Int. Adv. Otol. 2012; 8:(2) 217-222

ORIGINAL ARTICLE

Electron Microscopic Imaging of Osteonecrosis in Chronic Supurative Otitis Media

Ozgur Surmelioglu, Ulku Tuncer, Ozgur Tarkan, Suleyman Ozdemir, Sait Polat

Cukurova University School of Medicine Department of Otorhinolaryngology (OS, UT, OT, SO)
Çukurova University School of Medicine Department of Histology (SP)

Objective: In this study, we aimed to demonstrate electron microscopiccellular changes of bone necrosis developing due to chronic otitis media.

Material and Methods: This study was performed on the pathologic tissue samples of 20 cases that were operated due to chronic supurative otitis media in Çukurova University Ear Nose Throat Department. Collected cholesteatoma tissue samples, granulation tissue samples, incus and mastoid bone samples were examined by JEOL-JEM 1400 transmission electron microscopy after dyeing.

Results: Incus and mastoid bone were examined together in 16 of the operated patients. In the remaining 4 patients, only mastoid bones were examined as the ossicles were absent. Fourteen patients showed cholesteatoma in the middle ear and/or mastoid cellulae, and 6 patients showed granulation tissue. Middle ear ossicles were corroded in 13 of 14 (92.8%) patients with chronic otitis media and cholesteatoma versus 3 of 6 (50%) patients with chronic otitis media without cholesteatoma. In the samples of patients with chronic otitis media and cholesteatoma, ossicles were irregular, besides increase in vascularity; dense cellular infiltration was also observed. Swollen endoplasmic reticulum and cytoplasmic vacuoles are among the findings that support the enzymatic theory. Examination of incus samples revealed degenerative changes and large vacuoles in cells.

Conclusion: This study was not able to demonstrate enzymatic theory, it may shed light on further studies focusing on osteonecrosis.

Submitted : 11 October 2011 *Accepted : 22 April 2012*

Introduction

Chronic otitis media (COM) is an inflammatory disease characterized by clinical bone resorption[1]. Partial or total destruction of ossicles is seen in approximately 80 % of patients with cholesteatoma, whereas in chronic otitis media without cholesteatoma, ossicular chain corrosion can be seen in approximately 20% of the cases[1]. Cholesteatoma may cause bone erosion and result in intratemporal and intracranial complications, with high morbidity and mortality rates.

Studies on the etiology of bone destruction in chronic otitis media started in the beginning of the 1970s[1]. Although there are numerous theories to explain the destructive properties of cholesteatomas, bone resorption mechanism in chronic otitis media is not completely understood. Bone destruction mechanisms in cholesteatoma can be explained based on two theories: biophysical and biochemical. Biophysical action basis would be the pressure done by the cholesteatoma on the ossicular chain and on the middle ear walls on the other hand the biochemical theory is based on the destructive action of collagenases and inflammatory products[1].

As the exact mechanism of bone corrosion in cholesteatoma is not understood completely, surgical

Corresponding address:
Ozgur Surmelioglu
Dortyol State Hospital
Department of Otorhinolaryngology, Dörtyol / Turkey
Phone: +905376511501, Fax: +903267124759
E-mail: surmeli2004@yahoo.com

treatment is the only effective treatment of choice. Understanding the mechanism of bone erosion in chronic otitis media may be in future a medical treatment to inhibit inflammation and control bone destruction, which would reduce the morbidity and mortality of cholesteatoma.

In this study, bone necrosis were examined by using electron microscopy in the samples taken from mastoid bone and middle ear ossicles of 20 patients operated for chronic supurative otitis media. Purpose of the study was to demonstrate cellular changes in bone necrosis developed in relation with chronic otitis media.

Materials and Methods

This study was performed on the pathologic tissue samples of 20 cases that were operated due to chronic supurative otitis media in Çukurova University Ear Nose Throat Department between January 2007 and November 2007. Specimens of bone involved with cholesteatoma or granulation were taken from patients who underwent mastoid operations to treat chronic supurative otitis media. Specimens of normal mastoid bone were taken from five patients who had surgery on their normal mastoid (cochlear implantation, facial nerve decompression). Clinical data gathered in each case included the age of the patient, the indications for surgery, the hearing levels of the patients, and the type of surgical tecnique.

