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Inhibition of caspases alleviates gentamicin-induced cochlear damage in guinea pigs

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Abstract

The efficacy of caspase inhibitors for protecting the cochlea was evaluated in an in vivo study using guinea pigs, as the animal model system. Gentamicin (12 mg/ml) was delivered via an osmotic pump into the cochlear perilymphatic space of guinea pigs at 0.5 μl/h for 14 days. Additional animals were given either z-Val-Ala-Asp (Ome)-fluoromethyl ketone (z-VAD-FMK) or z-Leu-Glu-His-Asp-FMK (z-LEHD-FMK), a general caspase inhibitor and a caspase 9 inhibitor, respectively, in addition to gentamicin. The elevation in auditory brain stem response thresholds, at 4, 7, and 14 days following gentamicin administration, were decreased in animals that received both z-VAD-FMK and z-LEHD-FMK. Cochlear sensory hair cells survived in greater numbers in animals that received caspase inhibitors in addition to gentamicin, whereas sensory hair cells in animals that received gentamicin only were severely damaged. These results suggest that auditory cell death induced by gentamicin is closely related to the activation of caspases in vivo.

Keywords: Inner ear; Hair cell; Aminoglycoside; Caspase; Guinea pig

1. Introduction

The cochlea is unlikely, under normal circumstances, to be exposed to outside stresses, as the inner ear is enclosed by a bony wall and separated from blood by the blood–inner ear barrier. However, cochlear hair cells are quite easily damaged by specific chemical compounds [1,15], elevated noise levels [11,12,19] and ischemia [3]. There have been many cases of hearing loss as a result of these stresses. Aminoglycosides are a class of compounds that are well known as specific ototoxic agents [28,29], and recent research has suggested that hair cell death induced by these chemicals is closely related to apoptosis. In a 1985 study, Forge and co-workers [7,8] reported morphologic evidence of aminoglycoside-induced sensory hair cell apoptosis. In addition, the organ culture studies have provided evidence of an apoptotic cell death pathway in the inner ear. Matsui et al. [16] reported that caspase inhibitors suppressed apoptosis induced by aminoglycosides, and Cunningham et al. [6] reported that caspase 9 was more effective than caspase 8 at promoting apoptosis of hair cells. Recently, Matsui et al. [17] showed that either systemic or local application of general caspase inhibitor could protect chick vestibular sensory cells against the aminoglycoside-induced cell death in vivo. These evidences indicated that caspase signaling pathway has an important role in the aminoglycoside induced hair cell death, and that the inhibition of caspases may be one of the candidates for the treatment of inner ear diseases involving hair cell death. However, it has been unclear whether the effect of caspase inhibitor can protect hair cells of the mammals in vivo. Therefore, we planned to administrate the specific inhibitor of caspase 9 as well as the general caspase inhibitor into the cochlea of guinea pigs. The aim of this present study was to determine the effect of caspase inhibitors on cochlear hair cell death of mammals induced by aminoglycosides.

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2. Materials and methods

2.1. Animal subjects and experimental design

The experimental animals incorporated into this study were 15 male Hartley guinea pigs (350–400 g) with Preyer's reflexes and normally appearing tympanic membranes. All experimental protocols were reviewed by the Committee for Ethics on Animal Experiments at the Yamaguchi University School of Medicine. Experiments were carried out in accordance with the University Guidelines, Japanese Federal Law (No. 105) and Notification No. 6 of the Japanese Government.

