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Research paper

Blocking c-Jun-N-terminal kinase signaling can prevent hearing loss induced by both electrode insertion trauma and neomycin ototoxicity

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Abstract

Neomycin ototoxicity and electrode insertion trauma both involve activation of the mitogen activated protein kinase (MAPK)/c-Jun-N-terminal kinase (JNK) cell death signal cascade. This article discusses mechanisms of cell death on a cell biology level (e.g. necrosis and apoptosis) and proposes the blocking of JNK signaling as a therapeutic approach for preventing the development of a permanent hearing loss that can be initiated by either neomycin ototoxicity or electrode insertion trauma. Blocking of JNK molecules incorporates the use of a peptide inhibitor (i.e. D-JNKI-1), which is specific for all three isoforms of JNK and has been demonstrated to prevent loss of hearing following either electrode insertion trauma or loss of both hearing and hair cells following exposure to an ototoxic level of neomycin. We present previously unpublished results that control for the effect of perfusate washout of aminoglycoside antibiotic by perfusion of the scala tympani with an inactive form of D-JNKI-1 peptide, i.e. JNKI-1_{mut} peptide, which was not presented in the original J. Neurosci. article that tested locally delivered D-JNKI-1 peptide against both noise- and neomycin-induced hearing loss (i.e. Wang, J., Van De Water, T.R., Bonny, C., de Ribaupierre, F., Puel, J.L., Zine, A. 2003a. A peptide inhibitor of c-Jun N-terminal kinase protects against both aminoglycoside and acoustic trauma-induced auditory hair cell death and hearing loss. J. Neurosci. 23, 8596–8607). D-JNKI-1 is a cell permeable peptide that blocks JNK signaling at the level of the three JNK molecular isoforms, which when blocked prevents the increases in hearing thresholds and the loss of auditory hair cells. This unique therapeutic approach may have clinical application for preventing: (1) hearing loss caused by neomycin ototoxicity; and (2) the progressive component of electrode insertion trauma-induced hearing loss.

Keywords: Hearing loss; Electrode insertion trauma; Aminoglycoside ototoxicity; Otoprotection; JNK signaling; Hair cells; Apoptosis; D-JNKI-1 peptide (AM-111)

1. Introduction

Hearing deficits are often caused by death of the auditory hair cells. The human cochlea contains approximately 5,000 inner hair cells (IHCs) and 15,000 outer hair cells (OHCs), which do not posses an innate ability to regenerate themselves, since these hair cells (HCs) are only produced during embryonic development (Bredberg, 1968; Ruben, 1967). Once these HCs are damaged and not repaired or regenerated, a permanent hearing loss is the results. The ototoxic effects of aminoglycoside antibiotics are well known and further understanding of their mechanism(s) has been provided by recent studies indicating that

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the ototoxic effects of gentamicin requires an activated form of this drug. The activated form of this aminoglycoside then leads to the formation of a redux activated-gentamicin complex and the generation of reactive oxygen species (ROS; e.g. singlet oxygen molecules) (Sha and Schacht, 1999).

The effect of electrode insertion trauma (EIT) into the scala tympani is currently under investigation (Do et al., 2004; Eshraghi et al., 2005, 2006) and despite the fact that the molecular mechanism involved in the resulting loss of hearing post-implantation is not yet fully understood, there appears to be some similarities with the mechanism involved in the ototoxic effects of neomycin that induces loss of hearing (Wang et al., 2003a). The loss of sound trauma damaged auditory HCs is known to occur via a combination of both necrosis and apoptosis (Nicotera et al., 2003; Yang et al., 2004), and intermediate forms of cell death such as necrosis-like programmed cell death (Leist and Jaattela, 2001) and paraptosius (Sperandio et al., 2004). The histological characteristics of necrosis include both mitochondrial and nuclear swelling, dissolution of cellular organelles, and lyses of affected cochlear sensory cells with extensive degradation of DNA. Necrosis is generally thought to occur rapidly and therefore is thought to be very difficult to reverse with most otoprotection treatments, e.g. z-VAD-fmk, general caspase inhibitor. Reasonable approaches to the prevention of trauma induced necrosis of hair cells would be to either prevent the insult from occurring (e.g. modified surgical approach; Eshraghi et al., 2004) or to slow the metabolic response of a cell to injury as can be accomplished by the application of protective hypothermia (Balkany et al., 2005).