According to the type of pathology in patients, the tissue samples included cholesteatoma or granulation pieces with incus and cortical bone. The materials were taken by the surgeon, immediately embedded in Glutaraldehyde at 5 % and sent to Histology Department. After the specimens fixed in 5 % glutaraldehyde for 4 hours they were washed with Milloning phosphate solution. Dehydration in ethyl alcohol (50%-100%) in 4 ˚C and immersion by propylene oxide were performed. For the electronmicroscopic evaluation tissue samples processed by regular techniques and embedded in rezin. The specimens were serially sectioned in 500 A˚ widths. After dyeing of the tissue sections, a transmission electron microscopy was performed by JEOL-JEM 1400 electron microscope.

Results

In this study, electron microscopic results of the pathologic tissue samples of 20 cases who had operated due to chronic supurative otitis media were evaluated. Patient group consisted of 7 females and 13 males. The mean age was 25.1 years (range 10-42 years). Of the patients, 7 had bilateral chronic otitis media, and 13 had unilateral chronic otitis media.

Hearing threshold ranged between 1 and 80 dB in operated patients; the mean hearing threshold was 43.7 dB. In the pure tone test, the mean score of distinguishing speaking was 94.2%. In the pure tone sound threshold, air-bone interval ranged between 0-60 dB, and the mean value was 33.4 dB. One patient showed normal hearing threshold, eight patients had very low level hearing loss, five patients had low level hearing loss, four patients had moderate hearing loss, and two patients had severe hearing loss.

Of the operated patients due to chronic otitis media, 13 patients underwent radical mastoidectomy and 7 underwent open technique tympanomastoidectomy. Of the 13 radical mastoidectomy patients, 9 had cholesteatoma and 4 had granulation tissue. Of the 7 open technique tympanomastodiectomy patients, 5 had cholesteatoma and 2 had granulation tissue. Of the operated patients, 3 had patency of facial canal and 2 had patency of lateral semicircular canal. We observed corroded ossicles intraoperatively in 16 of the 20 patients operated for COM. Tissue sampling was performed from both incus and mastoid bone in 16 patients and only from mastoid bone in 4 patients due to lack of ossicles. Middle ear ossicle corrosion was present in 13 of the 14 (92.8%) patients with chronic otitis media and cholesteatoma and in 3 of 6 (50%) patients with chronic otitis media without cholesteatoma.

Pathologic tissue samples were collected for electron microscopic evaluation from left ear in 10 of the patients and from right ear in 10 of the patients. Cholesteatoma in the middle ear and/or mastoid cells

was present in 14 patients and granulation tissue was found in 6 patients.

Electron Microscopic Findings

In this study, during electron microscopic evaluation of cholesteatoma tissue, macrophages with plenty of large lysosomes in cytoplasm were observed. Furthermore, swollen mitochondria and many lipid particles were observed in the cytoplasm of macrophages. These findings support the enzymatic theory (Figure 1).

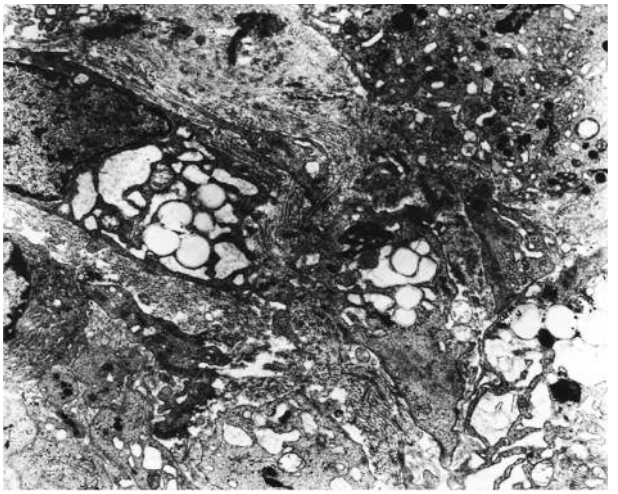

Figure 1. Macrophages with plenty of large lysosomes in cytoplasm are seen.

