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Involvement of retinoic acid-induced peroxiredoxin 6 expression in recovery of noise-induced temporary hearing threshold shifts

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ABSTRACT

All-trans retinoic acid (ATRA) is reported to reduce hair cell loss and hearing deterioration caused by noise-induced hearing loss (NIHL). The present study investigates the involvement of peroxiredoxin 6 (Prdx 6) in ATRA-mediated protection of temporary threshold shift of hearing. Mice fed with ATRA before or after exposure to white noise showed a faster recovery than untreated controls within 1 week, with a concomitant increase of cochlear Prdx 6 expression. Treatment of mouse auditory cells with ATRA induced Prdx 6 expression. A putative retinoic acid (RA)-response element (RARE) was identified in a murine Prdx 6 promoter region. Prdx 6 promoter activities were elevated in wild-type reporter plasmid-transfected cells, whereas no significant change in activity was in those with RARE-disrupted mutant reporter. RA receptor α (RARα) functions as a transactivator of Prdx 6 gene expression. These findings suggest that ATRA-induced Prdx 6 expression may be associated with rapid recovery from temporary NIHL.

1. Introduction

Noise-induced hearing loss (NIHL) is a common form of hearing loss in people who have certain occupations in industrial societies. Although many compounds have been developed to protect against hearing loss, approaches to prevent NIHL have mainly focused on the development of hearing conservation programs (Le Prell et al., 2007). Noise induces inner ear damage through physical and metabolic stresses (Henderson et al., 2006). In addition to mechanical damage, various

Abbreviations: NIHL, noise-induced hearing loss; ATRA, all trans retinoic acid; Prdx 6, peroxiredoxin 6; RARE, retinoic acid-response element; RAR, retinoic acid receptors.

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metabolic changes occur in the inner ear during noise exposure, resulting in cell death (Oishi and Schacht, 2011). Noise exposure drives intense metabolic activity in the major cellular system of the cochlea. This metabolic activity requires the mitochondria to produce abundant energy through the electron transport chain. As oxygen molecules are consumed as part of the mitochondrial aerobic respiratory process, excess superoxide anion is generated as an unwanted by-product, resulting in the accumulation of intracellular free radicals, including reactive oxygen and nitrogen species. Free radicals, small and chemically unique molecules each with an unpaired electron, can participate in chain reactions, leading to cellular damage by interacting with major cellular constituents, such as DNA, protein, and lipids.

Cochlear tissue labeling with an oxidation-sensitive dye revealed noise-induced increases in free radicals and lipid peroxidation products in the stria vascularis, outer hair cells, and auditory neurons. These accumulations triggered hair cell lesions due to apoptotic and necrotic cell death (Henderson et al., 2006; Le Prell et al., 2007). In animal models, the administration of exogenous antioxidant agents was shown to prevent noise-induced oxidative stress and associated functional loss by restoring the redox balance of sensory cells (Ohinata et al., 2003; Duan et al., 2004; Hight et al., 2003; Hou et al., 2003).

Vitamin A (retinol) is converted into its most active metabolite, retinoic acid (RA), by multistep enzymatic reactions inside cells. RA participates in various biological processes, including cell differentiation, proliferation, and development, by binding to RA receptors (RAR) and retinoid X receptors (RXR) that belong to the superfamily of nuclear hormone receptors. Upon RA binding in the nucleus, these proteins form heterodimers (RAR/RXR) or homodimers (RXR/RXR) and interact with RA-response elements (RARE) or retinoid X response elements (RXRE) within the promoters of target genes to regulate their transcriptional rates. In this context, heterodimers are generally considered to be more active than homodimers. In vitro binding studies have revealed that all-trans RA (ATRA) and its 9-cis isomers are high-affinity ligands for RARs, whereas RXRs only interact with 9-cis RA. Both retinol and RA have been suggested as potential therapeutic agents for many diseases, including leukemia, thyroid cancer, and xeroderma pigmentosum (Bastien and Rochette-Egly, 2004; Blomhoff and Blomhoff, 2006). With regard to acoustic trauma, ATRA administration was found to preserve the hearing threshold of mice from permanent hearing loss caused by consecutive noise exposure (Ahn et al., 2005). However, the protective mechanism of ATRA from NIHL at the molecular level remains to be elucidated.

