

New Bone Formation of the Guinea Pig Cochlea after Usage of Antiseptics Evaluated with a Micro-CT Scanner

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Abstract

Objectives: This study quantified new bone formation in the middle ear cavity of the guinea pig after topical antiseptic application.

Materials and Methods: Adult Hartley guinea pigs were used. Three different antiseptics that are used clinically to treat chronically discharging ears were studied: povidone-iodine, methylrosaniline chloride, and 13% (w/v) aluminum acetate.

Results: The temporal bones of the animals were scanned using *in-vivo* micro-tomography (micro-CT) before and 2 and 4 weeks after filling one of the middle ear cavities with antiseptic. Three-dimensional (3D) micro-CT images of the temporal bone were obtained and the thickest part of the bone defined as the region of new bone formation. After measurements were completed, the temporal bones were harvested for histopathological evaluation. Celloidin-embedded specimens were cut into 20- μ m-thick slices, stained, examined microscopically, and the results compared with the micro-CT findings.

Conclusions: The SkyScan 1178 enables repeated 3D observations of the temporal bone in the living guinea pig. The results were in good agreement with the histopathological findings.

Key words: New bone formation, Micro-CT scanner, Cochlea, Guinea pig

Introduction

This study quantified new bone formation in the guinea pig middle ear cavity after topical antiseptic application. The fact that new bone forms under such conditions is a relatively new finding, and histopathology has been the only method available to explore the existence and extent of new bone formation.

X-ray micro-tomography (micro-CT) is a new technique for visualizing the temporal bones with quasi-histological accuracy. The SkyScan 1178 (SkyScan, Kontich, Belgium) can be used to investigate small-animal microstructure non-invasively and repeatedly. We explored the utility of this instrument in the guinea pig.

Materials and Methods

Hartley guinea pigs weighing 250–300 g were used. Three different antiseptics were studied: povidone-iodine (Isodine, $n=5$), methylrosaniline chloride (gentian violet, $n=4$), and 13% (w/v) aluminum acetate (Burow's solution, $n=7$). These three antiseptics have been used clinically for the treatment of chronic otitis media. Although we previously found that two of these solutions were ototoxic^[1–3], new bone formation in the middle ear cavity of experimental guinea pigs is a relatively unknown side-effect of these antiseptic solutions.

The abovementioned antiseptics were freshly prepared by the Pharmacy Department of our university hospital; the

pH was measured and adjusted before each experiment.

The animals were anesthetized with sodium pentobarbital (30 mg/kg) and secured in a custom-made head holder. Xylocaine 0.5% was infiltrated into the surgical area before making the skin incision for access to the middle ear cavity.

The bulla was exposed using a retro-auricular incision. A 2-mm-diameter hole was made using a dental drill, and the round window membrane was visualized under a 40× operating microscope.

The middle ear cavity was filled with antiseptic solution (ca. 0.2 mL) and sutured. Two and 4 weeks later, the entire head was evaluated via SkyScan micro-CT.

The SkyScan 1178 (SkyScan, Kontich, Belgium) can reveal small animal microstructure non-invasively and repeatedly. A scan and reconstruction cycle can be performed in less than 2 min.

The temporal bones of the animals were scanned using *in-vivo* micro-CT before and 2 and 4 weeks after filling the middle ear cavities with an antiseptic solution. A three-dimensional (3D) scan of the temporal bone was obtained and the thickness of the new bone formation at the thickest part of the bone determined.

After the measurements were completed, the temporal bones were harvested for histopathological study. Celloidin-embedded specimens were cut into 20-µm-thick slices, stained, evaluated under a microscope, and the results compared with the micro-CT findings.

Data from the treated and untreated sides were compared using Student's paired *t*-test.

The study protocol was approved by the Fukuoka University Animal Ethics Committee.

Results

All three antiseptics studied caused massive new bone formation at both 2 and 4 weeks ($p < 0.05$ for Isodine at 2 weeks; $p < 0.01$ for all other comparisons) (Figs. 1-3). Gentian violet triggered the most new bone formation.

