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ORIGINAL ARTICLE

An Experimental Investigation into the Effects of Bacteria Exhibiting Acid Phosphatase Activity on Tympanosclerosis Plaques

Huseyin Katılmıs, Hale Aslan, Sedat Ozturkcan, Ilhan Afsar, Banu Senturk, Halil Emre Gogus, Filiz Gulustan

Department of Otorhinolaryngology and Head and Neck Surgery, Izmir-Turkey (HK, HA, SÖ, HG, FG)
Department of Clinical Microbiology Atatürk Research and Training Hospital, Ministry of Health, Izmir-Turkey (İA)
Department of Biochemistry Atatürk Research and Training Hospital, Ministry of Health, Izmir-Turkey (BŞ)

Objective; We aimed to weaken structure of sclerotic plaques in tympanic cavity by using bacteria and fungi that decompose calcium and phosphate in calcium and phosphate crystals.

Study Design; Experimental prospective in-vitro study.

Materials & Methods; We used the sclerotic plaques extracted from 10 patients who had undergone tympanoplasty due to advanced tympanosclerosis. These plaques and ossicles taken from fresh cadavers and approximately 20 mg promontorium bone were kept in separate tubes with E.coli, Bacillus subtilis and Aspergillus niger. These tubes which were also containing a brain\heart broth medium were incubated for two weeks. Calcium and phosphate amounts in the medium were then re-measured after 2 weeks.

Results; We found that calcium and phosphate departed in all groups, particularly in the E. coli group and that the plaque broke down. No change was seen in ossicles.

Conclusion; Naturally existing bacteria and fungi, particularly E. coli, can decompose tympanosclerotic plaques. We observed that no change occurred when we applied the same study on human middle ear ossicles. We believe that the effect of otitis media-causing, acid phosphatase-active bacteria on Ca-P metabolism might shine a light on tympanosclerosis etiology.

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Tympanosclerosis is characterized by homogenous masses formed by intra- and extracellular calcium phosphate crystals distributed in patches as wells as accumulation of collagen fibrils on the tympanic membrane and submucosa of the middle ear [1].

While etiology and pathogenesis are still not entirely understood [1], middle ear infections [2], serous otitis media [3], application of ventilation tubes [1,4], autoimmune deficiencies, and genetic disposition each have a role in etiology [1-6]. Incidence was found to be 11.6%-43% in patients with chronic otitis media [7].

Ear infections are the most prominent causes for tympanosclerosis. Aerobic and anaerobic microorganisms are responsible for half the cases with chronic otitis: 39% are aerobic microorganisms and 11% anaerobic. Among the aerobic microorganisms, P. aeuroginosa, S.aureus and S.pneumonia are the most frequent causes of chronic otitis, while the Bacteriodes group is also considered anaerobic [8]. Patients with tympanosclerosis generally do not complain of ear efflux. Autoscopic examinations generally reveal dry middle ear cavities.

The basic pathology for tympanosclerosis is traced to the sclerotic plaques formed by calcium phosphate crystals stored in submucosa. There is still no treatment for preventing accumulation of these calcium phosphate crystals. There are, though, some bacteria found in nature which can decompose calcium

Corresponding address:
Dr. Hale ASLAN
2084/7 sok.Seyhankent sitesi Nesli Apt F Blok 9.kat D:17
Bayraklı -İzmir/Turkey
Phone: +90 232 2444444 / 2580; Fax: +90 232 2431530; E-mail: drhaleaslan@gmail.com

phosphate crystals and enable use of phosphorus by plants [9]. These bacteria form 1-50% of the natural population in soils [10]. Bacteria exhibit this effect by producing acid phosphatase and are largely in the enterobactericea family. Bacteria in the Rhizobium [11], Serratia, Citrobacter, Proteus, Clebsiella [9], Pseudomonas and Bacillus [12] groups have acid phosphatase activity. E. coli has a phoA gene that regulates this phosphatase activity [13]. It has been determined that Aspergillus niger can produce citric acid and decompose phosphate [14].

