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Acute Effects of Combined Administration of Kanamycin and Furosemide on the Stria Vascularis Studied by Distortion Product Otoacoustic Emission and Transmission Electron Microscopy

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ALAM, S.A., IKEDA, K., KAWASE, T., KIKUCHI, T., KATORI, Y., WATANABE, K. and TAKASAKA, T. *Acute Effects of Combined Administration of Kanamycin and Furosemide on the Stria Vascularis Studied by Distortion Product Otoacoustic Emission and Transmission Electron Microscopy.* Tohoku J. Exp. Med., 1998, **186** (2), 79-86 —— Acute effects of kanamycin and/or furosemide administration on the stria vascularis of the guinea pig cochlea were assessed by distortion product otoacoustic emission (DPOAE) and transmission electron microscopy. Kanamycin alone failed to affect the DPOAE levels and ultrastructural changes. Furosemide alone caused a rapid but reversible fall of the DPOAE levels. No remarkable pathological changes in the strial vascularis were observed after a complete recovery of the DPOAEs. On the other hand, furosemide injection following kanamycin with a 2 hour interval resulted in two patterns of significant changes in the DPOAEs, namely, a sudden drop in the DPOAE levels 2 to 3 hours after furosemide injection and a gradual fall in the DPOAE levels immediately after the incomplete recovery from the furosemide-induced decrease of the DPOAE levels. Ultrastructural changes in the stria vascularis included numerous vacuoles in the strial marginal cells and increased electron density of the intermediate and basal cells. These physiological and morphological changes in the stria vascularis may imply new ototoxic features induced by kanamycin potentiated by furosemide. ———— kanamycin; furosemide; stria vascularis; distortion product otoacoustic emission; transmission electron microscopy

The stria vascularis is well known as a K^+-secreting non-sensory tissue and to be responsible for the generation of the endocochlear potential (Wangemann et al. 1995), which provides the driving force for the acoustic transduction current of the

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cochlear hair cells (Ikeda et al. 1994). Systemic administration of loop diuretics such as furosemide and ethacrynic acid induces significant but reversible changes in the stria vascularis. Quantitative analysis of each compartment of the stria vascularis revealed a shrinkage of the marginal cells and swelling of both intermediate cells and the intrastrial space (Santi and Lakhani 1983), which fully recovered 120 minutes after the injection of loop diuretics (Pike and Bosher 1980). On the other hand, no morphological alterations in the organ of Corti were recognized (Brown et al. 1979).

A primary lesion produced by aminoglycoside antibiotics appears in the outer hair cell, followed by delayed degeneration of the inner hair cells and the afferent nerve endings (Browning et al. 1982; Johnsson et al. 1981). A few reports have focused on the effect of aminoglycosides on the stria vascularis. Although acute changes after aminoglycoside administration alone were not observed in the cochlear tissue, chronic treatment resulted in a noticeable reduction in the overall thickness (Forge and Fradis 1985) and mitochondrial damage (Gratacap et al. 1985) in the stria vascularis. These studies suggest that the stria vascularis is one of the target tissue of aminoglycosides.

Brummett et al. (1975) reported that administration of kanamycin preceded by furosemide caused a marked increase in the number of missing outer hair cells from the organ of Corti, which was explained by the notion that loop diuretics facilitate the penetration of aminoglycosides into endolymph (Tran Ba Huy et al. 1983). As loop diuretics potentiate hair cell damage, an interaction between loop diuretics and aminoglycosides may also appear to occur in the stria vascularis, but such has not yet been proven.

Distortion product otoacoustic emission (DPOAE) measurement is a noninvasive technique to monitor energy-yielding processes of the cochlea. DPOAEs elicited by low-level stimuli are dependent upon the endocochlear potential and require normal outer hair cells (Whitehead et al. 1992). DPOAE measurement is a valuable and reliable tool to detect the fine pathology of both the stria vascularis and the outer hair cell. In this study, we attempted to examine the acute effects of combined administration of kanamycin and furosemide on stria vascularis studied by DPOAE and transmission electron microscopy.

Materials and Methods

Eight guinea pigs, weighing 250–350 g, with a normal Preyer's reflex, clean external ears and no sign of middle ear infection were used in this experiment. The guinea pigs were anesthetized with sodium pentobarbital (25 mg/kg, i.p.) and artificially ventilated after tracheostomy. Rectal temperature was maintained at 37°C by an automatically regulated heating system during the experiment. An indwelling catheter was placed in the internal jugular vein. The pinna and cartilaginous portion of the ear canal were removed to gain clear access to the

meatus.

