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ScienceDirect

Journal of Otology 9 (2014) 126–135

www.journals.elsevier.com/journal-of-otology/

Pattern of hair cell loss and delayed peripheral neuron degeneration in inner ear by a high-dose intratympanic gentamicin

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Received 10 October 2014; revised 11 October 2014; accepted 30 October 2014

Abstract

To gain insights into the ototoxic effects of aminoglycoside antibiotics (AmAn) and delayed peripheral ganglion neuron death in the inner ear, experimental animal models were widely used with several different approaches including AmAn systemic injections, combination treatment of AmAn and diuretics, or local application of AmAn. In these approaches, systemic AmAn treatment alone usually causes incomplete damage to hair cells in the inner ear. Co-administration of diuretic and AmAn can completely destroy the cochlear hair cells, but it is impossible to damage the vestibular system. Only the approach of AmAn local application can selectively eliminate most sensory hair cells in the inner ear. Therefore, AmAn local application is more suitable for studies for complete hair cell destructions in cochlear and vestibular system and the following delayed peripheral ganglion neuron death. In current studies, guinea pigs were unilaterally treated with a high concentration of gentamicin (GM, 40 mg/ml) through the tympanic membrane into the middle ear cavity. Auditory functions and vestibular functions were measured before and after GM treatment. The loss of hair cells and delayed degeneration of ganglion neurons in both cochlear and vestibular system were quantified 30 days or 60 days after treatment. The results showed that both auditory and vestibular functions were completely abolished after GM treatment. The sensory hair cells were totally missing in the cochlea, and severely destroyed in vestibular end-organs. The delayed spiral ganglion neuron death 60 days after the deafening procedure was over 50%. However, no obvious pathological changes were observed in vestibular ganglion neurons 60 days post-treatment. These results indicated that a high concentration of gentamycin delivered to the middle ear cavity can destroy most sensory hair cells in the inner ear that subsequently causes the delayed spiral ganglion neuron degeneration. This model might be useful for studies of hair cell regenerations, delayed degeneration of peripheral auditory neurons, and/or vestibular compensation. In addition, a potential problem of ABR recording for unilateral deafness and issues about vestibular compensation are also discussed.

Keywords: Cochlea; Vestibular system; Gentamicin; Local medication; Ototoxicity

1. Introduction

Loss of sensory hair cells and their connecting neurons in the inner ear often leads to permanent hearing loss and/or vestibular disorders. Although permanent hearing loss and vestibular dysfunction are not life threatening, the quality of life will be heavily influenced by the permanent disability. Since the destruction of sensory hair cells and peripheral ganglion neurons in the inner ear are unrepairable because the

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Peer review under responsibility of PLA General Hospital Department of Otolaryngology Head and Neck Surgery.

http://dx.doi.org/10.1016/j.joto.2014.12.001

injured hair cells and peripheral neurons cannot naturally regenerate, it is difficult to rescue the sensorineural hearing loss and balance dysfunction. The cochlear implant is widely applied in clinic for profound deafness patients to bypass the dysfunctional cochlea and to substitute for the missing hair cells. The cochlear implant provides electrical signals to spiral ganglion neurons and their axons projecting to the cochlear nucleus. Therefore, sufficient spiral ganglion neuron survival is a fundamental prerequisite for efficient work of cochlear implant (Shibata et al., 2011; Pfingst et al., 2011). Thus, there is a strong clinical need for establishing ways of promoting spiral ganglion neuron survival following hair cell death. Kanamycin or gentamicin selectively destroys sensory hair cells in the inner ear followed by a slow progress of secondary damage to spiral ganglion neurons due to the lack of electrical stimulation and neurotrophic factors. This secondary degeneration of spiral ganglion neurons is referred to as "delayed spiral ganglion neuron death" (Ding and Salvi, 2005; McFadden et al., 2004; Ding et al., 2010a). In order to better understand the interdependence of sensory hair cells and peripheral ganglion neurons in the inner ear, as well as the hair cell death pathways, hair cell regeneration, or delayed spiral ganglion neuron death, numerous animal deafing models have been studied in the field of hearing research. The studies would benefit by the use of animal models with well-defined cell damage in the inner ear.

