Background: Basic fibroblast growth factor (b-FGF) is recently being used by Korver et al.4 Twenty cochleae from 10 animals in group A and twenty one in group 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, EP was measured in both ears, and then both cochleae were removed. Twenty cochleae from 11 animals were processed for scanning electron microscopy (SEM) study. In group B (n=11), after myringotomy was performed carefully as a control. One week later, the endocochlear DC potential (EP) was measured 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 electrophysiological and morphological techniques.

Results

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 stapedectomy (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 11 animals were processed for scanning electron microscopy. There was no significant difference in either outer or inner hair cell counts between the b-FGF side and the control side for any (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=0.350, apical p=1.000).

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)

Quantitative 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 basilar turn, middle turn and apical turn were selected. When the preparation went smoothly under a surgical microscope, stapes was placed into the tympanic cavity, and b-FGF preparation was infused once every two days for four times. 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.

3.1. Electrophysiological findings

In group A, the EP values of experimental and control ears were 90.0 ± 8.4 mV, 99.0 ± 6.1 mV, and 97.4 ± 8.1 mV, respectively (Figure 1). This was no significant difference between the both sides in this group (p=0.771). In group B, the EP values of experimental ears and control ears were 86.5 ± 11.4 mV and 87.5 ± 6.1 mV, respectively (Figure 2). EP value was apparently low on the experimental ear in one animal (53.1 mV), 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).

3.3.2. Group B

The outer hair cell survival rates on the experimental side were 91.9 ± 0.8, 100% and 97.4 ± 6.1% 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%, 99.1 ± 2.8 and 100% in basal, middle and apical turn, respectively, on the experimental side, while they were 100%, 100% and 99.9 ± 1.0% 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 (outer hair cell; basal turn p=0.396, middle turn p=0.201, apical turn p=0.275, inner hair cell; basal turn p=0.377, middle turn p=0.357, apical turn p=1.000).

4. Discussion

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 animal 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, we set the duration of administration of this agent one week in this study. It is becoming clear, therefore, that RWM is isolated within a week in most experimental studies instilling the agents into the middle ear.1,4,5 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.

Bibliography


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

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Methods and Materials

Introduction

Basic fibroblast growth factor (b-FGF) 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 repairing a perforation of the tympanic membrane and for postoperative mastoid cavity problems.1,2 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 electrophysiological and morphological techniques.

Results

3.3.2. Group B

The outer hair cell survival rates on the experimental side were 91.9 ± 0.8, 100% and 97.4 ± 6.1% in basal, middle and apical turn, respectively, whereas they were 100%, 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%, 99.1 ± 2.8 and 100% in basal, middle and apical turn, respectively, on the experimental side, while they were 100%, 100% and 99.9 ± 1.0% 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 (outer hair cell; basal turn p=0.396, middle turn p=0.201, apical turn p=0.275, inner hair cell; basal turn p=0.377, middle turn p=0.357, apical turn p=1.000).

Discussion 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, we set the duration of administration of this agent one week in this study. It is becoming clear, therefore, that RWM is isolated within a week in most experimental studies instilling the agents into the middle ear.1,4,5 We set the interval of administration of this agent two days because the biological half life of this agent is 48 hours.

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