The Journal of Laryngology and Otology
February 1994, Vol. **108**, pp. 113–115

Solubilization of keratin debris in conservative treatment of middle ear cholesteatoma: an *in vitro* study

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Abstract

A variety of solutions were tested *in vitro* to find a suitable solvent which could be used in clinical practice for cholesteatoma debris. Though a little weak as a solvent, a liquid soap composed mainly of plant oil did not cause irritation of the middle ear mucosa, and was thought to be a promising solvent with which to rinse away tenacious debris, especially when used in combination with hydrogen peroxide.

Key word: Cholesteatoma

Introduction

Although middle ear cholesteatoma is properly treated only by surgical procedures, the advantages of conservative treatment cannot be underestimated.Use of appropriate antibiotics and removal of the contents in the cholesteatoma sac may hinder progress of the disease and thereby prevent complications. Such procedures are inevitable especially when surgery is contraindicated due to very advanced age or to poor general condition. Although rare, conservative treatment can cure some types of small cholesteatoma (Georgopoulos, 1989). Removal of keratin debris may be a key procedure in conservative treatment, since the debris offers a favourable culture medium for various bacteria and its accumulation causes destruction of the surrounding bones (Ruedi, 1957; Orisk and Chole, 1987). This is usually accomplished by the use of a microsurgical pick, curette and forceps, together with irrigation–suction. However, the removal often turns out to be incomplete because of difficulties in the approach due to anatomical structure, especially when a cholesteatoma extends deep into the mastoid cavity. In such a situation, the use of a suitable detergent to loosen the debris will facilitate its removal. The purpose of this study was to evaluate the efficacy of various solutions *in vitro* with which to dissolve the debris.

Materials and methods

Cholesteatoma debris was removed during surgery from the otitis media of 30 patients with cholesteatoma. The debris was divided into pieces and approximately 20 mg (wet weight) was placed in separate test tubes. The debris in each tube was then mixed with 2 ml of one of the test solutions and incubated at 37°C for 48 hours. Debris from one patient was sufficient for three to seven test solutions. As there was individual variation in solubility of the debris, at least three specimens, from different origins, were tested for each solution.

Various solutions were used for example physiological saline, 1 N hydrochloric acid, 1 N sodium hydroxide, 30 per cent hydrogen peroxide, 8 N urea, 20 per cent acetylcysteine, 1 per cent chymotrypsin, etc. Protein denaturants such as 0.1 M sodium dodecyl sulphate, 0.1 M cholic acid and 0.1 M diiodosalicylic acid, were included in the study. A kitchen detergent (Cherina®, 50 per cent), a liquid soap composed mainly of rape oil and palm oil (Sekken®: 0.01, 0.1, 1 per cent) and a laundry synthetic detergent which contains anionic interfacial active agents and a proteolytic enzyme (Hi-Top®: 1 per cent) were also tested. The concentration of each test solution was fairly high, since the aim of this study was to evaluate the possible effectiveness of various reagents and the experiments were therefore conducted *in vitro*.

The solubility of the debris was estimated after 48 hours of immersion in the test solution. It was classified as: (1) none; (2) weak; (3) moderate; and (4) strong. Strong was judged to be when more than two-thirds of the debris was dissolved; moderate was indicated when the solubility was between one third and two thirds; weak was defined when it was below one third.

Results

Figure 1 shows a comparison of the solubility of debris, from the same origin, in three different test solutions. Solubility was very strong in 1 per cent Hi-Top®, weak in 8 N urea and not demonstrated in physiological saline.

Results of the present study are summarized in Table I. Hydrochloric acid, sodium hydroxide and another seven test solutions had no substantial solvent effect on the debris. Hydrogen peroxide made the debris swell but could not dissolve it. Urea, acetylcysteine and another

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Presented at the Fourth International Conference on Cholesteatoma and Mastoid Surgery (Niigata, Japan), September 9, 1992.
Accepted for publication: 19 August 1993.

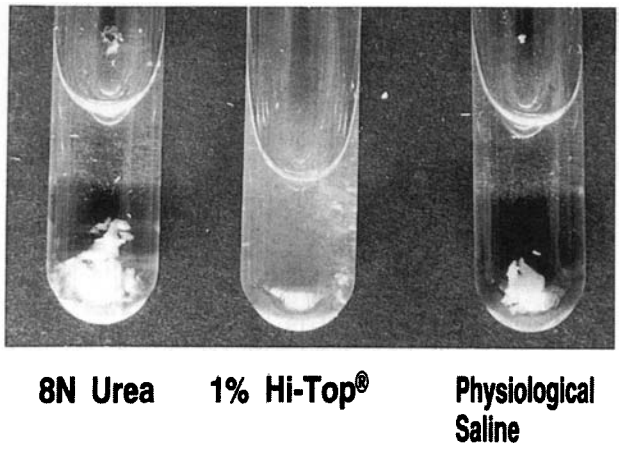

FIG. 1

Comparison of solubility of keratin debris in three representative test solutions. The results were judged as very strong in Hi-Top®, weak in urea and none at all in physiological saline.