DPOAE responses at 2f1-f2 were measured using a system from Etymotic Research (earphone, ER-2; microphone, ER-10B; IBM PC based DSP board, Ariel DSP 16+; software, CUBDIS 2.4; Elk Grove Village, IL, USA) as described in the previous study (Zheng et al. 1997). The equilevel primaries (P1 = P2 = 65, 55, 45 dB SPL) at a frequency ratio of f2/f1 = 1.2 were used. DPOAE measurements were carried out at 13916 Hz of f2, in which the frequency area corresponds to the anatomical region observed in electron microscopy. Five animals received a combined treatment with kanamycin and furosemide. As controls, kanamycin alone was given in one animal while furosemide alone was administered in two animals. An initial measurement was made before drug administration. Kanamycin (400 mg/kg) was given intravenously within one minute. DPOAEs were measured immediately after kanamycin injection and then every 15 minutes intervals. Two hours after kanamycin injection furosemide (70 mg/kg) was administered intravenously. The animals were sacrificed 2–3 hours after furosemide administration.

Cardiac perfusion was performed by 0.9% saline solution containing 0.1% sodium nitrite, followed by 50 ml of fixative consisting of 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) at room temperature. Then the animals were decapitated rapidly, and the stapes was removed. The cochleas were perfused with fixative by two routes, through an apical perforation and the round window. The excised cochleas were immersed in the same fixative over night at 4°C and rinsed with 0.1 M cacodylate buffer (pH 7.4). Decalcification was performed by 0.12 M disodium ethylenediamine tetraacetic acid (EDTA, pH 7.0) for 1 week at room temperature. EDTA solution was changed daily. The decalcified cochleas were postfixed in ice-cold 1.0% osmium tetroxide in the same buffer for 2 hours after washing in 0.1 M cacodylate buffer (pH 7.4) for 30 seconds. Specimens were dehydrated in graded ethanol solutions, cleared in propylene oxide and embedded in Epon 812 (TAAB Laboratories Equipment Ltd., Berks, England). Ultrathin sections of the basal turn of each cochlea were prepared using a diamond knife on a Sorvall MT-5000 ultramicrotome (Dupont, New York, NY, USA), stained with uranyl acetate and lead citrate, and examined with a JEOL JEM-100S or JEM-100C transmission electron microscope (JEOL, Tokyo) operated at 80–100 kV.

Results

An animal treated with kanamycin alone showed normal DPOAE responses for 2 hours after administration (Fig. 1a). Intravenous injection of furosemide alone caused a rapid and transient fall in DPOAEs, followed by a complete recovery within 30 to 40 minutes and the subsequent maintenance of normal DPOAE levels (Fig. 1b). Combined administration of kanamycin and furosemide resulted in two patterns of DPOAE changes. Furosemide administration following kanamycin caused DPOAEs evoked by 45–65 dB stimuli to suddenly

