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Reduced expression of sialoglycoconjugates in the outer hair cell glycocalyx after systemic aminoglycoside administration

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Abstract

In this study we investigated the effect of systemic aminoglycoside administration on the expression of sialoglycoconjugates in the outer hair cell (OHC) glycocalyx of the adult guinea pig. Sialoglycoconjugates were visualized by means of ultrastructural lectin cytochemistry, using *Limax flavus* agglutinin (LFA) and wheat germ agglutinin (WGA) as probes. Labelling densities were determined for the apical membranes (including the stereocilia and stereociliary cross-links) and basolateral membranes of OHCs in the respective (basal, middle and apical) cochlear turns from animals that had been treated with gentamicin or neomycin for 5 or 15 consecutive days. Our results indicate that: (1) sialoglycoconjugate expression in the OHC glycocalyx demonstrates an intracochlear gradient decreasing towards the apical turn; (2) OHCs demonstrate a polarity in sialoglycoconjugate expression, in that the basolateral membranes contain more sialoglycoconjugates per surface area than the apical membranes; (3) aminoglycoside administration results in reduced expression of sialoglycoconjugates in the OHC glycocalyx; in this respect, basal-turn OHCs are more susceptible than those in the middle and apical turns; (4) reduction in sialoglycoconjugate expression after aminoglycoside administration is more prominent in the basolateral membranes; and (5) the difference in ototoxic potencies between gentamicin and neomycin is not reflected at the level of sialoglycoconjugate expression. The present data support our earlier hypothesis that aminoglycosides, already at an early phase of intoxication, interfere with the function of the endoplasmic reticulum and/or the Golgi apparatus, implying that these organelles play a crucial role in the initial phase of aminoglycoside-induced OHC degeneration.

Keywords: Cochlea; Outer hair cells; Glycocalyx sialoglycoconjugates; Aminoglycoside ototoxicity; Gentamicin; Neomycin

1. Introduction

Cochleotoxicity is one of the most prevalent adverse effects that are encountered during or after prolonged treatment with aminoglycoside antibiotics. Clinically, aminoglycoside-induced cochleotoxicity is manifested as a permanent sensorineural hearing loss with an initial high-frequency deficit that may continue to progress to the lower frequencies. Hearing loss results from a progressive and irreversible loss of cochlear hair cells and subsequent degeneration of the organ of Corti.

The histopathological lesions observed in the organ of Corti following aminoglycoside administration have been well documented in various mammalian species: Morphological studies have demonstrated that the outer hair cells (OHCs) are more vulnerable than the inner hair cells, and that the OHCs show signs of degeneration well before morphological changes are observed in any other cell

Abbreviations: GlcNAc, *N*-acetyl-D-glucos- amine; LD, labelling density; LFA, *Limax flavus* agglutinin; NAcGlucase, β-*N*-acetylglucosaminidase; NANA, *N*-acetylneuraminic acid; NANase, neuraminidase; OHC, outer hair cell; PBS, phosphate-buffered saline; SEM, standard error of the mean; WGA, wheat germ agglutinin

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type, within or outside the organ of Corti (for reviews, see Lim, 1986; Govaerts et al., 1990; Garetz and Schacht, 1996; Forge and Schacht, 2000). OHCs undergo a number of distinct morphological alterations in response to chronic aminoglycoside administration. An increase in the number of secondary lysosomes in the infracuticular region, vesiculation and dilatation of the subsurface cisternae of the endoplasmic reticulum as well as the Golgi saccules, formation of Hensen's bodies, and disorganization of the glycocalyx lining the apical and basolateral membranes are changes that are ultrastructurally observed in OHCs during an early phase of intoxication (Lim, 1986; De Groot and Veldman, 1988; De Groot et al., 1991).

