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Over-expression of X-linked inhibitor of apoptosis protein slows presbycusis in C57BL/6J mice

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Abstract

Apoptosis of cochlear cells plays a significant role in age-related hearing loss or presbycusis. In this study, we evaluated whether over-expression of the anti-apoptotic protein known as X-linked Inhibitor of Apoptosis Protein (XIAP) slows the development of presbycusis. We compared the age-related hearing loss between transgenic (TG) mice that over-express human XIAP tagged with 6-Myc (Myc-XIAP) on a pure C57BL/6J genetic background with wild-type (WT) littermates by measuring auditory brainstem responses. The result showed that TG mice developed hearing loss considerably more slowly than WT littermates, primarily within the high-frequency range. The average total hair cell loss was significantly less in TG mice than WT littermates. Although levels of Myc-XIAP in the ear remained constant at 2 and 14 months, there was a marked increase in the amount of endogenous XIAP from 2 to 14 months in the cochlea, but not in the brain, in both genotypes. These results suggest that XIAP over-expression reduces age-related hearing loss and hair cell death in the cochlea.

Keywords: X-linked inhibitor of apoptosis protein; Apoptosis; Presbycusis; Mouse

1. Introduction

Age-related hearing loss (AHL), or presbycusis, is a common neurodegenerative disorder which affects approximately 40% of the population by 65 years of age (Seidman et al., 2002). Many factors such as noise exposure and miscellaneous ototoxic insults can injure receptor hair cells (HC) and spiral ganglion neurons (SGNs) in the cochlea, and collectively these are thought to contribute to AHL. At present, it is difficult to distinguish between the effects of aging per se from the cumulative action of these environmental factors. The HCs and SGNs are terminally differentiated cells and cannot be replaced by mitosis. Despite intense efforts to promote regeneration of HCs and SGNs in mammals, it is not yet possible to prevent the loss of these cells and the ensuing impairment of hearing.

A large body of evidence implicates apoptosis in age-related cochlear cell death (Alam et al., 2001; Iwai et al., 2001; Pickles, 2004; Spicer and Schulte, 2002; Zheng et al., 1998). During the process of aging, caspase-mediated apoptosis can be triggered by a variety of factors (Spicer and

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Schulte, 2002). Activation of these cysteinyl proteases in the cochlea causes the death of HCs and SGNs (Zheng et al., 1998). Hence, caspase inhibition as a method of preventing cochlear cell death may be a novel treatment strategy. In support of this approach, caspase inhibitors such as z-DEVD-fmk (caspase-3) and z-LEHD-fmk (caspase-9) have been shown to protect cochlear hair cells from cisplatin-induced death (Liu et al., 1998; Wang et al., 2004; Wu et al., 2005; Zhang et al., 2003). In addition, direct caspase inhibitor application to the inner ear protects vestibular hair cells against aminoglycoside toxicity (Matsui et al., 2003). Other interventions that partially prevent ototoxin-induced hair cell loss include the use of minocycline (Wei et al., 2005), neurotrophins (Ding et al., 1999a; Ernfors et al., 1996; Zheng et al., 1995), calpain inhibitors (Wang et al., 1999) and anti-oxidant therapy (Garetz et al., 1994; Lautermann et al., 1995; Ohinata et al., 2003). These treatments all block apoptosis. Unfortunately, the short duration of action of these chemical inhibitors or anti-oxidants limits their clinical utility in the treatment of presbycusis.

Members of the Inhibitor of Apoptosis Proteins (IAPs) such as X-linked IAP (XIAP), human-IAP1 (HIAP1/cIAP2) and human-IAP2 (HIAP2/cIAP1) inhibit apoptosis by blocking both the intrinsic (caspase-9; XIAP) and extrinsic (caspase-8; HIAP1/2) pathways that converge on the executioner caspases-3 and -7, which, in turn, are both inactivated directly by XIAP (Deveraux et al., 1998; Deveraux et al., 1997; Roy et al., 1997; Suzuki et al., 2001). As a result, elevating IAP expression increases the survival of many cells types when challenged with a variety of apoptotic triggers (Liston et al., 1996; Robertson et al., 2000). In the central nervous system (CNS), virally mediated over-expression of XIAP reduces the loss of *Cornu Ammonis* (CA)1 hippocampal neurons and preserves spatial navigation memory after transient forebrain ischemia (Xu et al., 1999). Virally mediated IAP expression also delays the death of cultured cerebellar granule neurons following potassium withdrawal (Simons et al., 1999). In hepatocytes, over-expression of HIAP2 inhibits apoptosis induced by various cytokines (Schoemaker et al., 2002).