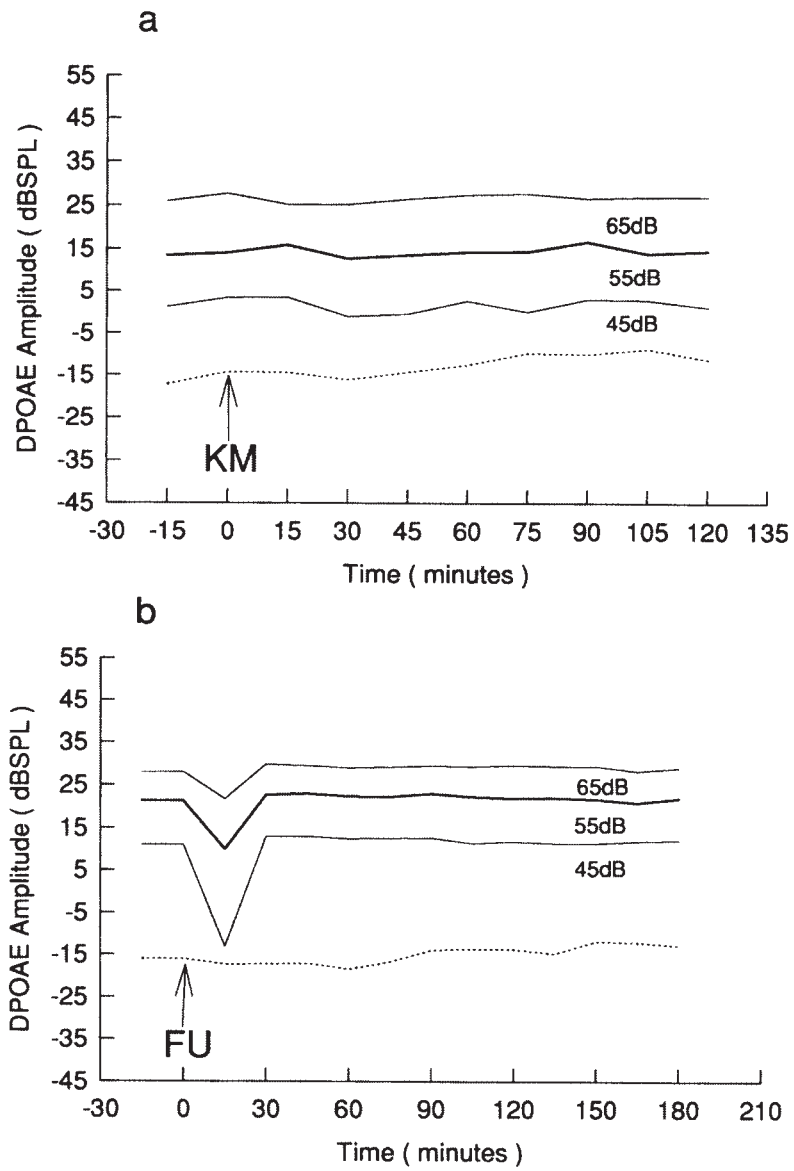

Fig. 1. Effects of kanamycin (KM) or furosemide (FU) on DPOAEs at 45 to 65 dB SPL. (a) Note that kanamycin alone failed to affect the DPOAEs. (b) Note that furosemide alone induced a rapid and transient fall in the DPOAEs. Dotted line denotes noise floor levels.

drop 2 to 3 hours after the complete restoration to the initial level in three animals (Fig. 2a). In the other 2 animals (Fig. 2b), furosemide resulted in a rapid drop in the DPOAEs, followed by a significant but incomplete recovery. The DPOAEs evoked by low-level stimuli gradually and slightly declined until the end of the observations.

Transmission electron microscopic observations were carried out in all animals after the DPOAE measurements. Each animal treated with kanamycin or furosemide alone showed no abnormal intracellular spacing, marginal cell shrinkage, or intermediate cell swelling in the stria vascularis (Figs. 3 and 4). Normal cytoplasmic structures were seen in the marginal cells. Although expansion of intracellular spaces and shrinkage of the marginal cells as commonly observed at