In contrast to necrotic cell death, the apoptotic death of an oxidative stress injured cell uses a series of biochemical intracellular signaling events that occur in response to injury to activate cell death molecules present within the cell in an inactive pro-form (e.g. conversion of Bid to truncated Bid). The cytoplasm and nuclear chromatin of the injured cell condenses, ribosomes and mitochondria aggregate, and the cell begins to die by forming cellular fragments called apoptotic bodies. The process of apoptosis often results in the creation of additional ROS and other free radicals, which cause additional injury, intracellular acidification, activation of caspases, and externalization of phosphatidyl serine residues (Lefebvre et al., 2002; Van De Water et al., 2004). Activation of procaspase molecules to activated caspases is a hallmark of the apoptosis form of programmed cell death. The following caspase molecules have been implicated in the apoptotic death of auditory hair cells, i.e. caspases 3, 5, 6, 8, and 9 (Van De Water et al., 2004). It is now known that intracellular events involved in the process of apoptosis can occur almost immediately after an insult (Hu et al., 2006) and/or also over a span of several days after the initial insult with apoptosis often the result of cumulative damage caused by the accumulation of ROS and other free radicals within the injured cell.

Aminoglycosides have been found to activate intracellular signal transduction pathways within an injured hair cell which contribute to its elimination via apoptosis (Wang et al., 2003a). The MAPK/JNK signal transduction pathway causes phosphorylation of the transcription factor c-Jun (Kyriakis et al., 1994). This happens in response to cellular stress induced by events such as aminoglycoside and acoustic trauma generation of ROS. In response, activated c-Jun contributes to AP-1 transcriptional complexes, and causes signal cascades that lead to apoptosis. The identification of this system, and others such as cysteine proteases (caspases) (for a review see Van De Water et al., 2004), has opened the door to the testing of inhibitors (e.g. z-VAD-fmk) that can block these signaling pathways (Wang et al., 2004).

The period of time between an initial insult and the onset of apoptosis of a damaged auditory HC, provides a window of opportunity for an interventional otoprotective therapy that specifically aims at lessening the ototoxic damage produced by an aminoglycoside and/or electrode insertion trauma. This report looks at these mechanisms at the cell biology level and proposes that the same therapeutic approach may be used to prevent the hearing loss that can result from either of these insults.

2. Aminoglycoside induced ototoxicity

Aminoglycosides are important antibiotics used to treat life threatening gram-negative bacterial infections and in the treatment of cystic fibrosis (Schacht, 1999). However, with the benefit of their efficacious antimicrobial action, comes the limiting factor of their ototoxic side-effect. Aminoglycosides are known to damage auditory hair cells, by a variety of proposed mechanisms, in such a way that 2–5% patients treated with this class of antibiotic develops an irreparable hearing loss.

2.1. Generation of free radicals and otoprotection by antioxidant molecules

Generation of ROS and other free radicals is one of the mechanism by which aminoglycosides cause apoptosis of auditory and vestibular sensory cells (Priuska and Schacht, 1995; Schacht, 1999; Sha and Schacht, 2000). It has been documented that gentamicin, a commonly used aminoglycoside, possibly causes formation of free radicals by forming complexes with iron, which is vital for normal mitochondrial function (Priuska and Schacht, 1995). Several reports have placed mitochondrial homeostasis at the core of aminoglycoside induced ototoxicity (Dehne et al., 2002; Fischel-Ghodsian, 2003). Although, it has only been postulated that free-radical formation causes ototoxicity, this hypothesis has strong support from the results of experiments which demonstrate that treatment with antioxidants attenuates aminoglycoside-induced hearing loss (Forge and Schacht, 2000; Sha and Schacht, 2000).