The study protocol is described schematically in Fig. 1. Osmotic pumps (Model 2002, Alza Co., Palo Alto, CA, USA) filled with sterile saline were implanted into each animal for the infusion of agents into the right cochlear perilymphatic space. The procedures for implantation of osmotic pumps were performed on the right ears, and the intact left ears were used for control. Seven days after pump implantation each osmotic pump was newly replaced with a pump containing the specific agent to be delivered in combination with gentamicin. The animals were then divided into three groups ($n = 5$ in each) according to the contents of the replacement pumps (agents were infused at a rate of 0.5 μl/h for 14 days in each case); GM only group received a 12 mg/ml gentamicin solution (Schering-Plough K.K., Kenilworth, NJ, USA) in 1% dimethyl sulfoxide (DMSO) (Sigma, St. Louis, MO, USA) with saline; GM + general caspase inhibitor group received a gentamicin solution (12 mg/ml) containing 250 μM of the general caspase inhibitor (z-Val-Ala-Asp (Ome)-fluoromethyl ketone (z-VAD-FMK) (Enzyme System Products, Livemore, CA, USA) also in 1% DMSO with saline; GM + caspase 9 inhibitor group received a gentamicin solution (12 mg/ml) containing 250 μM of the caspase 9 inhibitor (z-Leu-Glu-His-Asp-FMK (z-LEHD-FMK) (Kamiya Biomedical Company, Seattle, WA, USA) in 1% DMSO with saline. To evaluate cochlear function, auditory brain stem response (ABR) thresholds were assessed before and at 4-, 7- and 14-day time points following the exchange of osmotic pumps. Following the final ABR examination, each of the animals was sacrificed for histological examination.

2.2. Implantation and exchange of osmotic pumps

For the infusion of specific agents into the inner ears of the experimental animals, we used osmotic pumps connected to 10 cm polyethylene catheters (i.d. = 0.28 mm, o.d. = 0.61 mm) and a 1 mm Teflon catheter (i.d. = 0.18 mm, o.d. = 0.3 mm) with plastic glue. Both pumps and catheters were infused with sterile saline and were pretreated in saline until implantation. The surgical procedures for the pump implantations were performed under general anesthesia with ketamine (16 mg/kg, intraperitoneal injection (i.p.)) and xylazine (16 mg/kg, i.p.) and local anesthesia (1% lidocaine HCl, 1.5 ml). The mastoid bulla was exposed via postauricular incision at the right ear, and two incisions were then made into it with a small electric drill for both the procedure and subsequent observations. A tiny hole was then made on the cochlear bony wall at a distance of 1 mm from the round window with a fine needle. The hole was sized to fit the catheter. The tip of the catheter, which was connected to the osmotic pump, was inserted into the perilymphatic space of the cochlear basal turn via this tiny hole. We subsequently confirmed that the perilymph did not leak out. The catheter was fixed to the mastoid bulla with dental cement (GC Fuji1, GC Co., Tokyo, Japan). After the skin incision was closed, antibiotic ointment was applied. All animals received sterile saline at 0.5 μl/h via right cochlear perilymph after implantation of osmotic pumps.

Osmotic pumps were exchanged for new pumps containing specific experimental reagents 7 days after implantation. The procedures for the exchange of pumps were performed under the same anesthesia used for the initial pump implantation. A new incision was then made at the back of the animals, and the first osmotic pump and catheter were

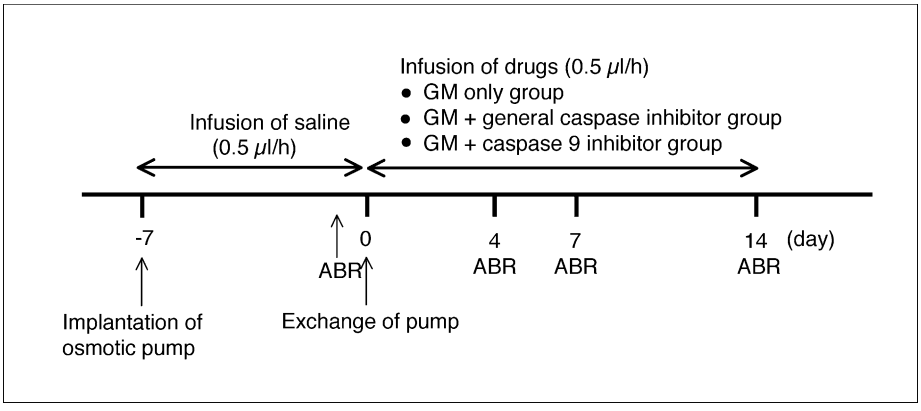

Fig. 1. Schematic depiction of the experimental protocol. Solutions containing gentamicin either alone or in combination with specific caspase inhibitors were administered for up to 14 days after the exchange of osmotic pumps. An ABR examination was performed prior to the exchange of the osmotic pump and at 4-, 7- and 14-day timepoints following the start of the infusion of agents.