In our in vitro study we investigated the effects and solubility of microorganisms with acid phosphatase activity on calcium and phosphate crystals in sclerotic plaques obtained from patients operated on due to tympanosclerosis.

Materials & Methods

Sclerotic plaques were obtained from operations on 10 patients with advanced tympanosclerosis (Wielinga-Kerr type 3-4) and were placed in sterile tubes. The bacteria E.coli and Basillus subtilis were used as well as the fungus Aspergillus niger. 2 tubes were designated for each microorganism and a 5cc brain\heart broth medium was put into each tube to maintain the viability of the bacteria. This medium was dehydrated and included 37.09 1 of distilled water, 27.5g/l of brain extract + heart extract + peptone as well as 2.09 g/l glucose, 5 g/l NaCl and 2.5 gr/l Na2HPO4. Its pH was approximately 7, 4.

About 20 mg of sclerotic plaque was put in each tube. The amount of microorganisms to be cultured in the medium was set at 0.5 McFarland (1x107 CFU/ml bacterium). The mixture was kept at 37 0C in an incubator for 2 weeks. Calcium and phosphate amounts in the medium were reexamined after 2 weeks using a phosphate phosphomolydate method with calcium arsenazo 3 stain reaction in an Abbott Architect C800 autoanalyser (Abbott Lab.USA). Calcium 0.3 mg/l and phosphate 5 mg/l were found in medium content. To investigate the effect of this mixture on middle ear ossicles, malleus + bacillus, incus + E.coli and promontorium bone + Aspergillus niger were taken from fresh cadavers and were put in the tube. The bacteria and medium amounts were kept at the same levels. We investigated amount of calcium and phosphate before and after incubation for each of tubes and results were compared statistically using the Wilcoxon signed ranks test in SPSS 15.0 program . We accepted that $p<0.05$ value is statiscally significant.

Results

The differences in calcium and phosphate levels in each tube were measured following a two week interval (Table 1). After two weeks the presence of calcium was measured at 1.79 mg/l and phosphate was 7 mg/l in the first tube with an E. coli culture. In the second tube, calcium was found to be 1.66 mg/l and phosphate was 14.3 mg/l.

Table 1. Calcium and phosphate amounts in the medium (measured after 2 weeks).

Microorganisms		Calcium (mg/l)	Phosphate (mg/l)
E.coli	1st tube	1.79	7
+ plaque	2nd tube	1.66	14.3
	Mean value	1.725	8.65
B.subtilis	1st tube	0.04	7.9
+ plaque	2nd tube	0.06	7
	Mean value	0.032	7.45
Aspergillus	1st tube	0.13	0
+ plaque	2nd tube	0.26	1.8
	Mean value	0.195	0.9

At the same time, calcium was measured 0.04 mg/l, and phosphate 7.9 mg/l in the first tube with the Bacillus subtilis culture. In the second tube calcium was measured at 0.06 mg/l and phosphate was present at the level of 7.0 mg/l.

In the first tube with the Aspergillus niger culture, calcium amount was 10.13 mg/l while phosphate was not present (as in the medium.) Calcium was measured 0.26 mg/l and phosphate was 1.8 mg/l in the second tube.

We observed that the calcium and phosphate levels remained unchanged (that is, they did not differ from the medium) in the tubes into which we placed malleus, incus and promontorium bones.

