleus (DCN) and the ventral cochlear nucleus (VCN). They receive the output of the auditory portion of the cochlear and set up parallel analysis and perception (Frisina and Walton, 2006). According to the previous studies, although age-related degeneration of peripheral and central auditory system (CAS) is well described, the exact pathogenesis of presbycusis is largely unknown.

Oxidative stress-induced oxidative damage to various biological molecules has been proposed as important factors in the process of aging (Ozawa, 1997). In the previous study, it has been demonstrated that the NADPH oxidase 3 (NOX3) might be an important source of the reactive oxygen species (ROS) in the cochlear of D-galactose (D-gal)-induced aging rats and revealed that chronic injection of D-gal can increase NOX3-dependent oxidative stress, mitochondrial damage and apoptosis in the peripheral auditory system (PAS) (Du et al., 2012b). However, the effects of NADPH oxidases (NOXs) in the CAS were still obscure. NOXs, including NOX1, NOX2, NOX3, NOX4, NOX5, DUOX1 and DUOX2, are an important source of ROS production, NADPH oxidase 2 (NOX2) is not restricted to phagocytic cells, but it is present in a wide variety of nonphagocytic cells and tissues, including neurons (Serrano et al., 2003; Quinn et al., 2006). NOX2 and its corresponding subunits P22^{phox},

*Corresponding author.
E-mail address: entcsgao@163.com (C. Gao).
† Z. Du and Q. Yang contributed equally to this work.

Abbreviations: 8-OHdG, 8-hydroxy-2-deoxyguanosine; CAS, central auditory system; CD, common deletion; cyt c, cytochrome c; DAPI, 4′,6-diamidino-2-phenylindole; D-gal, D-galactose; MDA, malondialdehyde; MMP, mitochondrial membrane potential; mtDNA, mitochondrial DNA; NOXs, NADPH oxidases; NOX2, NADPH oxidase 2; PAS, peripheral auditory system; PBS, Phosphate-buffered saline; RE, relative expression; ROS, reactive oxygen species; TBS, Tris-buffered saline; TEM, transmission electron microscopy; T-SOD, total superoxide dismutase; TUNEL, terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate (dUTP) nick-end-labeling; VCN, ventral cochlear nucleus.

http://dx.doi.org/10.1016/j.neuroscience.2014.11.061
0306-4522/© 2014 IBRO. Published by Elsevier Ltd. All rights reserved.

$P47^{phox}$ and $P67^{phox}$ form the active NOX2 enzyme complex that transports electrons from cytoplasmic NADPH to extracellular or phagosomal oxygen to generate superoxide (Bedard and Krause, 2007).

Oxidative damage to mitochondria and the accumulation of mitochondrial DNA (mtDNA) mutations are heavily implicated in the process of aging (Hamilton et al., 2001; Hiona and Leeuwenburgh, 2008). In humans, a 13-bp direct repeat (at 8470–8482 and 13,447–13,459) in the mtDNA leads to the frequent occurrence of a 4977 bp deletion (also known as the "common deletion", CD) by recombination between the repeats. A similar common mtDNA deletion occurs in rats due to a 16-bp direct repeat at 8103–8118 and 12,937–12,952; recombination between these repeats leads to a 4834-bp deletion. Therefore, CD has been used as a biomarker for aging (Yowe and Ames, 1998; Nicklas et al., 2004; Meissner et al., 2008; Markaryan et al., 2009). D-Gal has been used to induce oxidative stress *in vivo* to mimic natural aging in rats and the levels of mtDNA CD were significant increased in the CAS of D-gal-treated rats (Chen et al., 2010a,b; Zhong et al., 2012). However, there is no direct evidence to demonstrate that the accumulation of mtDNA CD caused by oxidative damage in the CAS of D-gal-induced aging rats.