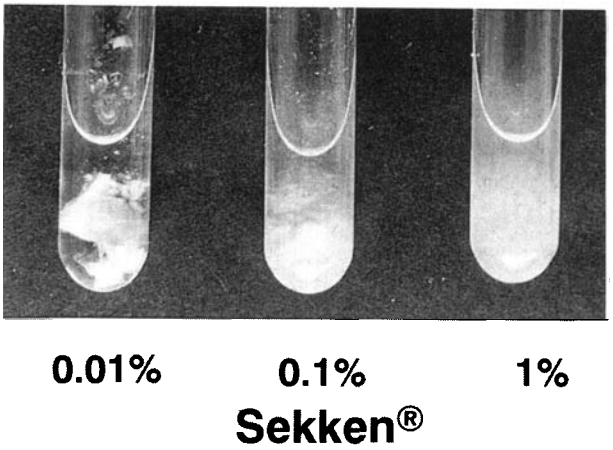

FIG. 2

Solubility of the debris in liquid soap (Sekken®) at three different concentrations.

four test solutions had a weak solvent effect. Protein denaturants and liquid soap exhibited a stronger effect when compared to the previous solutions. However laundry detergent, nitric acid and formic acid proved to be very effective in dissolving the debris, although it was apparent that nitric acid and formic acid were too strong to be used in clinical practice.

Solubility in liquid soap was tested at three different levels of concentration. It was moderate at 1 per cent (pH 10.5), weak at 0.1 per cent (pH 9.2) and at 0.01 per cent (pH 8.1), (see Figure 2).

Discussion

Cholesteatoma debris is composed mainly of soft keratin which is derived from desquamated epithelium. Keratin is a fibrous protein with a rich cysteine residue and is very resistant to various solvents. This feature is due to the biochemical conformations of keratin which have the following characteristics: (a) richness in disulphide cross bridges, (b) an alpha-helix structure which allows intrachain hydrogen bonding between each peptide, and (c) richness in water insoluble alkyl bases which are arranged facing the outside of the alpha-helix structure. According to Lehninger (1975), keratin can be dissolved by strong acid or alkali, or by proteolytic enzymes after treatment with a protein denaturant. However, most of these types of reagents cannot be applied in clinical cases because they cause severe irritation of the live tissues.

A suitable solvent for use in conservative treatment must fit the following criteria: (1) dissolve the accumulated debris for easy removal, hopefully in a short period of time; (2) facilitate exfoliation of the desquamated layer of cholesteatoma; (3) not irritate the ear canal skin or the middle ear mucosa; and (4) not damage the inner ear function when entering the middle ear. At present, such an ideal reagent or combination of reagents has not been found.

In our outpatient clinic, irrigation of the ear is usually performed using warm saline or acrinol solution. However, complete removal of the debris is often difficult, since these solutions have no loosening effect on debris. Use of hydrogen peroxide is recommended to loosen the debris, as it is also useful in the removal of crust and cerumen due to the action of the oxygen-free radical (Goodhill, 1979). Acetylcysteine, urea, and *N*-(2-mercaptopropionyl)-glycine have also been reported as effective in dissolving the keratin debris (Kluyskens *et al.*, 1979, 1981). Some of these reagents were tested in this study and found to be weak in their effects.

Three solutions, however, were very effective in dissolving the debris but it is apparent that nitric acid and formic acid are too strong to be utilized in clinical practice. According to our preliminary experiment using guinea pigs, the laundry detergent caused severe inflammation of the middle ear mucosa when injected into the otic bulla. Solutions which showed a moderate dissolving effect such as cholic acid, diiodosalicyltic acid and sodium dodecyl sulphate, also caused middle ear inflammation. The liquid soap composed solely of plant oil (Sekken®) did not cause such severe irritation, and may be suitable

TABLE I

SOLUBILITY OF THE DEBRIS IN VARIOUS SOLUTIONS

None	Weak	Moderate	Strong
Physiological saline	Urea (8 M)	Cholic acid (0.1 M)	Hi-Top® (1%)***
Hydrochloric acid (1 N)	Acetylcysteine (20%)	Diiodosalicylic acid (0.1 M)	Nitric acid (69%)
Acetic acid (99.5%)	Chymotrypsin (1%)	Sodium dodecyl sulphate (0.1%)	Formic acid (90%)
Trichloroacetic acid (1 N)	Protease (2%)	Sekken® (1%)**	
Sodium hydroxide (1 N)	Waxnate® (100%)†		
Toluene (99.9%)	Cherina® (50%)‡		
Benzene (99.5%)			
Michalon® (50%)*			
Hydrogen peroxide (30%)			

*Dandruff shampoo; †ear wax solvent; ‡kitchen detergent; ** body shampoo; ***laundry detergent.

https://doi.org/10.1017/S0022215100126040 Published online by Cambridge University Press

for use in removing the debris. The liquid soap is alkaline and can neutralize organic acids produced by microorganisms in the cholesteatoma sac. These organic acids are thought to be partly responsible for bone destruction (Iino *et al.*, 1982).

We attempted to use diluted liquid soap (0.01 per cent) for irrigation in two patients with attic retraction cholesteatoma and wide opening of the sac. Their chief complaints were continuous discharge with bad odour and that hearing in the involved ears was completely lost. Before irrigation, the tympanic membrane was carefully observed under an operating microscope to ensure absence of a perforation. After removal of the visible debris in the pocket, irrigation–suction of that area was carried out using hydrogen peroxide in order to make the invisible debris loosen and swell. Then, the pocket was irrigated with the liquid soap several times, followed by irrigation with physiological saline for safety. Local ablation using a curved microsurgical pick during irrigation facilitated removal of the desquamated layer of debris. Residue of the debris was checked by use of a rigid endoscope. These procedures, together with the use of antibiotics for control of infection, have proved effective in controlling cholesteatoma in these patients.

Although removal of the keratin debris alone cannot cure cholesteatoma, importance of a conservative treatment should not be underestimated in order to prevent aggravation of the disease. So far as we know, both experimental and clinical data concerning solvents which can be applied safely in the ear to dissolve cholesteatoma debris are still lacking. Any advance in this field will be an advantage in the conservative treatment of cholesteatoma.

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