Influence of Topical Application of Basic Fibroblast Growth Factor upon Inner Ear

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Abstract

BACKGROUND: Basic fibroblast growth factor (b-FGF) is recently reported to show a good effect on the treatment of postoperative mastoid cavity problem, but its ototoxicity has not been investigated yet.
OBJECTIVE: To investigate the effect of b-FGF on the inner ear of guinea pigs.
STUDY DESIGN AND SETTING: In group A (n = 10) and B (n=11), b-FGF were applied into the left external auditory canal and the middle ear, respectively. The right ear served as a control. One week later the endocochlear DC potential (EP) was measured and morphology of the cochleae was examined using scanning electron microscopy.
RESULTS: In group A, the EP values of experimental ears and controls were 90.0 ± 8.4 (mV, mean ± SD) and 89.4 ± 4.3 (P > 0.05). In group B, those were 86.5± 11.4 and 87.5 ± 6.1 (P > 0.05). Morphologic findings showed no damage on the cochlear.
CONCLUSION: b-FGF application to the external and middle ears does not seem to have an apparent risk of ototoxicity.
Key words: Fibroblast growth factor (FGF); Endocochlear DC potential (EP), Guinea pig, Ototoxicity

Introduction

Basic fibroblast growth factor (b-FGF) preparation is a spray-type agent locally applied for treatment of bedsores, cutaneous ulcers, and other. Recently it has been used for the treatment of ear diseases including repair of a perforation of the tympanic membrane and for postoperative mastoid cavity problems.[1,2,3] However, to our knowledge, there have been no experimental studies investigating the possibility of the ototoxicity of this agent when it is applied to the external or middle ear. In the present study, we investigate the influence of b-FGF preparation on the inner ear of guinea pigs using the electorophysiological and morphological techniques.

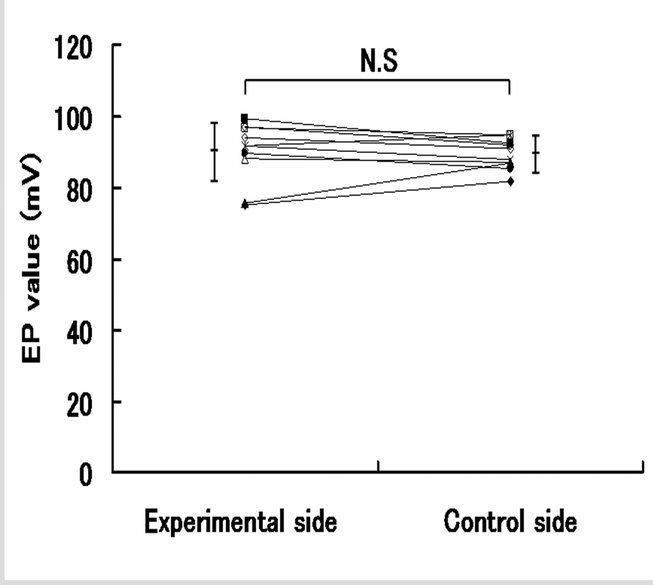
Methods and Materials

2.1. **Animals**
Twenty one healthy male guinea pigs (weight 260–340 g), free of external or middle ear disease, were used in this study.
2.2. Drug administration
Guinea pigs were divided into two groups. In group A(n=10), after spongel (Astellas Pharma Inc.) was put into the external ear, approximately 100 μg of b-FGF preparation (2,000 μg/ml, Kaken Pharmaceutical Co., Ltd) was applied into the left external auditory canal with a syringe once every two days for four times in total, and the right ear served as a control. This amount and concentration of b-FGF administered into the animal ears correspond to one hundred times as much in the amount and twenty times as much in the concentration comparing with those clinically used for patients. One week later, endocochlear DC potential (EP) was measured in both ears and then both cochleae were removed. Twenty cochleae from ten animals were processed for scanning electron microscopy (SEM) study. In group B (n=11), after myringotomy was performed carefully under a surgical microscope, spongel was placed into the tympanic cavity, and b-FGF preparation was infused once every two days four times in total. Approximately 100 μg of b-FGF preparation was needed to fill the external and middle ears. The right ear served as a control. One week later, EP was measured in both ears, and then both cochleae were removed. Twenty one cochleae from 11 animals were processed for SEM. One cochlea on the control side was broken when the cochlea was removed.
2.3. Electrophysiological recordings
The EP was recorded from the basal turn of the cochlea. The glass microelectrode was advanced into the scala media through the round window membrane (RWM) until a positive EP was obtained.
Differences in the EP between the control and the experimental ears were analyzed statistically using paired Student's two-tailed t-test. Difference was considered significant when the P value was less than 0.05.
2.4. Hair cell count (SEM)
Semiquantitative hair cell counts were performed with a modified version of the method used by Korver et al.[4] Twenty cochleae from 10 animals in group A and twenty one cochleae from 11 animals in group B were used. Representative areas of the basal turn, middle turn and apical turn were photographed. In each area, inner or outer hair cells were counted in a section that contained 10 pillar cell heads. The results were shown as the average survival percentage rates compared to control. Statistical analysis was performed using non-paired Student's two-tailed t-test. Difference was considered significant when the P value was less than 0.05.

