Effects of Antibiotic Nasal Nebulizer Treatments on the Auditory Sensory Epithelium in Guinea Pigs

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Abstract. The effects of antibiotics administered by intramuscular injection or nasal inhalation on the auditory sensory epithelium of guinea pigs were examined using auditory brainstem response (ABR), scanning and transmission electron microscopy. The threshold of the ABR increased, and auditory hair cells were severely damaged in guinea pigs treated with gentamycin, an antibiotic of the aminoglycoside group, administered by intramuscular injection at a dose of 400 mg/kg/day for 2 weeks. On the other hand, the threshold of the ABR was unchanged, and auditory hair cells remained intact, in guinea pigs treated with gentamycin or cefmenoxime, an antibiotic of the cephem group, administered by nasal inhalation for 30 min/day for 3 weeks. This finding suggests that antibiotic nasal nebulizer treatment is safe, while aminoglycoside antibiotic intramuscular injection is very dangerous to the auditory sensory epithelium.

Key words: Auditory sensory epithelium - Aminoglycoside antibiotic (gentamycin) - Cephem antibiotic (cefmenoxime) - Nasal nebulizer - Intramuscular injection - Guinea pig

INTRODUCTION

Nasal inhalation treatments with a heated mist have been proposed for many years as treatments for severe congestion due to viral infection, sinusitis, the common cold or allergic rhinitis *. In these treatments, various antibiotics such as aminoglycoside and others from the cephem group have been used in the mist solutions. Aminoglycoside antibiotics, including gentamycin and kanamycin, have, however, been shown to cause side effects, such as hearing loss and auditory hair cell damage by both intramuscular injection * and intratympanic injection *. The effects on the auditory epithelium of aminoglycosides administered by nasal inhalation, however, had not yet been determined. In the present study, we investigated the fine structural changes occurring in the auditory epithelium of guinea pigs induced by either intramuscular injection or by nasal inhalation of gentamycin or cefmenoxime, employing scanning, as well as transmission electron microscopy, and recorded the auditory brainstem response (ABR).

MATERIALS AND METHODS

1. Experimental procedure

Thirty-six adult guinea pigs, 300-400 g in body weight, were divided into 6 groups of 6 animals each. Group 1: Animals were sacrificed without antibiotic administration (normal). Group 2: Animals were given gentamycin (400 mg/kg body weight/day) dissolved in saline by intramuscular injection once a day for 14 days. Group 3: Animals were given cefmenoxime (400 mg/kg body weight/day) dissolved in saline by intramuscular injection once a day for 14 days. Group 4: Animals inhaled a spray of 4% gentamycin solution for 30 min/day for 3 weeks. Group 5: Animals inhaled a spray of 1% cefmenoxime solution for 30 min/day for 3 weeks. Group 6: Animals served as controls by inhaling a spray of saline. Molecular water vapor was supplied by an Omlon molecular nebulizer (Omlon, Tokyo, Japan). After injection or inhalation, the animals were treated for physiological and subsequent morphological analyses as described below.

2. Auditory brainstem response (ABR)

In all the animals used, the ABR was measured under general anesthesia by an intramuscular injec-
tion of pentobarbital (35 mg/kg), and was recorded using an MEM-4104 (Nihon Kohden, Tokyo, Japan).

3. Scanning electron microscopy (SEM)

Animals were perfused through the left cardiac ventricle with 2.5% glutaraldehyde and 2% paraformaldehyde in 0.05 M cacodylate buffer (pH 7.4) for 5 min. After decapitation, the organ of Corti was removed from the temporal bone, and immersed in the same fixative at 4°C for 3 hr. The specimens were post-fixed in 1% buffered osmium tetroxide solution at 4°C for 2 hr, dehydrated through a graded ethanol series, immersed in t-butyl alcohol, and freeze-dried in a VFD-20 freeze-drying apparatus (Vacuum Device Inc., Ibaraki, Japan). They were then coated with platinum in an IB-5 ion coater (Eiko, Ibaraki, Japan) and examined with an S-800 scanning electron microscope (Hitachi, Ltd., Tokyo, Japan).

4. Transmission electron microscopy (TEM)

The organ of Corti, fixed and post-fixed as described above, was stained en block with 3% aqueous uranyl acetate, dehydrated in ethanol, and embedded in Epon 812 epoxy resin. Ultrathin sections cut with a Reichert Ultracut-M ultramicrotome (C. Reichert Optische Werke AG, Vienna, Austria) were stained with uranyl acetate and lead citrate, and examined with a 1200EX (JEOL Ltd., Tokyo, Japan) transmission electron microscope.

RESULTS

1. Control guinea pigs

The ABR pattern of the normal guinea pigs was composed of four positive large waves, and the threshold level of the normal guinea pigs was 30 dB SPL.

The auditory sensory epithelium of the normal guinea pig consisted of two types of sensory hair cells (inner and outer hair cells) and supporting cells, as reported previously.

2. Intramuscular injection group

The threshold of the ABR of the intramuscular cefmenoxime-treated guinea pigs was 30 dB SPL, which did not lead to hearing loss (Fig. 1a). The auditory sensory epithelium of the intramuscular cefmenoxime-treated guinea pigs had no damage (Fig. 1b, c).

3. Nasal inhalation groups

The threshold of the ABR of the gentamycin-treated guinea pigs was 90 dB SPL, which was much higher than that in the control group (Fig. 2a). The amplitude of each wave was smaller than that in the control group.

SEM revealed severe damage in outer hair cells from the basal to apical turn of the auditory sensory epithelium of the gentamycin-treated guinea pigs (Fig. 2b). The hair bundles of the outer and inner hair cells were completely missing. The supporting cells, the apical surfaces of which were covered with microvilli, were enlarged remarkably. Transmission electron microscopy of thin sections also showed degenerative changes in hair cells and swelling in supporting cells (Fig. 2c). The outer hair cells had disappeared completely, and swollen supporting cells occupied the region (Fig. 2c). Damaged hair cells were sometimes observed, and the cytoplasm of the hair cells possessed many large vacuoles, including variously-sized dense bodies (Fig. 2d). The vacuoles were surrounded by a limiting membrane.

DISCUSSION

1. Effects of intramuscular injection of aminoglycoside

Aminoglycoside antibiotics such as kanamycin and gentamycin have been widely used for the treatment of bacterial infections with Gram-negative bacilli and staphylococci. It is well-known that intramuscular injections from this group of antibiotics can cause such side effects as hearing loss and renal disturbance. It has been reported that auditory hair cells were damaged by aminoglycoside antibiotics in guinea pigs and in birds. However, the stria vascularis was not damaged by aminoglycoside antibiotics. In the present study, both hair cell