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Effects of *Ginkgo biloba* Extract on the Cochlear Damage Induced by Local Gentamicin Installation in Guinea Pigs

Investigations evaluating the protective effect of *Ginkgo biloba* extract (EGb) on gentamicin (GM) ototoxicity were undertaken. Guinea pigs treated with 5 mg/kg gentamicin sulfate on the round window niche (RWN) showed acute changes on electrocochleogram and hair cell or microvilli damage on scanning electron microscopy (SEM). There was accumulation of GM in the whole cochlea, especially in the organ of Corti, stria vascularis, and type III fibrocyte on immunohistochemical study. However, the guinea pigs pretreated with local or systemic EGb revealed no significant changes by local GM installation. From these results, we concluded that EGb has a protective effect on the development of GM ototoxicity in the cochlea.

Key Words : *Ginkgo biloba; Plant extract; Gentamicin; Drug toxicity, ototoxicity; Guinea pig; Cochlea*

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INTRODUCTION

Ototoxicity is an adverse effect of aminoglycoside antibiotics affecting both the auditory and vestibular functions of the ear. Gentamicin (GM) is a potent and specific ototoxic agent. The lipid binding specificity of GM inhibits the enzymatic reactions; therefore, the acute toxicity of GM can be explained by a displacement of calcium and inhibition of calcium-dependent membrane functions (1).

The *Ginkgo biloba* extract (EGb), which is made from the leaves of the ginkgo tree, is therapeutically used in an attempt to increase peripheral and cerebral blood flow. A significant increase in skin perfusion and a decrease in blood viscosity and elasticity are well documented (2). In addition, a calcium-stabilizing role for EGb has been suggested (3).

It was, therefore, the aim of this study to investigate the effects of local as well as systemic EGb application in a well-defined experimental GM ototoxic cochlear model.

MATERIALS AND METHODS

Guinea pigs weighing 350-450 g received 5 mg/kg GM on the round window niche (RWN) by a posterior approach. The guinea pigs were divided into 4 groups with each group containing 5 animals (Table 1): two groups were evaluated with electrocochleogram (ECoG) & scanning electron microscopy (SEM) (local GM only and local EGb with local GM groups). Two groups were evaluated with immunostaining (24 hour-local GM only and systemic EGb with 24 hour-local GM groups).

The local EGb with local GM group received EGb with a dosage of 10 mg/kg on RWN for 30 minutes, and received GM with a dosage of 5 mg/kg on RWN for 45 minutes. The EGb step was omitted in the local GM group (1). The ECoG was registered for each step and both groups were immediately sacrificed for SEM (Table 1).

The systemic EGb was injected intraperitoneally, 60 minutes before local installation of GM in the systemic EGb with 24 hour-local GM group, whereas only GM was applied in the 24 hour-local GM only group. Both groups were sacrificed for immunostaining after 24 hours (Table 1).

ECoG was checked with an active electrode at the round window niche. Tone pips, 3 kHz, 95 dB sound pressure level (SPL) were used for ECoG. SEM preparations were made in the conventional manner. Immunostainings to GM antigen were performed with the modified ABC method (4).

Table 1. Experimental groups

Experimental group	EGb treatment	GM on RWN	Evaluation
Local GM only (n=5)	No	5 mg/kg for 45 min	ECoG, SEM
Local EGb with local GM (n=5)	EGb 10 mg/kg for 30 min	5 mg/kg for 45 min	ECoG, SEM
24-hour local GM only (n=5)	No	5 mg/kg for 24 h	Immunostaining
Systemic EGb with 24-hour local GM (n=5)	EGb 100 mg/kg, 1 h before local GM application	5 mg/kg for 24 h	Immunostaining

EGb, *Ginkgo biloba* extract; GM, gentamicin; RWN, round window niche; ECoG, electrocochleogram; SEM, scanning electron microscopy.

RESULTS

Changes in electrocochleogram

ECoG responses to 3 kHz, 95 dB SPL tone pips did not change with the phosphate buffer solution (mixing solution for EGb) applied to RWN. However, the responses were eliminated after 45 minutes with GM on RWN in five animals investigated in this study. EGb pretreated guinea pigs showed no significant changes on ECoG to the same exposure period of GM on RWN (Fig. 1). This finding was observed in all five animals.

SEM observations

In all five animals studied, SEM of the local GM only group represented damage of the outer and inner hair cell stereocilia and of the Deiter cell microvilli. The damage was greatest at the basal turn (Fig. 2-1). However, the EGb pretreated group showed well-preserved hair cells and supporting cells in all five animals (Fig. 2-2).