Discussion

The merit of computed tomography (CT) over conventional histopathological studies of the temporal bone is obvious. CT is an *in vivo* modality that is rapid and repeatable. In comparison, temporal bone histopathology is expensive and time-consuming. A drawback of CT is that the images are coarse and lack detail. Our equipment has a minimum slice thickness of only 80 µm.

(1) Povidone-Iodine (N=5)

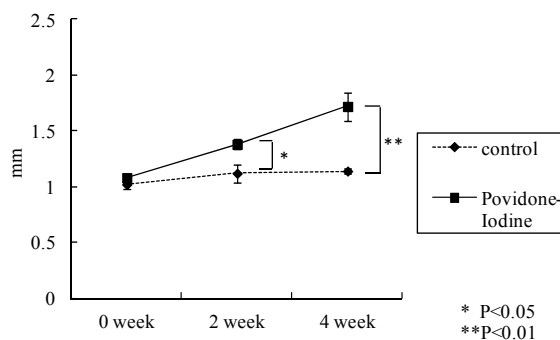


Fig. 1 Thickness of bone after usage of Povidone-iodine.

(2) Gentian Violet (N=4)

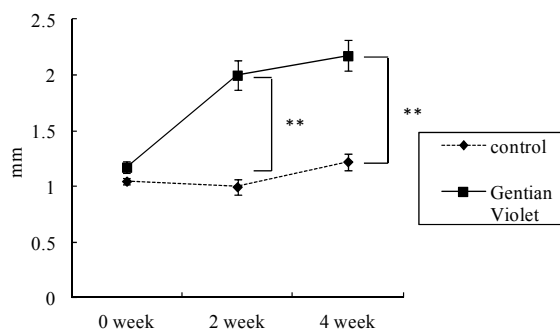


Fig. 2 Thickness of bone after usage of Gentian violet.

(3) Burow's solution

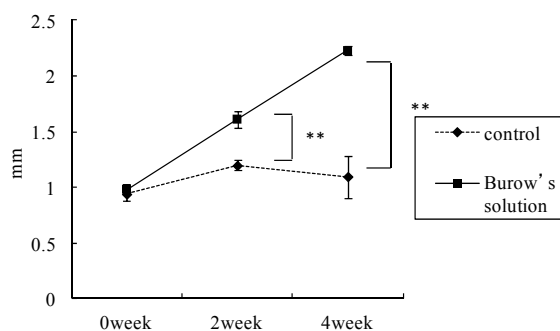


Fig. 3 Thickness of bone after Burow's solution.

Many authors have sought to visualize cochlear structure using CT. Postnov et al. [4] attempted to evaluate positioning of the cochlear implant electrode in cylindrical blocks of human temporal bone. This was the first application of micro-CT for visualization of the inner ear structures in human temporal bones and for evaluation of the surgical positioning of the cochlear implant electrode relative to the intracochlear soft tissues.

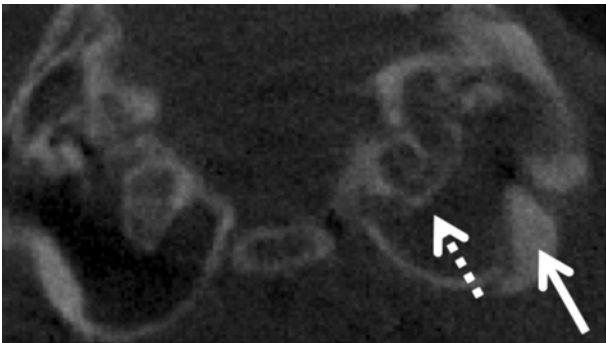


Fig. 4 Axial view of the cochlea 4 weeks after usage of Gentian violet. Left side is control., broken line of the right side indicates the cochlea, the thick line indicates thick bone tissue.

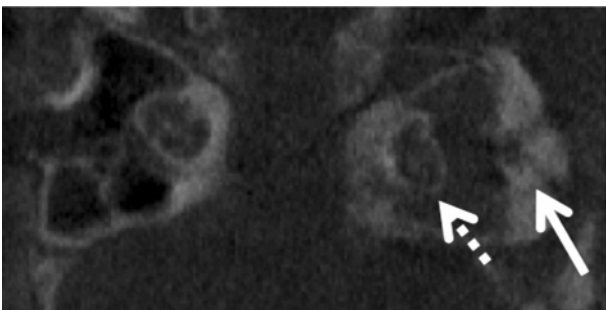


Fig. 5 Coronal view of the cochlea 4 weeks after usage of Gentian violet. Left side is control., broken line of the right side indicates the cochlea, the thick line indicates thick bone tissue.