The mean of two tubes (one mean for each bacterium) was calculated for statistical analysis. The calcium rate was 1.725 in group 1 (E. coli), 0.032 in group 2 (B. subtilis) and 0.195 in group 3 (A.niger). When these 3 groups were compared with each other in terms of calcium rates, differences between the E. coli and B. subtilis groups and also between the E. coli and A. niger groups were statistically significant (p=0.031 , p=0.032) but not those between B.subtilis and the A. niger groups (p=:0.839). (Table 2)

The PO4 rate was 8.65 for group 1, 7.45 for group 2 and 0.9 for the group 3. When the PO4 rates of these three groups were compared with one another, difference between groups 1 and 2 was statistically significant (p=0.044). The differences were also significant between groups 1 and 3 (p=0.043) and groups 2 and 3 (p=0.043). (Table 2)

Discussion

Tympanosclerosis is a complication of chronic otitis media in which acelluler hyaline and calcified deposits accumulate within the tympanic membrane and the submucosa of the middle ear [8]. Studies are being conducted concerning the prevention of dystrophic calcifications using calcium antagonists, membrane stabilizers, corticosteroids and prostaglandins taking advantage of their anti-inflammatory effects [15].

Mann et al. created tympanosclerosis experimentally by infecting the ears of rats with S. pyogenes and intoxicating them with IV vitamin D3. They then treated the condition with a calcium antagonist administration. Finally, they observed that there was a positive effect on secondary calcification in the group treated with calcium antagonists [16].

Brodie et al. administered dexamethazon to mice with dysplastic bone lesion disease. The disease manifested around bone ossicles, otic capsules with a histology similar to tympanosclerosis in humans. As a result, they observed that less dysplastic bone lesions developed compared to the control group in mice [17].

In an experimental study conducted with albino guinea pigs, Adin et al. found that the development of tympanosclerosis was more evident in the ears in which a topical calcium channel blocker were not dropped. Their findings supported the hypothesis that calcium channel blockers have a preventive effect on tympanosclerosis [18].

Bacteria with acid phosphatase activity are not among the frequently encountered pathogens in chronic otitis media. Pajor et al. took efflux samples from 274 ears with chronic otitis media and found the presence of bacteria at the following rates: 42.9% S. aureus, 19.8% P. aeruginosa, less frequently 4.1% Acinetobacter spp, 3.7% P. Mirabilis, 3.3% P.vulgaris, and 1.5% metycilline resistant S. aureus. Among the patients with a fungal agent, 37.1% had aspergillus spp and 22.9% had C. albicans [19]. In a study conducted by Gul et al., 70 patients with chronic otitis media were shown to have various bacteria present at the following rates: 23% P. aeuroginosa, 18% S. aureus and 17% proteus spp [20].

Table 2. Statistical analysis of Ca and PO4 among the groups.

	Groups 1-2	Groups 1-3	Groups 2-3
Ca	p=0.031	p=0.032	p=0.839
PO4	p=0.044	p=0.043	p=0.043

In light of the literature, we can say the microorganisms that give rise to chronic and acute otitis media (H.influenza, S.pneumonia, P.aeruginosa, S.aureus) also show acid phosphatase activity, but the effects of these microorganisms on Ca-P metabolism have not been investigated [21, 22].

Ten patients receiving surgery at our clinic were found not to have infections at all. The lack of tymanosclerotic plaques in infected ears and the lack of infection in ears with tympanosclerosis are striking. This raises the question of whether or not these conditions lead to a reduction of microorganisms, causing an over-accumulation of calcium phosphate crystals. We did not find any studies conducted on middle ear microbiology in tympanosclerotic ears . Therefore, there is need for further studies on the microbial flora in tympanosclerotic ears.

In this study, we aimed to weaken the structure of sclerotic plaques by using bacteria and fungi found in nature that decompose calcium and phosphate in calcium and phosphate crystals. Naturally existing bacteria and fungi, particularly E. coli, can decompose tympanosclerotic plaques. Each sclerotic plaque can be in a different stage and also can have a different Ca and P04 amount. Fort his reason and also not to do a mistake, we used 2 test tubes for each bacteria and took the mean values. We observed that no change occurred when we applied the same study on human middle ear ossicles. The reason why there was no change in ossicles which were in tube was; there was no dystrophical calcification, physiological one and they did not have Ca and P04 only. We believe that the effect of otitis media-causing, acid phosphatase-active bacteria on Ca-P metabolism might shine a light on tympanosclerosis etiology.

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