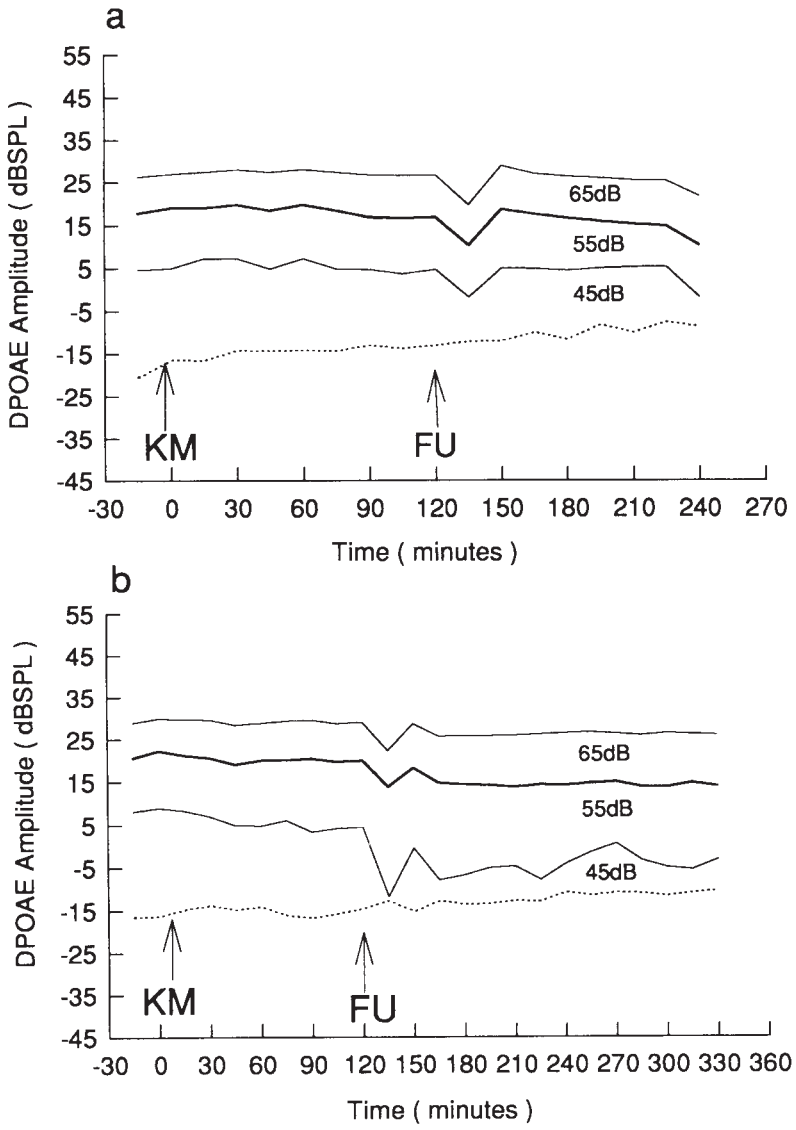

Fig. 2. Effects of the combination of kanamycin (KM) and furosemide (FU) on DPOAEs at 45 to 65 dB SPL. (a) Note a sudden drop in the DPOAE levels 2 to 3 hours after furosemide injection. (b) Note a gradual fall in the DPOAE levels immediately after the incomplete recovery from the furosemide-induced decrease of the DPOAE levels. Dotted line denotes noise floor levels.

the early stage of furosemide administration were not recognized under the combination of kanamycin and furosemide, the strial cells showed a variety of discrete structural changes. In some animals, the electron density of both the intermediate and basal cell cytoplasm was considerably increased, resulting in a poor contrast between the marginal cells and the other two types of cells within the stria vascularis (Fig. 5a). Some animals showed numerous vacuoles in the marginal cell cytoplasm (Fig. 5b). There was no correlation between DPOAE findings and the morphological changes.