In electron microscopic evaluation, we also demonstrated increase in intercellular space between the cells, irregular cellular surface and amorphous matter accumulation into intercellular space of incus bone samples electron microscopically. Fibrous tissue was increased in bone tissue. In addition, a large amount of capillary vessels were observed. In bony matrix, a net composed of collagen fibers together with osteocytes with their tiny long cytoplasmic extensions located into lacunae were characterized. In the cytoplasm, bundles composed of a few organelle and filamentous structures were extending from nuclear periphery to cytoplasmic extensions. In the periphery of bony matrix, osteoblast cells with typical nucleus and cytoplasm, and fibroblasts around these cells and capillary vessels were determined. Bone regions in the vicinity of cholesteatoma showed dense osteocytic activity. This situation suggests that due to the effect of cholesteatoma, osteocytes in the bone differentiate and cellular changes take place (Figure 2).

Figure 2. In bony matrix, a net composed of collagen fibers together with osteocytes with their tiny long cytoplasmic extensions located into lacunae

In this study, edema regions characterized by broadening of intercellular space were also observed. The degenerative changes of osteocytes in bone matrix, vacuolar appearance of cytoplasm resulting from swollen endoplasmic reticulum cisternae in the cytoplasm of these cells, lytic regions due to destruction of organelles were also monitored. This shows enzymatic changes due to increase in osteoclastic activity. Cytoplasmic vacuoles may be accepted as the index of enzymes produced by cells (Figure 3). Tiny collagen fibers were observed on the granulation tissue. In bone matrix, besides osteoblasts characterized by long spindle-shaped nucleus and wide cytoplasm, presence of a loosely formed collagen fiber web was noticed. Osteocytes showed hyper chromatic nucleus and vacuolization due to swollen endoplasmic reticulum cisternae and mitochondria in cytoplasm. In addition, lysosomal increase and lytic regions were observed in cytoplasm.

Mastoid tissue sample taken together with granulation tissue showed widespread bleeding areas, cellular infiltration including monocytes, lymphocytes and neutrophils and edema. Some of the osteocytes in bony matrix showed chromatin increase and lytic lesions in cytoplasm. Besides, cellular structures were mostly

intact and normal, the structure and organization of the matrix including collagen fibers were also normal (Figure 4).

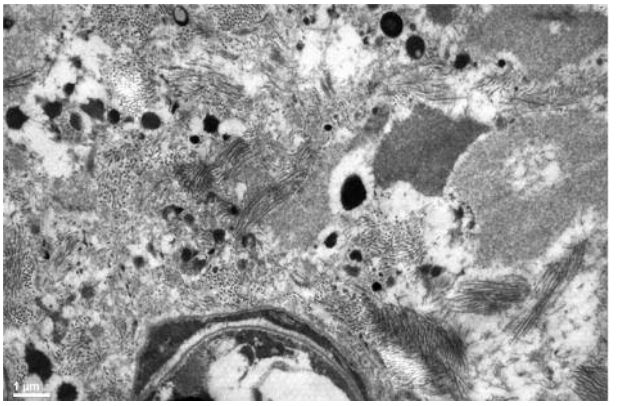

Figure 3. Degenerative changes of osteocytes in bone matrix

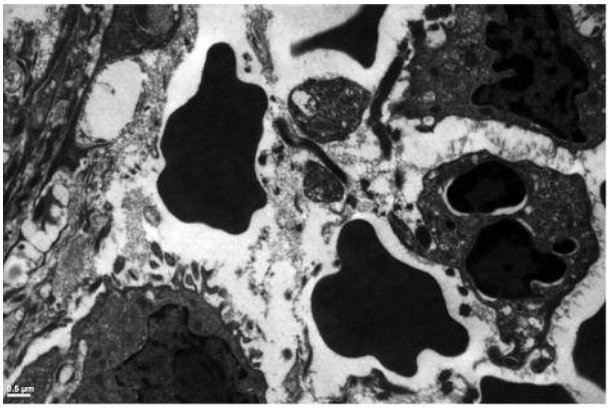

Figure 4. Widespread bleeding areas, cellular infiltration including monocytes, lymphocytes and neutrophils and edema are seen.