Shibata et al. [5] used micro-CT to visualize the internal fine structure of the human fetal cochlea, including Reissner's membrane and the spiral ganglion. Richard et al. [6] viewed and quantified morphological features and mineralization of the developing fetal human bony labyrinth using 3D micro-CT imaging. Suzuki [7] studied 350 human temporal bones collected at autopsy and found that 171 (49.1%) had otitis media, of which 165 (97.1%) also

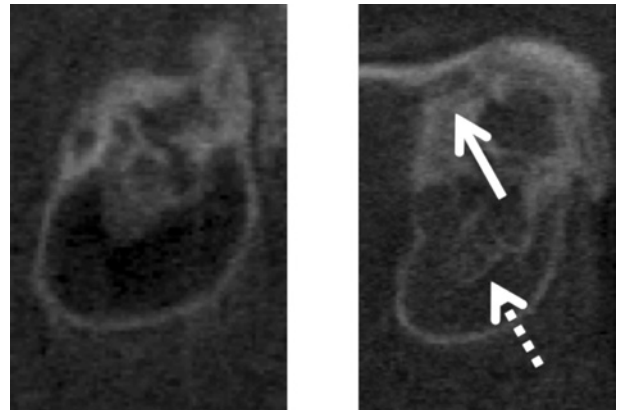


Fig. 6 Sagittal view of the cochlea 4 weeks after usage of Gentian violet. Left side is control., broken line of the right side indicates the cochlea, the thick line indicates thick bone tissue.

had bony changes, particularly new bone formation and reconstruction. Bone formation was closely associated with chronic inflammation of the middle ear cavity. Osseous destruction was rare in cases of chronic otitis media. The bony pathology that we found was most likely attributable to chronic inflammation caused by the antiseptics instilled into the middle ear cavity.

We repeatedly observed histopathological changes in the temporal bones of guinea pigs without sacrificing the animals. We studied only bony tissues, but future improvements in CT should also allow visualization of soft cochlear tissues. A combination of electro-physiological and histopathological methodology will greatly advance our knowledge of the pathogenesis of drugs ototoxic to the middle ear cavity.

We studied guinea pigs *in vivo* for 4 weeks after application of antiseptics, and found that new cochlear bone formation commenced at 2 weeks. Such bone formation is a novel finding, and its effects on auditory function require further study.

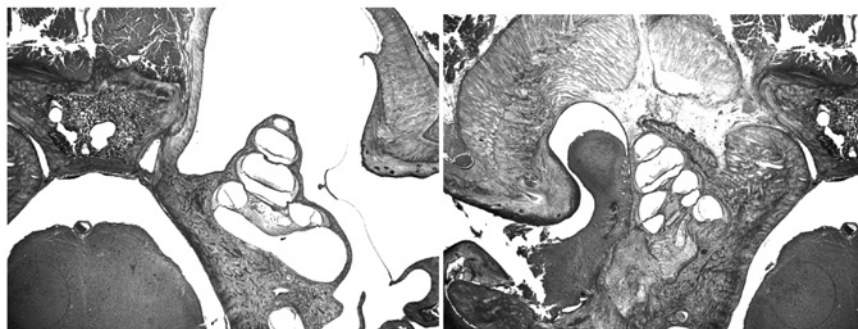


Fig. 7 Histopathology of the cochlea 4 weeks after usage of Gentian violet. Left side is control which shows normal tympanic membrane and clean middle ear cavity. Right side cochlea is surrounded by thick bony tissues.

Conclusions

The SkyScan 1178 facilitated repeated 3D observations of the temporal bone in the living guinea pig. The results were in good agreement with the histopathological findings. Since the sections obtained were 80 μm thick, we were unable to analyze the detailed cochlear ultrastructure.

Acknowledgment

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[The authors declare no conflict of interest.]