The cellular mechanisms underlying the cytotoxic effect of aminoglycosides are incompletely understood. Since the effects aminoglycosides have on the metabolic pathways in OHCs are so manifold (cf., Lim, 1986; Rybak, 1986; Govaerts et al., 1990; Garetz and Schacht, 1996; Forge and Schacht, 2000), the precise trigger for (outer) hair cell degeneration remains elusive. However, it is now well established that aminoglycosides, after their endocytotic uptake, are sequentially transported to endosomes and then lysosomes via vesicular transport (Lim, 1986; De Groot et al., 1990; Hiel et al., 1992; Dulon et al., 1993; Fikes et al., 1994; Aran et al., 1995; Hashino and Shero, 1995; Hashino et al., 1997; Steyger et al., 2003). Also, it has been shown that cellular uptake and lysosomal accumulation of aminoglycosides largely precedes, even by days, the development of functional and cellular damage in hair cells (Hiel et al., 1993; Dulon et al., 1993; Hashino and Shero, 1995; Hashino et al., 1997; Imamura and Adams, 2003a). It is thought that this delay in the emergence of the drug's cytotoxic effect is related to the storage capacity of the lysosomes, and that lysosomal disruption with release of the aminoglycoside molecules into the cytosol could be a direct trigger for (outer) hair cell degeneration (Lim, 1986; De Groot et al., 1990; Hashino et al., 1997). Once spilt into the cytosol, the drug could react with potential cytosolic targets or interfere with crucial signalling pathways and, hence, initiate the chain of molecular events that ultimately lead to (outer) hair cell degeneration. Oxidative stress resulting from an overproduction of reactive oxygen species (Takumida et al., 1999; Sha and Schacht, 2000; Bertolaso et al., 2001, 2003; Dehne et al., 2002), up-regulation of calcium-dependent proteases (Ding et al., 2002), and activation of intracellular signalling pathways that are involved in apoptosis (Ylikoski et al., 2002; Kalinec et al., 2003; Bodmer et al., 2003) are but some of the mechanisms that have recently been implicated in aminoglycoside-induced (outer) hair cell degeneration.

However, this "lysosomal-accumulation-and-disruption" model cannot, in itself, explain the subcellular changes that take place during an early phase of intoxication. De Groot and Veldman (1988) observed that the colloidal thorium reactivity of the glycocalyx lining the apical and basolateral surfaces of the OHCs is diminished as early as 24 h after the first application of gentamicin, and is even completely abolished after five consecutive days of chronic administration. At this point of time, however, the lysosomes display a normal ultrastructural appearance and immunogold labelling for gentamicin is not yet scattered throughout the cytosol (De Groot et al., 1990; Hashino and Shero, 1995; Hashino et al., 1997; Imamura and Adams, 2003a). These observations suggest that aminoglycoside molecules, after their endocytotic uptake, are not only targetted to the lysosomes but also to other intracellular organelles, from where they can interfere with hair cell physiology. Likely, but hitherto ignored, candidates are the endoplasmic reticulum and the Golgi apparatus, which play a pivotal role in the de novo synthesis and subsequent modification (e.g., glycosylation) of proteins and lipids that are destined to the cell surface. The oligosaccharide sidechains of these membrane glycoconjugates form the glycocalyx which covers the basolateral and apical membranes, including the stereocilia and the stereociliary cross-links.

In previous publications, we have attributed the loss of colloidal thorium reactivity of the OHC glycocalyx after aminoglycoside administration to aberrant synthesis or glycosylation of membrane glycoconjugates (De Groot and Veldman, 1988; De Groot et al., 1990). It should be noted that aberrant glycosylation or altered expression patterns of cellular glycoconjugates are common findings in cells and tissues undergoing pathological changes (Damjanov, 1987; Roth, 1993), and sialic acids are the most ubiquitous oligosaccharides in the glycocalyx of OHCs (Tachibana et al., 1987, 1990; Gil-Loyzaga and Brownell, 1988; Katori et al., 1996). Furthermore, it is evident from enzymatic digestion experiments that colloidal thorium reactivity of the OHC glycocalyx is mainly due to the presence of sialic acid residues (Van Benthem et al., 1992, 1993). In the light of these observations, it is logical to hypothesize that aminoglycosides interfere with the function of the endoplasmic reticulum and/or the Golgi apparatus and, hence, with the expression of sialoglycoconjugates in OHCs.

In order to verify this hypothesis, we have quantified the effect of chronic systemic administration of two aminoglycosides with different cochleotoxic potencies (gentamicin and neomycin) upon OHC glycocalyx reactivity for *Limax flavus* agglutinin (LFA), which has a specific affinity for the sialic acid *N*-acetylneuraminic acid, and wheat germ agglutinin (WGA), which reacts with the monosaccharides *N*-acetyl-D-glucosamine and *N*-acetylneuraminic acid. For this purpose, we used ultrathin cryosections of microdissected organ of Corti samples taken from