Peroxiredoxins (Prdxs) comprise a superfamily of thiol-independent antioxidant proteins. These antioxidants catalyze the reduction of peroxides through conserved cysteine residues, without requiring cofactors such as metals or prosthetic groups. They are widely distributed in all phyla, and are classified as 1- and 2-cys Prdxs according to the number of cysteine residues involved in the catalytic mechanism (Rhee et al., 2005). Of the six mammalian Prdxs, Prdx 6 is a sole 1-cys enzyme with both phospholipase A_2 and peroxidase activities. Unique features of Prdx 6 include the use of glutathione (GSH) instead of thioredoxin as the physiological reductant, heterodimerization with glutathione S-transferase π (GST π) as the catalytic recycle, and maintenance of phospholipid turnover (Fisher, 2011). The overexpression of Prdx 6 protects cells from membrane damage associated with phospholipid peroxidation (Manevich et al., 2001), whereas the blockade of endogenous Prdx 6 expression with an antisense oligonucleotide results in the accumulation of lipid peroxidation products, leading to apoptotic cell death (Pak et al., 2002). The antioxidant defensive function of Prdx 6 is further supported by mouse model phenotypes. Prdx 6-null mice were shown to exhibit increased sensitivity to various oxidative stresses, with increased mortality and tissue damage (Wang et al., 2003, 2004). By contrast, transgenic Prdx 6-overexpressing mice were more resistant to hyperoxia (Wang et al., 2006). Analyses of the proximal promoter of the Prdx 6 gene have identified various putative regulatory elements (Lee et al., 1999), binding sites for Pax5 (Lee et al., 2008), and several redox-active transcription factors (Fatma et al., 2001, 2009; Chowdhury et al., 2009). These results suggest that complex transcriptional machinery regulates Prdx 6 gene expression.

In the present study, we examined the effect of ATRA administration on hearing recovery in mice after noise exposure. We investigated the temporal expression of the cochlear Prdx 6 protein during the recovery period, and the regulatory mechanism of the Prdx 6 gene in ATRA-treated cochlear hair cells.

2. Materials and methods

2.1. Materials

All components of the cell culture media were purchased from Life Technologies (Gaithersburg, MD), unless otherwise noted. Complete protease inhibitor cocktail and ATRA were obtained from Sigma–Aldrich (St. Louis, MO). Polyclonal antibodies to Prdx 6 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were from AbFrontier Co. (Seoul, Korea). Horseradish peroxidase (HRP)-conjugated secondary antibody was from Jackson ImmunoResearch Laboratory (West Grove, PA). An immortalized auditory cell line (HEI-OC1) that was derived from long-term cultures of Immortomouse cochleae was kindly provided by Dr. Federico Kalinec (Dept. of Cell and Molecular Biology, House Ear Institute, Los Angeles, CA). The RARα-overexpressing plasmid (pCMX-RARα) was generously provided by Dr. Sang-Beom Seo (Dept. of Life Sciences, Chung-Ang University, Seoul, Korea). All other chemicals were of biotechnology grade and were purchased from Amresco Inc. (Solon, OH).

2.2. Animals

Six-week-old BALB/c mice with normal Preyer's reflexes (Orient Charles River Technology, Seoul, Korea) received food and water ad libitum and were maintained on a 12 h light/dark cycle. All experimental protocols were performed in compliance with the guidelines of the National Institutes of Health and the Declaration of Helsinki. Protocols were approved by the Committee on the Use and Care of Animals of the University of Ulsan. Animal care was performed under the supervision of the Laboratory Animal Unit of the Asan Institute for Life Sciences.