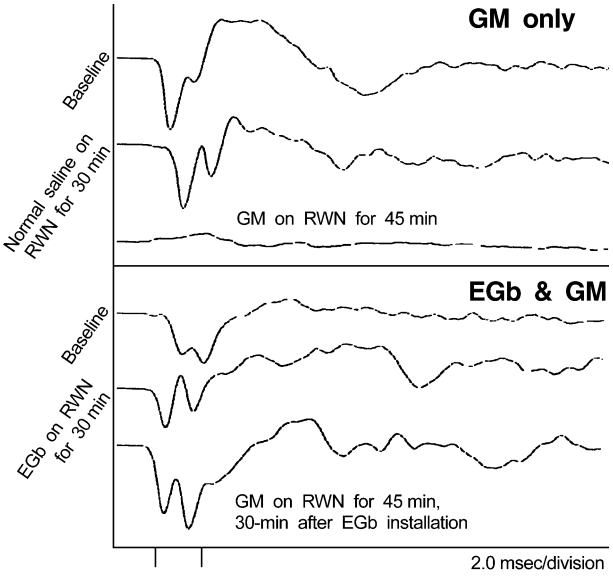

Fig. 1. Electrocochleogram responses to 3 kHz, 95 dB SPL tone pips in both the local gentamicin (GM) only group and the local *Ginkgo biloba* extract (EGb) with local GM treated ear group. RWN, round window niche.

Immunostaining

No immunostaining was observed in negative control specimens (Figs. 3-1A and 3-2A). In the local GM with-

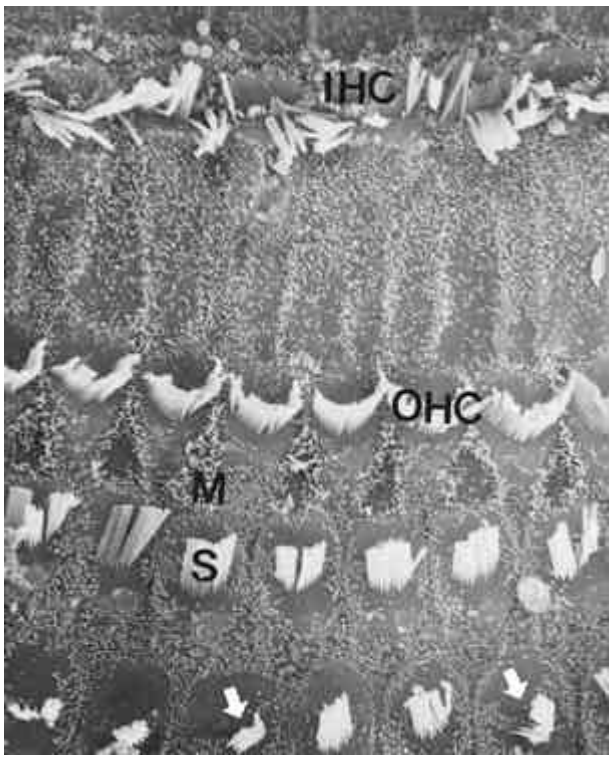

Fig. 2-1. Scanning electron microscopic findings in the local gentamicin only group. Loss and damage of the hair cell stereocilia and Deiter cell microvilli throughout the whole cochlea turns are evidently observed. White arrows (bottom) indicate loss of outer hair cells. Stereocilia (S) show loss of stiffness, clamping, and destruction. Microvilli (M) show shrinking, shortening and decrease of population (×1,500).

out EGb administration group, locations of immunostaining to GM by GM antibody were diffuse in all five animals; however, the Organ of Corti, stria vascularis and fibrocytes of the spiral ligament (especially, type III fibrocyte) showed strong positive reactions (Fig. 3-1B). In the systemic EGb with local GM group (three out of five animals), weak reactions to the GM antibody were observed in the organ of Corti, stria vascularis, and spiral ligament (Fig. 3-2B). In other two animals, no reactions were observed in the cochlea.