Cochlear and vestibular hair cell damage can be induced by numerous factors, such as acoustic trauma, aging, ototoxic chemicals, blast wave, irradiation, infection, heavy metals, pesticide, herbicide, organic solvent, and gene mutations, etc (Ding and Salvi, 2005; Ding et al., 2012, 2011a, 2011b, 2004, 2010b, 2007; Ding et al., 1999a; Ding et al., 2002a, 2013, 2011c, 1999b; McFadden et al., 1999; Qi et al., 2008; Wang et al., 1999; Wu et al., 2011a, 2011b; Someya et al., 2009; Nicotera et al., 2004; Kane et al., 2012; Guan, 2011; Federspil et al., 1976). These various aetiologies have led to many attempts to develop appropriate animal models for different study purposes. Since the ototoxic effects and neurotoxic effects are presented together in most toxic chemicals, such as platinum-based antineoplastic drugs, antimalarial drugs, and heavy metals, those chemicals should not be used for the study of delayed neuron death. However, aminoglycoside antibiotics such as kanamycin or gentamicin directly destroy sensory hair cells while preserving the ganglion neurons in the inner ear during its injury, although the spiral ganglion neurons finally will indirectly afterwards. Therefore, kanamycin or gentamicin-induced direct injury to sensory hair cells in the inner ear is considered an ideal experimental models of delayed spiral ganglion neuron death.

In order to establish the experimental model of sensory hair cell damage and delayed neuron death, several strategies or approaches were attempted. One approach is systemic treatment of kanamycin or gentamicin using consecutive daily doses, because a single dose failed to induce inner ear damage due to obstruction of the blood-labyrinth barrier, except in the case of acute renal failure or genetic defects (Guan, 2011; Federspil et al., 1976). Continued kanamycin or gentamicin systemic treatment for several days often causes severe but incomplete damage to the cochlear hair cells and vestibular hair cells (Ding and Salvi, 2005). To overcome the obstruction of the blood-labyrinth barrier and enhance the entry of ototoxic drugs into the inner ear, loop-inhibiting diuretics, such as ethacrynic acid or furosemide, which can abolish the blood flow in vessels supplying the lateral wall of the cochlea and damage the stria vascularis, temporarily open the blood–cochlear barrier thereby enhancing the entry of ototoxic drugs into the cochlea (Ding and Salvi, 2005; McFadden et al., 2004; Ding et al., 2010a, 2011a, 2004, 2010b, 2007, 2002a; Ding et al., 2003; Li et al., 2011; Liu et al., 2011; McFadden et al., 2002). Co-administering loop diuretics with kanamycin or gentamicin can result in a complete hair cell loss in the cochlea, but cannot injure the vestibular hair cells (Ding and Salvi, 2005; Ding et al., 2010a, 2004; Liu et al., 2011; McFadden et al., 2002; Ding et al., 1995). Therefore, this approach is only able to wipe out all hair cells and allowing for the study of delayed spiral ganglion neurons death in the cochlea. To destroy the sensory hair cells in both the cochlear and vestibular system while bypassing the blood-labyrinth barrier, an alternative approach for gentamicin ototoxicity is via the round window membrane which is permeable to many agents (Ding and Salvi, 2005; Ding et al., 2011a; He et al., 2009), or direct delivery into the cochlear or vestibular cavity (Li et al., 2004; Swan et al., 2008; Wagner et al., 2005). Since the local application of gentamicin by tympanic injection is simple and effective to damage both cochlear and vestibular sensory hair cells, we applied gentamicin to one ear to create a unilateral loss of sensory hair cell loss in both the cochlear and vestibular system. The functional and pathological changes in the cochlear and vestibular system were observed after gentamicin treatment, and the delayed peripheral ganglion neurons degeneration was also evaluated.

2. Materials and methods

2.1. Animal treatment

Twenty 250–300 g adult pigmented guinea pigs with normal Preyer's reflexes were used in the present study. Animals were anesthetized with a mixture of ketamine (30 mg/kg i.p.) and chloropromazine (15 mg/kg, i.p.), and placed on a temperature-controlled heating pad with a rectal probe to maintain core temperature at 37 °C. Guinea pigs were placed in a right lateral position, and the left meatus of the external ear was sterilized by filling with 70% ethanol for 5 min. The meatus of the external ear was then dried by aseptic cotton swab. Under a surgical microscope and sterile conditions, approximate 200 μl gentamicin sulfate dissolved in normal saline at a concentration of 40 mg/ml in 1 ml syringe was penetrated through tympanic membrane, and slowly injected into the middle ear cavity until the solution completely filled the middle ear cavity and overflowed into the external ear canal as described previously (He et al., 2009).