Results

3.1. Electrophysiological findings
In group A, the EP values of experimental and control ears were 90.0±8.4mV (mean±standard deviation) and 89.4±4.3mV, respectively (Figure 1). There was no significant difference between the both sides in this group (p=0.778). In group B, the EP values of experimental ears and control ears were 86.5±11.4mV and 87.5±6.1mV, respectively (Figure 2). EP value was apparently low on the experimental ear in one animal (53.1mV), but there were also two control ears showing low EPs in this group. There was no significant difference between the both sides in this group either (p=0.771).
3.2. Electron microscopic findings
Figure 3 shows SEM micrographs of a guinea pig cochlea from an experimental ear in group B. Almost normal stereociliary arrangements and surface structure on the inner and outer hair cells and an normal number of microvilli on the surface of the inner pillar cells can be seen in the basal (Figure 3A), middle (Figure 3B), and apical (Figure 3C) turns. No notable changes were seen in any turns in either group A or B.
3.3. Hair cell count
3.3.1. Group A
The outer hair cell survival rates on the experimental side were 98.8±2.0, 100±0 and 99.4±1.7% in basal, middle, and apical turn, respectively, whereas they were 98.0±5.4, 99.3±1.6 and 98.1±5.6% in basal, middle and apical turn, respectively, on the control side. The inner hair cell survival rates were 100±0, 100±0 and 100±0% in basal, middle and apical turn, respectively, on the experimental side, while they were 95.2±12.6, 98.0±6.3 and 100±0% in basal, middle and apical turn, respectively, on the control side. There were no significant differences in either outer or inner hair cell counts between the b-FGF side and the control side for any turn (outer hair cell; basal turn p=0.724, middle turn p=0.201, apical turn p=0.549, inner hair cell; basal turn p=0.377, middle turn p=0.357, apical turn p=1.000).

3.3.2. Group B
The outer hair cell survival rates on the experimental side were 99.1±0.8, 100±0 and 97.4±5.8% in basal, middle and apical turn, respectively, whereas they were 100±0, 99.3±1.6 and 99.5±1.5% in basal, middle and apical turn, respectively, on the control side. The inner hair cell survival rates were 100±0, 99.1±2.8 and 100±0% in basal, middle and apical turn, respectively, on the experimental side, while they were 100±0, 100±0 and 100±0% in basal, middle and apical turn, respectively, on the control side. There were no significant differences in either outer or inner hair cell counts between the b-FGF side and the control side for any turn (outer hair cell; basal turn p=0.396, middle turn p=0.201, apical turn p=0.275, inner hair cell; basal p=1.000, middle p=0.305, apical p=1.000).