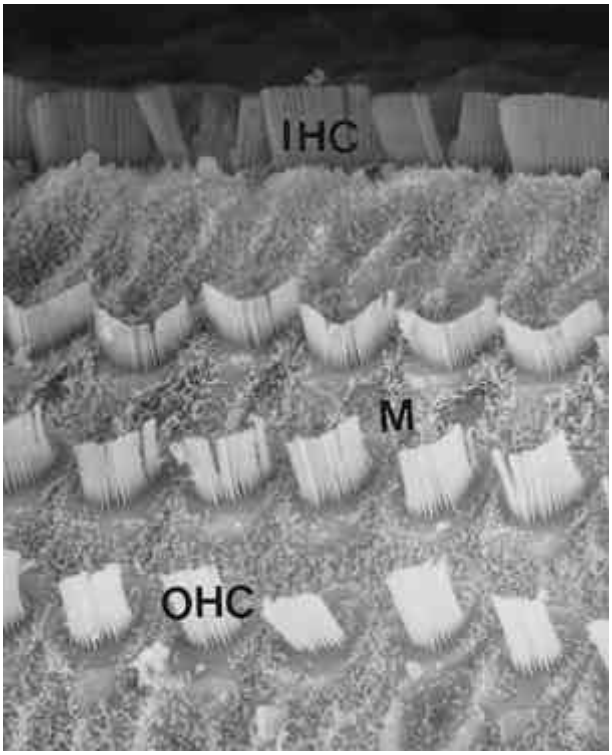

Fig. 2-2. Scanning electron microscopic findings in the local *Ginkgo biloba* extract with local GM group. Normal structure of the hair cell stereocilia, Deiter cell microvilli, and supporting cells is relatively well preserved. IHC indicates inner hair cell, OHC outer hair cell, and M microvilli of Deiter cell (×2,000).

DISCUSSION

In this study, we demonstrated that local installation of GM on RWN always damaged the cochlea both morphologically and functionally. This is consistent with the result of the previous reports (1, 5). There have been several proposals regarding the mechanism of aminoglycoside ototoxicity. Now, the direct influence of aminoglycosides on the hair cells of the cochlea and vestibular apparatus has been strongly suggested (1, 5, 6). In addition, Tachibana et al. proposed interference with cell membrane lipids by the binding of aminoglycoside to the plasma membrane, which can be antagonized by calcium, as a major factor underlying aminoglycoside injury of the inner ear (1). Other experiments have shown that GM inhibits polyphosphoinositide metabolism, which is essential for controlling cell membrane permeability and maintenance of membrane structure (1, 5). Therefore, a displacement of calcium and inhibition of calcium-dependent membrane functions can be the initial mechanism of GM ototoxicity (1). Formation of complexes with phos-

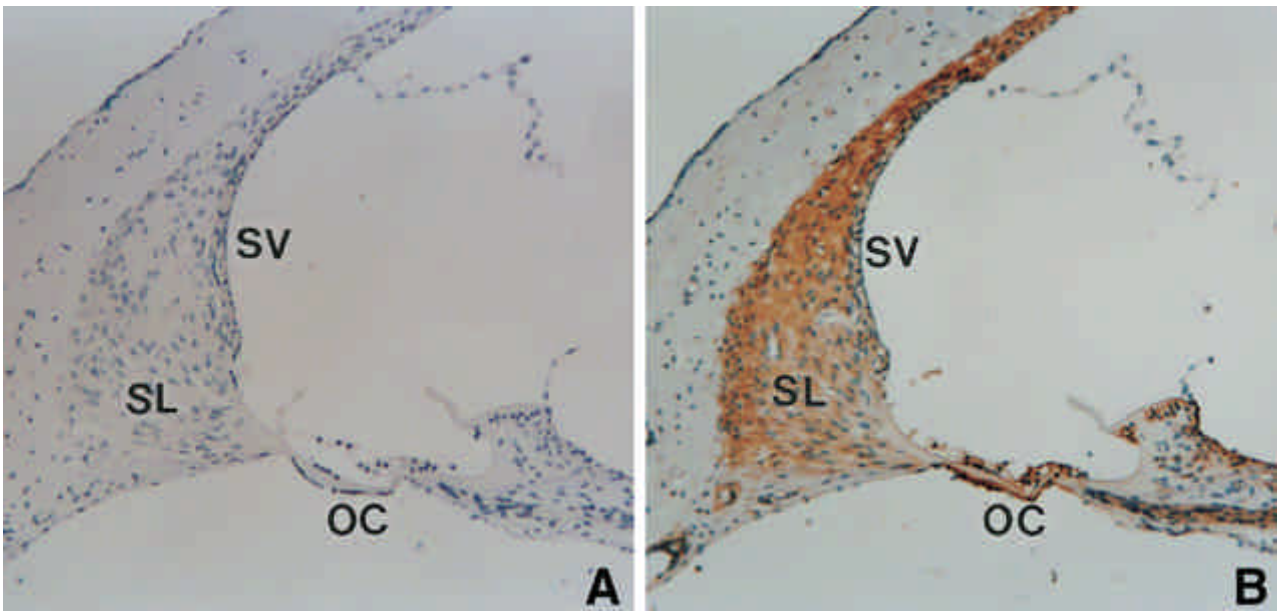

Fig. 3-1. Immunostaining to the gentamicin (GM) antigen in negative control (A) and in the 24-hour local GM only group (B). The organ of Corti (OC), stria vascularis (SV) and spiral ligament (SL), especially in type III fibrocytes are strongly stained with GM antibody (×100).