Apoptosis may also play a key role in the age-related decline of physiological function in multiple organs (Youle and Strasser, 2008), including aging in the peripheral and central components of the auditory system (Someya et al., 2007, 2008; Chen et al., 2010a). Although the previous studies demonstrated that apoptosis cells were significantly increased in the CAS of D-gal-induced aging rats (Chen et al., 2010a,b), the cell apoptosis pathway has not been fully elucidated. In this study, we investigated the levels of H_2O_2 and the expression of NOX2 and its corresponding subunits $P22^{phox}$, $P47^{phox}$ and $P67^{phox}$, 8-hydroxy-2-deoxyguanosine (8-OHdG), cytosolic cytochrome c (cyt c) and cleaved caspase-3, the accumulation of mtDNA CD, the alteration of mitochondrial ultrastructure, the levels of ATP and mitochondrial membrane potential (MMP) and the occurrence of apoptosis in the VCN of D-gal-induced aging rats. Furthermore, we also explored the possible mechanism involved in presbycusis using D-gal-induced aging rats.

EXPERIMENTAL PROCEDURES

Animals and treatments

One hundred and seventy-six 1-month-old male Sprague–Dawley rats were obtained from the Experimental Animal Centre of the Guangxi Medical University. The rats were individually housed in a temperature-controlled (20–22 °C) room with a 12-h light–dark cycle and had free access to food and drinking water. The body weight of the experimental animals was monitored during the experiment as a general measure of health. The injection of D-gal to induce aging was administered following an established method. After acclimation for 2 weeks, the rats were randomly divided into four groups ($n = 44$ for each group) depending on the dosage of D-gal (Sigma, St. Louis, MO, USA): low, medium, high and a control group. Each day rats were injected subcutaneously with 150 mg/kg (low-dose), 300 mg/kg (medium-dose) and 500 mg/kg (high-dose) D-gal for 8 weeks; control rats were given the same volume of the vehicle (0.9% saline) for 8 weeks. After the experiment termination, the rats were anaesthetized with ketamine (30 mg/kg, i.p.) and chlorpromazine (15 mg/kg, i.m.), and blood was taken from the heart. Serum was obtained by centrifugation at $800 \times g$ for 15 min at 4 °C and stored at −80 °C until the measurements of H_2O_2, total superoxide dismutase (T-SOD) activity and malondialdehyde (MDA) were performed. The VCN were dissected and used for the determination of H_2O_2 and ATP levels and the extraction of total RNA, genomic DNA, protein and mitochondria. Alternatively, they were perfused with 2.5% glutaraldehyde for morphological studies by transmission electron microscopy (TEM) or with 4% paraformaldehyde for immunohistochemical analysis and terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate (dUTP) nick-end-labeling (TUNEL) staining. All experiments were carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Guangxi Medical University (Permit Number: SYXK2006-0003).

Serum H_2O_2, T-SOD activity and MDA assay

Using the serum from forty-eight rats ($n = 12$ per group), the H_2O_2, T-SOD activity and MDA were quantified using colorimetric kits (Jiancheng, Nanjing, China) according to the manufacturer's instructions.

Tissue H_2O_2 assay

After the last injection, twenty-four rats ($n = 6$ per group) were killed, and both sides of VCN from each rat were rapidly removed and homogenized in cold saline. The homogenate was centrifuged at $4000 \times g$ for 15 min at 4 °C, and the supernatant was used for H_2O_2 assay. Protein concentrations were determined using an Enhanced BCA Protein Assay Kit (Beyotime, Haimen, China). H_2O_2 in the VCN was quantified using colorimetric kits (Jiancheng, Nanjing, China) according to the manufacturer's instructions.

RNA preparation and quantitative real-time polymerase chain reaction (RT-PCR)

The mRNA expression levels of NOX2, $p22^{phox}$, $p47^{phox}$ and $p67^{phox}$ were determined by quantitative real-time SYBR Green PCR. After the last injection, twenty-four rats ($n = 6$ per group) were killed, and both sides of VCN from each rat were rapidly removed. One side of the VCN was used for RNA extraction, and the other side of the VCN was used for mtDNA analysis (see below). Total RNA was extracted with TRIzol reagent (TaKaRa, Dalian, China) according to the manufacturer's protocol. cDNA was reverse transcribed using a PrimeScript RT reagent Kit (TaKaRa, Dalian,

Download English Version:

https://daneshyari.com/en/article/6273111

Download Persian Version:

https://daneshyari.com/article/6273111

Daneshyari.com