In the inner ear, blocking caspase activity by XIAP over-expression exhibits at least two advantages over the use of exogenous inhibitors. Firstly, virally mediated XIAP expression in the inner ear produces more prolonged caspase inhibition than chemical inhibitors (Chan et al., 2007; Cooper et al., 2006). Secondly, XIAP also blocks non-caspase-mediated cell death, such as that produced by activation of the c-Jun terminal kinase pathway, which is also implicated in cochlear hair cell loss (Kaur et al., 2005; Suckfuell et al., 2007). Thirdly, XIAP by inhibiting caspase-3 and -7, XIAP blocks both extrinsic and intrinsic cell death pathways. These latter two features make XIAP the most potent of all known inhibitors of apoptosis (Deveraux and Reed, 1999; Deveraux et al., 1999; Kaur et al., 2005). Yet another advantage over small molecule caspase inhibitors such as z-VAD-fmk or DEVD-fmk, is that these small inhibitors are not selective caspase inhibitors (Schotte et al., 1999) but may block cell death by inhibiting a variety of cysteinyl proteases. The non-selective and irreversible cysteinyl protease inhibition produced by z-VAD-fmk or -DEVD-fmk increases the potential for toxicity.

Here we report the protective effects of XIAP over-expression in the inner ear in slowing the development of hearing loss that are potentially related to aging, using a transgenic mouse in which expression of the human XIAP gene is under control of the ubiquitin promoter (ubXIAP). The ubXIAP transgene was engineered to produce XIAP that contains a 6-Myc tag (Myc-XIAP), which is expressed in most cells types in the cochlea. Consistent with slow presbycusis, XIAP over-expression also reduced hair cell loss in the cochlea.

2. Materials and methods

2.1. Subjects and procedures

Transgenic founders were generated by microinjection of a linearized plasmid construct consisting of the Ubiquitin C promoter, 6 repeats of the 9E10 Myc epitope tag fused to the amino terminus of the human XIAP coding region, and a polyadenylation signal from SV40. The construct was microinjected into the male pronucleus of C57BL/6J X C3H F1 zygotes. C57BL/6J X C3H F1 offspring were backcrossed over 15 generations against wild-type (WT) C57BL/6J mice to obtain ubXIAP transgenic animals on a pure C57BL/6J genetic background. To obtain wild-type littermates for experimentation, ubXIAP animals were crossed with WT C57BL/6J mice. Transgenic status within the colony was determined by PCR targeting the 6-Myc tag. All transgenic mice used in this experiment were heterozygous.

The Myc-XIAP C57 transgenic (TG) mice and wild-type (WT) littermates were bred in the animal facility at Dalhousie University. In total, 48 mice were used in this study and longitudinally observed for development of hearing loss with time; 24 animals comprised each of the WT and TG groups with a matched number of mice of each gender in the two groups. Over the 14-month duration of the experiment, a few mice died, so that by the end of the experiment, 17 TG and 15 WT mice had survived. Hearing status was evaluated using frequency-specific auditory brainstem responses (ABR) that were performed every 2 months from the ages of 2–14 months. After the final ABR testing, the animals were sacrificed and both cochleas were harvested; one was used for evaluation of hair cell loss and the other for the quantification of both endogenous and transgenic XIAP. In total, 19 cochleas were taken from each group for constructing cytocochleograms (two mice from TG and 4 from WT groups contributed two cochleas). Western blotting was used for the quantification of both Myc-XIAP (65 kDa) and endogenous XIAP (endo-XIAP; 55 kDa). A piece of brain tissue was also taken from the temporal lobe of each animal for measurement of endo-XIAP and Myc-XIAP. Western blotting was success-