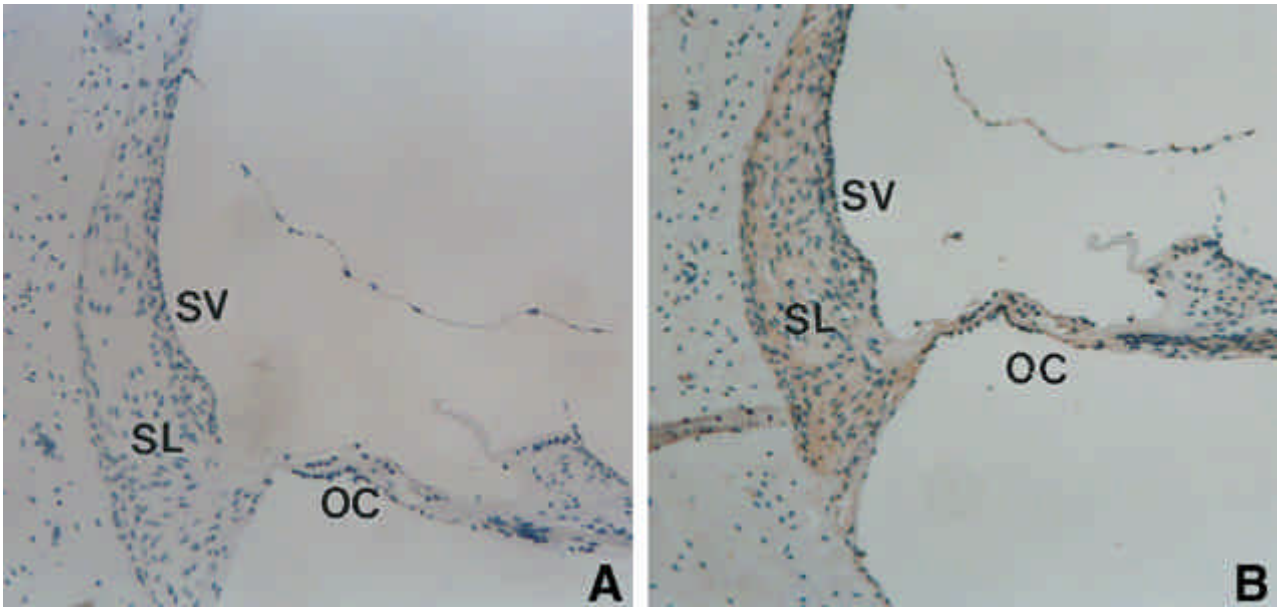

Fig. 3-2. Immunostaining to the gentamicin (GM) antigen in negative control (A) and in the systemic *Ginkgo biloba* extract with 24-hour local GM group (B). The organ of Corti (OC), stria vascularis (SV) and spiral ligament (SL) are weakly stained with GM antibody (×100).

phatidylinositol bisphosphate may then lead to structural disturbances of the membrane affecting cellular homeostasis and allowing penetration of GM into the cell.

Attempts to find an agent to antagonize or prevent aminoglycoside-induced injury have been disappointing to date. EGb has been known to be a potent vasodilating agent and EGb has also been suggested to be a platelet activating factor antagonist, a potential calcium stabilizer and radical scavenger (2, 7, 8). On the basis of the calcium mediating ototoxicity of GM and the calcium stabilizing and radical scavenging effects of EGb, we attempted the investigation of systemic and local EGb administration in a GM induced ototoxic cochlear model.

The ECoG changes by GM on RWN started 30 minutes after GM application. The EGb, pretreated for 30 minutes, blocked the changes on ECoG caused by GM for the same duration. SEM examination confirmed the results of ECoG. Immunostaining to GM antigen also revealed a significant difference between the systemic EGb intraperitoneal injection group and the group without EGb treatment. In systemic EGb with local GM group, no GM staining was observed on the cochlea in 2 of 5 guinea pigs while 3 of them showed weak accumulation of GM on the cochlea. This finding suggests that EGb may delay the uptake of GM or rapidly excrete GM. However, we can not explain whether EGb blocked GM uptake or whether EGb plays some role in excreting the uptaken GM at this point. However, with our preliminary results, we suggest that there are some protective roles of EGb for GM ototoxicity.

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