Discussion

The present study demonstrated for the first time the functional and mor-

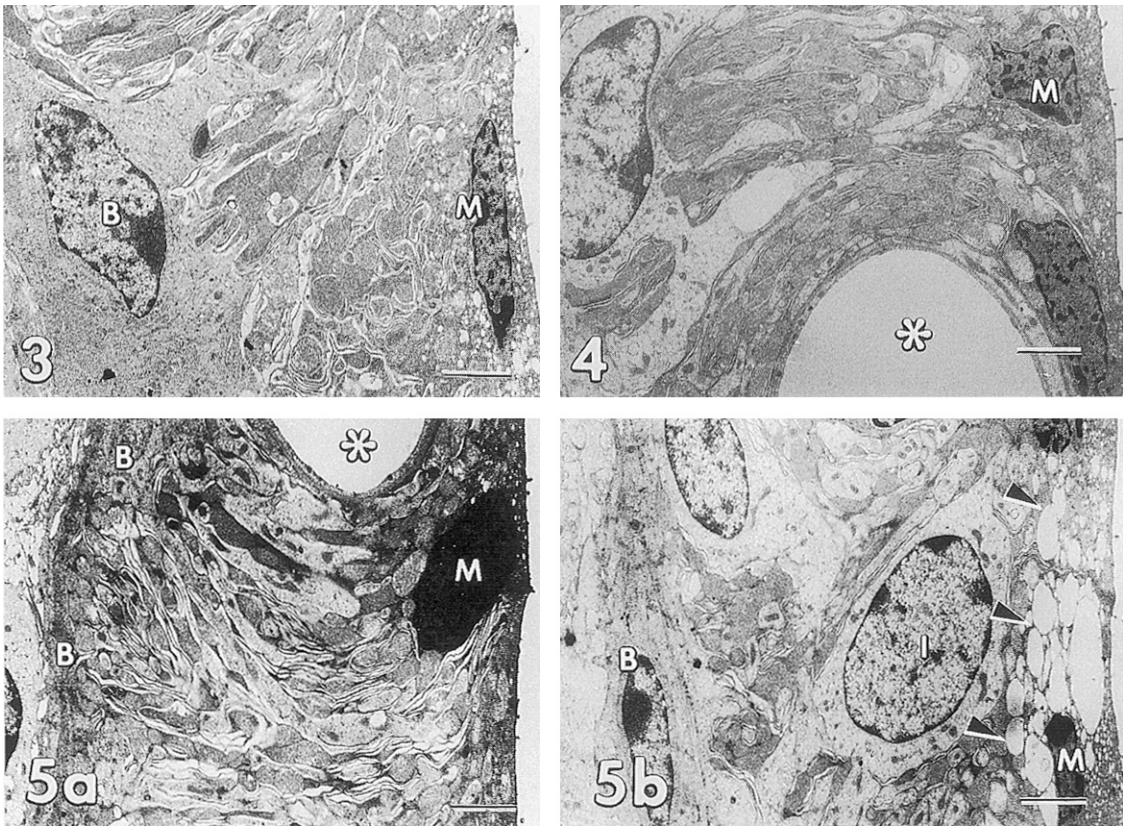

Fig. 3. Ultrastructural observations of the stria vascularis of the basal turn of the cochlea after kanamycin injection. No remarkable pathological changes were observed. M, marginal cell; B, basal cell. Bar: 2 μm.

Fig. 4. Ultrastructural observations of the stria vascularis of the basal turn of the cochlea after furosemide injection. Intact morphology was observed. M, marginal cell; $*$, capillary. Bar: 2 μm.

Fig. 5. Ultrastructural changes in the stria vascularis of the basal turn of the cochlea after the combination of kanamycin and furosemide injection. (a) Note the increased electron density in both the intermediate and basal cell cytoplasm. (b) Note the numerous vacuoles (arrowheads) in the marginal cell. M, marginal cell; I, intermediate cell; B, basal cell; $*$, capillary. Bar: 2 μm.

phological changes in the stria vascularis resulting from the acute effects of combined intravenous administration of kanamycin and furosemide. A variety of studies have provided abundant evidence of an interaction between aminoglycosides and loop diuretics, indicating that the hair cell damage induced by aminoglycoside was augmented by the additional administration of loop diuretics (Mathog and Klein 1969; West et al. 1973; Brummett et al. 1975; Russell et al. 1979; Hayashida et al. 1989). However, few reports have referred to the strial changes resulting from this interaction. Russell et al. (1979) showed in guinea pigs that subcutaneous administration of kanamycin followed by ethacrynic acid at an interval of 2 hours induced a transient but more severe edema of the stria vascularis than that induced by ethacrynic acid alone. They did not observe the long-term changes after treatment. In the present study, the edema of the stria

vascularis disappeared a few hours after furosemide followed by kanamycin whereas some ultrastructural changes were detected in the strial cells. The difference from that of Russell et al. (1979) may have been due to differences in the periods of observation as well as that of the route of drug administration.

Vacuolization in the marginal cell and changes in electron density in the strial cells have been observed in chronic treatment with aminoglycosides (Forge and Fradis 1985). The combined administration of furosemide with kanamycin may explain why the acute effects of kanamycin were similar to those observed in chronic treatment. The ototoxic potentiation of aminoglycosides by loop diuretics observed in sensory cell damage has been proposed to result from facilitation of the transport of aminoglycosides into endolymph (Tran Ba Huy et al. 1983). According to this hypothesis, strial changes similar to those in chronic treatment may be brought about by excessive accumulation of kanamycin in the strial cells potentiated by furosemide.

The substantial and underlying mechanisms of such cytological changes in the strial cells are unknown, but these pathological changes were accompanied by distinct changes in DPOAEs, namely, a sudden drop in the DPOAE levels 2 to 3 hours after furosemide injection and a gradual fall in the DPOAE levels immediately after the incomplete recovery from the furosemide-induced decrease in the DPOAE levels. DPOAEs evoked by low-level stimuli are affected by both the outer hair cells and the endocochlear potential (Whitehead 1992). Physiological methods besides DPOAEs have been used to monitor the endocochlear potential originating from the stria vascularis. The microelectrode method that reaches the scala media is able to measure precisely the endocochlear potential, but may destroy the fine structure of the stria vascularis. Thus, we chose DPOAE measurements as a noninvasive and reliable tool to physiologically monitor the strial cells. The present findings of DPOAEs imply fine alterations of the strial cells at the ultrastructural level.

In conclusion, both physiological and morphological assessment of the strial cell function using DPOAEs and transmission electron microscopy revealed new ototoxic features which may be brought about by kanamycin potentiated by furosemide.

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