Discussion

Chronic otitis media is defined as the presence of irreversible inflammatory tissue changes in the middle ear[2]. The presence of cholesteatoma with chronic otitis media, causes more morbiditiy due to bone destruction. Chole and coworkers, in their study, reported that bone destruction was present in COM without cholesteatoma but it was more frequent in COM with cholesteatoma[3]. Some other studies reported damage to the ossicles in 80% of the patients with COM and cholesteatoma, whereas this rate was 10-20% in simple COM without cholesteatoma[2]. The mechanisms leading to this increase in bone degradation in the presence of cholesteatoma are still unclear[2-4].

In our study, of the 20 patients in the study group, 14 operated with a diagnosis of chronic otitis media plus cholesteatoma and in accordance with the literature we found partial or complete destruction of middle ear ossicles in 13 (92.8%) patients. In patients operated because of granulation and KOM, ossicle destruction rate was 50%. This ratio seems to be higher than those reported in the literature.

Although there are many studies to explain the pathophysiology of bone resorption in cholesteatoma, the real course of the pathology and contributing factors are not clear yet[2-4]. Understanding the mechanism of bone corrosion in chronic otitis media may be in future a medical treatment to inhibit inflammation and control bone destruction, which would reduce the morbidity and mortality of cholesteatoma.

As the cause of destruction, pressure due to accumulated keratin was initially proposed by Kirchner[5]. According to this theory, due to the mass effect of cholesteatoma, capillary circulation impairs in contact areas and osteonecrosis may develop as a result of impaired blood supply. Physical effects of cholesteatoma lead to transient electrical potentials and sub epithelial monocyte accumulation. These monocytes activate cellular events of bone destruction. Activated monocytes may produce PGE2 which stimulates bone formation. It has been shown that these inflammatory mediators stimulate the development of epidermal basal cells of cholesteatoma[4-6].

However, many recent studies showed that a local inflammatory reaction, combined with proteolytic

activity of numerous matrix-degrading enzymes, plays a major role[7-13]. Among these enzymes, matrix metallo proteinases (MMP) including gelatinase A and B (MMP-2 and MMP-9), stromelysin-1 (MMP-3), and neutrophil collagenase (MMP-8) are of special interest

[8,11,12]. A biochemical theory eventually was postulated, in which enzymes and cytokines released by cholesteatomas would cause bone lysis and destruction[6]. Lautenschlager was the first who suggested cholesteatoma might produce enzymes to corrode bone. Abramson et al.[4] demonstrated that cultured human cholesteatoma may destruct collagen tissue in guinea pig. Another study that supports enzymatic theory came from Yuasa et al. They showed keratin debris of cholesteatoma may demineralization of bone hydroxyapatite due to its acidic pH.[14]

In this study, during electron microscopic evaluation of cholesteatoma tissue, macrophages with plenty of large lysosomes in cytoplasm were observed. Furthermore, swollen mitochondria and many lipid particles were observed in the cytoplasm of macrophages. These findings support the enzymatic theory. The degenerative changes of osteocytes in bone matrix, vacuolar appearance of cytoplasm resulting from swollen endoplasmic reticulum cisternae in the cytoplasm of these cells, lytic regions due to destruction of organelles were also monitored. This shows enzymatic changes due to increase in osteoclastic activity. Cytoplasmic vacuoles may be accepted as the index of enzymes produced by cells.

Additionally, in electron microscopic evaluation, we also demonstrated increase in intercellular space between the cells, irregular cellular surface and amorphous matter accumulation into intercellular space of incus bone samples electron microscopically. Fibrous tissue was increased in bone tissue. In addition, a large amount of capillary vessels were observed. According to the most studies, incus was the most affected ossicle in the middle ear with supurative otitis. Dornellos et al. reported some histopathological change on incus. In their study, they found moderate and several inflammations on incus[15].