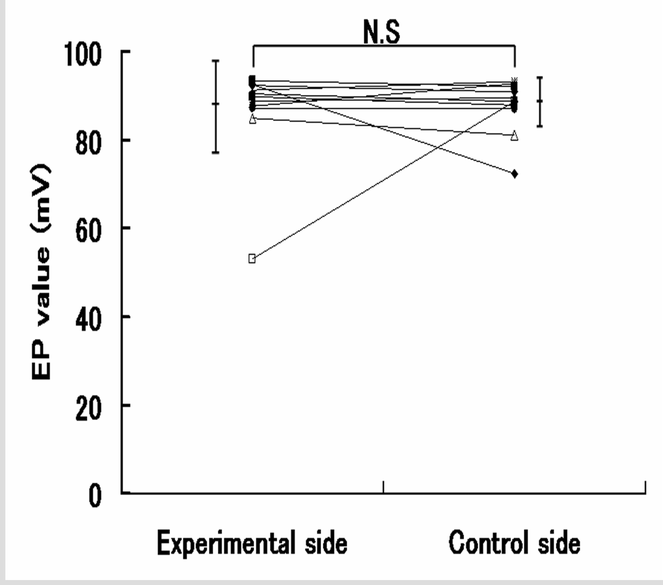


Figure_1
EP values of animals in group A are shown (n=10). There was no significant difference between experimental and control ears. Error bar =SD, N.S. = no statistically significant difference.

Figure_2
EP values of animals in group B are shown (n=11). There was no significant difference between experimental and control ears. Error bar =SD, N.S. = no statistically significant difference.

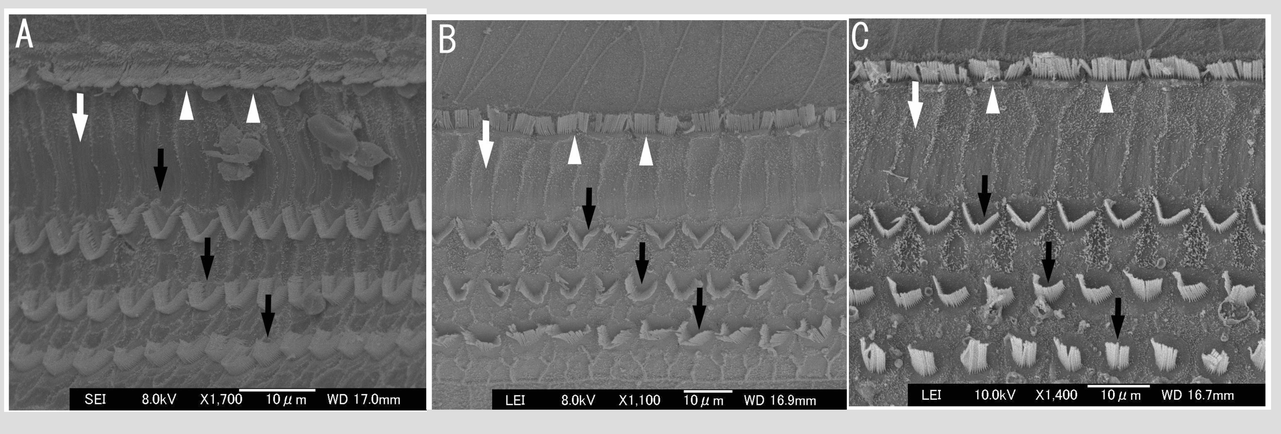


Figure_3
Representative SEM photomicrographs of the organ of Corti from a guinea pig in group B at 1 week after treatment (b-FGF preparation in the left middle ear). Intact inner (arrowheads) and outer hair cells (black arrows) and inner pillar cells (white arrows) can be seen in the basal (A), middle (B), apical (C) turns. Scale bar=10um.

Discussions and Conclusions

As described in the method, b-FGF preparation given to the animals in this study was apparently far greater in the amount as well as in the concentration than that usually used in human. We, therefore, consider that RWM is sufficiently exposed to b-FGF preparation in the middle ear group animals.
One concern was that an ear on the experimental side in group B (middle ear group) had a low EP. When we opened the bulla to expose the middle ear of this animal, we could not find any damages on the stapes or perilymph leakage. Thus, the cause of the decrease in EP in this animal is uncertain.
Although a literature reported an experience of using this agent to human ears for approximately two months,[1] we set the duration of administration of this agent one week in this study. It is because ototoxicity, if there is, is manifested within a week in most experimental studies instilling the agents into the middle ear.[4,5,6,7] We set the interval of administration of this agent two days because the biological half life of this agent is 48 hours.
In this study, topical b-FGF preparation did not cause either significant reduction in EP or any degenerative changes to the structures in the organ of Corti even when applied into the middle ear. From the results of the present study, it was concluded that, as long as the EP and electron-microscopic morphology, b-FGF application to the external and middle ears, which is recently becoming prevalent as a good tool of conservative treatment for middle ear diseases, did not seem to have an apparent risk of ototoxicity.

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