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Research Report

Reversible neurotoxicity of kanamycin on dorsal cochlear nucleus

Guo-Run Fan[a,1], Ze-Deng Yin[d,1], Yu Sun[a,1], Sen Chen[a], Wen-Juan Zhang[a], Xiang Huang[c], Wei-Jia Kong[a,e,*], Hong-Lian Zhang[b,**]

[a]Department of Otorhinolaryngology, Union Hospital of Tongji Medical College, Huazhong University of Science and Technology, Jiefang Avenue 1277, Wuhan 430022, PR China
[b]Department of Preventive Medicine and Public Health, Union Hospital of Tongji Medical College, Huazhong University of Science and Technology, Jiefang Avenue 1277, Wuhan 430022, PR China
[c]Institute of Otorhinolaryngology, Union Hospital of Tongji Medical College, Huazhong University of Science and Technology, Jiefang Avenue 1277, Wuhan 430022, PR China
[d]Department of Otorhinolaryngology, The Affiliated Hospital of Luzhou Medical College, Taiping Avenue 25, Luzhou 646000, PR China
[e]Key laboratory of Neurological Disease, Ministry of Education, China

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ABSTRACT

The time course of aminoglycoside neurotoxic effect on cochlear nucleus is still obscure. We examined dynamic pathological changes of dorsal cochlear nucleus (DCN) and investigated whether apoptosis or autophagy was upregulated in the neurotoxic course of kanamycin on DCN after kanamycin treatment. Rats were treated with kanamycin sulfate/kg/day at a dose of 500 mg by subcutaneous injection for 10 days. Dynamic pathological changes, neuron density and neuron apoptosis of the DCN were examined at 1, 7, 14, 28, 56, 70 and 140 days after kanamycin treatment. The expressions of JNK1, DAPK2, Bcl-2, p-Bcl-2, Caspase-3, LC3B and Beclin-1 were also detected. Under transmission electron microscopy, the mitochondrial swelling and focal vacuoles as well as endoplasmic reticulum dilation were progressively aggravated from 1 day to 14 days, and gradually recovered from 28 days to 140 days. Meanwhile, both autophagosomes and autolysosomes were increased from 1 day to 56 days. Only few neurons were positive to the TUNEL staining. Moreover, neither the expressions of caspase-3 and DAPK2 nor neurons density of DCN changed significantly. LC3-II was drastically increased at 7 days. Beclin-1 was upgraded at 1 and 7 days. P-Bcl-2 increased at 1, 7, 14 and 28 days. JNK1 increased at 7 days, and Bcl-2 was downgraded at 140 days. LC3-B positive neurons were increased at 1, 7 and 14 days. These data demonstrated that the neurons damage of the DCN caused

Abbreviations: ABR, auditory brainstem response; ALs, autolysosomes; APs, autophagosomes; CMA, chaperone-mediated autophagy; Cvt, cytoplasm to vacuole targeting; DCN, dorsal cochlear nucleus; FC, Fusiform cell; LC3, microtubule-associated protein light chain 3; MMP, Mitochondrial membrane permeabilization; PVCN, posteroventral cochlear nucleus; ROS, reactive oxygen species; TEM, transmission electron microscopy

*Corresponding author at: Department of Otorhinolaryngology, Union Hospital of Tongji Medical College, Huazhong University of Science and Technology, Jiefang Avenue 1277, Wuhan 430022, PR China. Fax: +86 27 857 76343.
**Corresponding author at: Department of Preventive Medicine and Public Health, Union Hospital of Tongji Medical College, Huazhong University of Science and Technology, Jiefang Avenue 1277, Wuhan 430022, PR China.
E-mail addresses: entwjkong@yahoo.com.cn (W.-J. Kong), zhl_bjk@whuh.com (H.-L. Zhang).
[1]These authors are joint first authors.

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by kanamycin was reversible and autophagy was upregulated in the neurotoxic course of kanamycin on DCN through JNK1-mediated phosphorylation of Bcl-2 pathway.