Conclusion

In conclusion, pathophysiology of osteonecrosis in COM is not been known clearly yet. The purpose of this studywas to demonstrate electron microscopic cellular changes of bone necrosis developing due to chronic otitis media. In the electron microscopic examination, we observed increased vascularity, vacuolar appearance in monocyte and macrophages, and impaired morphology of cellular elements in the bone tissue samples of cholesteatoma. In the bone samples with granulation, cellular structures showed better morphology, but there were lysosomes and vacuolar appearance in cells. The findings of this study support the osteoclastic enzymatic activity for COM with cholesteatoma. Quantitative enzyme assay is not possible in pathologic tissues, but there are different evidences that support enzymatic activity. The present study which was performed on COM patients may shed light on further studies that will investigate osteonecrosis.

References

1. Chole RA. The molecular biology of bone resorption due to chronic otitis media. Ann New York Acad Sci 1997; 830:95-109.

2. Kurihara A, Toshima M, Yuasa R, Takasaka T. Bone destruction mechanism in chronic otitis media with cholesteatoma: specific production by cholesteatoma tissue in culture of bone resorbing activity attributable to interleukin-1 alpha. Ann. Otol. Rhinol. Laryngol 1991; 100:989-998.

3. Chole AR, McGinn MD, Tinling SP. Pressure-induced bone resorption in the middle ear. Ann Otol Rhinol Laryngol 1985; 94:165-170.

4. Abramson M, Moriyama H, Huang C. Pathogenic factors in bone resorption in cholesteatoma. Acta Otolarygol 1983; 97:437-442.

5. Kirchner FR, Proud GO. Epidermoid cholesteatoma. GP. 1963; 28:97-103.

6. Cummings C, Fredrickson J, Harker L, Krause CJ. Otolaryngology-Head and Neck Surgery. Second Edition. Mosby-year Book, Inc. Missouri, 1993: 4:2823-2832.

7. Chole RA. Cellular and subcellular events of bone resorption in human and experimental cholesteatoma: the role of osteoclasts. Laryngoscope 1984; 94:76–95.

8. Moriyama H, Honda Y, Huang CC, Abramson M. Bone resorption in cholesteatoma: epithelial-mesenchymal cell interaction and collagenase production. Laryngoscope 1987; 97:854–9.

9. Abramson M, Huang CC. Localization of collagenase in human middle ear cholesteatoma. Laryngoscope 1977; 87:771–9.

10. Amar MS, Wishashi HF, Zakhary MM. Clinical and biochemical studies of bone destruction in cholesteatoma. J Laryngol Otol 1996; 110:534–9.

11. Schönermark M, Mester B, Kempf HG, Bläser J, Tschesche H, Lenarz T. Expression of matrix-metalloproteinases and their inhibitors in human cholesteatomas. Acta Otolaryngol (Stockh) 1996; 116:451–6.

12. Schmidt M, Grünsfelder P, Hoppe F. Induction of matrix metalloproteinases in keratinocytes by cholesteatoma debris and granulation tissue extracts. Eur Arch Otorhinolaryngol 2000; 257:425–9.

13. Bujia J, Kim C, Boyle D, Hammer C, Firestein G, Kastenbauer E. Quantitative analysis of interleukin-1-alpha gene expression in middle ear cholesteatoma. Laryngoscope 1996; 106: 217–20.

14. Yuasa R, Kaneko Y, Ise I, Lino Y. Bone destruction due to the rupture of a cholesteatoma sac: a pathogenesis of bone destruction in aural-cholesteatoma. Laryngoscope. 1980; 90:1865-71.

15. Cristina Dornelles, Letícia Petersen Schmidt Rosito, Luíse Meurer, Sady Selaimen da Costa. Hystology findings' correlation between the ossicular chain in the transoperative and cholesteatomas. Rev Bras Otorrinolaringol. 2007; 73:738-43.