1. Introduction

Aminoglycoside antibiotics are polycationic compounds and were first introduced into clinical practice in the 1940s, especially in the treatment of Gram-negative infections and tuberculosis (Schacht, 1993). However, shortly thereafter, the side-effects of aminoglycosides became evident, such as permanent hearing loss, vestibular deficits and nephrotoxicity (Rybak and Ramkumar, 2007; Selimoglu et al., 2003). The antibacterial mechanism of aminoglycoside antibiotics is that they can interfere with microbial ribosomes (Warchol, 2010). Considering that mitochondria of mammals contain ribosomal RNAs, therefore, they can act as a target of aminoglycosides. Intervening mitochondrial ribosomes of mammals could result in disruption of the electron transport chain and formation of reactive oxygen species (ROS), which play a role in promoting the opening of the mitochondrial permeability transition pore (Jacotot et al., 1999), and damage to cell constituents from DNA to lipids and proteins (Wang et al., 2003; Ylikoski et al., 2002). ROS can activate the JNK pathway, resulting in apoptosis by activating caspase-3 (Zhang et al., 2012). Meanwhile, mitochondrial membrane dysfunction and increased steady-state levels of superoxide can also elevate the pro-apoptotic DAPK activation (Shang et al., 2005), ultimately leading to cell death through caspase-3 activation (Kishino et al., 2004). Several studies indicated that aminoglycosides have a direct neurotoxic effect on the cochlea nucleus (Koitchev et al., 1986; Theopold, 1976), but others found that the cell volume and total cell numbers of cochlea nucleus did not exhibit significant change after aminoglycoside treatment (Fleckeisen et al., 1991; Galasinska-Pomykol et al., 1975). The mammals dorsal cochlear nucleus (DCN) is considered to contribute to the localization of the sound sources by integrating acoustic and somatosensory information (Oertel and Young, 2004; Ryugo et al., 2003; Shore, 2005), and project mainly to the contralateral inferior colliculus (Ryugo and Willard, 1985). Fusiform cells (FCs) are the most numerous of the principal cell types and play a key role in the function of the DCN (Irie and Ohmori, 2008). However, up to now, the chronic dynamic ultrastructural changes of the DCN after kanamycin administration are still unknown. Apoptosis is a host defense mechanism which plays a key role in the maintenance of tissue homeostasis by removing damaged cells. Whether the kanamycin treatment could induce neurons apoptosis of DCN also remains unclear.

Autophagy, as an intracellular degradation process of cellular most long-lived proteins and organelles or aberrant cellular components, is a highly conserved and ubiquitous lysosomal degradative process (Mizushima et al., 2008). There are several forms of autophagic degradation routes, including macroautophagy, chaperone-mediated autophagy (CMA), mitophagy, pexophagy and cytoplasm to vacuole targeting (Cvt) (Dunn et al., 2005; Kim et al., 1997; Massey et al., 2006; Mizushima, 2009; Tolkovsky, 2009). Macroautophagy is the major degradation pathway for cytoplasmic components turnover and henceforth referred to as 'autophagy', and plays an important role in cell homeostasis in physiological and/or pathological conditions, such as normal growth settings, nutrient depletion, excitotoxic stimuli and oxidative stress (Barth et al., 2011; Kiffin et al., 2004; Wang et al., 2008). In mammalian systems, autophagy is constitutively activated at low basal levels and can be upregulated by stress like nutrient depletion (Barth et al., 2011), oxidative stress (Clement et al., 2009), excitotoxic stimuli (Wang et al., 2008), spinal cord injury (Kanno et al., 2009), focal cerebral ischemia (Rami et al., 2008) or hypoxia-ischemia induced brain injury (Carloni et al., 2008). Both apoptosis and autophagy are fundamental cellular pathways, which may share some common regulatory mechanisms (Levine and Yuan, 2005). As mentioned above both JNK and DAPK pathway can result in apoptosis by activating caspase-3. But JNK-mediated Bcl-2 phosphorylation (Ham et al., 2003) and DAPK2-mediated phosphorylation of Beclin-1 can promote autophagy (Zalckvar et al., 2009). However, whether the autophagy takes part in the kanamycin induced possible neurotoxic course on DCN, and, if so, which pathway may be involved in, remains unclear.

The aims of this study are to investigate the chronic dynamic pathological change of DCN and examine whether apoptosis or autophagy is upregulated in the possible neurotoxic course of kanamycin to the neurons of DCN after systemic kanamycin treatment. We used the formation of autophagosomes (APs) and enhanced expression of Beclin 1 and LC3 as hallmarks of autophagy (Klionsky et al., 2008). The neurons density of DCN was also detected. As for apoptosis investigation, we detected the expression of caspase 3 and processed TUNEL staining. The immune-staining specific to kanamycin was performed to determine the uptake of kanamycin in DCN. The expressions of JNK1, DAPK2, Bcl-2 and p-Bcl-2 were also detected to investigate the possible pathway of autophagy. These results indicated that the ultrastructural changes of DCN might, at least in part, due to the neurotoxicity of kanamycin. Our results also demonstrated that the neurons damage of the DCN caused by systemic application of kanamycin was reversible and autophagy was upregulated significantly in the early stage after kanamycin treatment, which indicated that autophagy was upregulated in the possible neurotoxic course of kanamycin on DCN through JNK1-mediated phosphorylation of Bcl-2 pathway.

2. Results

2.1. *Auditory function and the daily body weights*

There was no statistical difference in auditory brainstem responses (ABR) threshold among all groups before treatment ($P>0.05$, Fig. 1). The average threshold of ABR in the control group and experimental groups at 1, 7, 14, 28, 56, 70 and 140