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Antimicrobial activity of cerumen in the ear: Article review

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Abstract
Introduction: Cerumen contains various antimicrobial factors which exhibit a wide spectrum of activity against various pathogenic bacteria and form a complex of innate biochemical protection. Antimicrobial peptides are thought to have an important role in defense against infection.
Objective: to know the antimicrobial activity of ear ear wax.
Methods: The literature study used *in vivo, in vitro,* in silico research articles, and literature review articles from national and international journals accessed from Google Scholar, Elsevier, Science Direct, and Pubmed using the keywords antimicrobial, cerumen, ear. We used 13 relevant articles in this literature review.
Results: The natural defenses of cerumen involve innate immunity including a large number of antimicrobial peptides and proteins. These antimicrobial peptides and proteins include human B-Defensin (hBD), LL-37, dermcidin, lysoferrin, lactoferrin, secretory secretory leukocyte proteases (SLPI, antileukoprotease), and alpha 1-antitrypsin. These proteins and peptides exhibit broad spectrum activity against various microorganisms.
Conclusion: The peptides and proteins contained in cerumen show broad spectrum activity against various microorganisms.

Keywords: antimicrobial, wax, ear

Introduction

Cerumen or ear wax is a mixture of secretions from the sebaceous (fat) and apocrine (ceruminous) glands with epithelial debris. This combination of these compositions forms an acidic layer (normal pH of cerumen 6.8) which helps prevent ear canal infections. The shape and consistency of cerumen differs genetically and racially. It is thought to be related to the content of immunoglobulins and lysozyme. The ear canal has the ability to self-cleansing by migrating the epithelium from the tympanic membrane out through the ear canal. Cerumen is removed from the ear by a natural mechanism of ear cleaning in the form of epithelial migration of the umbo of the tympanic membrane laterally. Jaw movement when chewing helps the process of removing cerumen from the external auditory canal (Pivi M et al., 2018) [10].

Cerumen (ear wax) is produced by the sebaceous glands, ceruminous glands and apocrine glands located in the lateral third of the human ear canal. Two forms of cerumen were identified as 'wet' and 'dry'. Both forms are related to race and controlled by two autosomal alleles. The wet phenotype is dominant and the dry is recessive. The mongoloid population has a dry phenotype, whereas the wet is common among the Caucasian and African. Cerumen is believed to protect the external ear canal against infection. Apart from providing a physical barrier against infection, it is believed that cerumen has antibacterial and antifungal properties.

Cerumen serves to lubricate the external auditory canal and trap dust with the small particles and other insects, thereby preventing them from reaching and damaging the eardrum. Cerumen also provides protection from infecting bacteria such as bacteria and fungi, as well as antimicrobial activity caused by fatty acids, lysosomes, and acidity of cerumen (Adegbiji WA, et al., 2014) [1].

Cerumen contains various antimicrobial factors that exhibit a broad spectrum of activity against various bacterial pathogens and form an innate biochemical protective complex. Antimicrobial peptides are considered to have an important role in defense against infection. Natural defenses involving innate immunity include a large number of antimicrobial peptides and proteins. These antimicrobial peptides and proteins consisted of human B-Defensin (hBD), LL-37, dermcidin, lysoferrin, lactoferrin, and leukocyte secretory proteases (SLPI, antileukoprotease), and alpha 1-antitrypsin. These proteins and peptides exhibit broad-spectrum activity against a wide range of microorganisms, including bacteria, fungi, viruses, yeasts, and protozoa (Yong JY et al., 2008) [13]. The most common microorganisms found in the human ear are Staphylococcus aureus (24.6%) and Pseudomonas (9.0%) (Naqi SA, 2016) [9].

Methods

The literature study used research articles *in vivo*, *in vitro*, in silico, and literature review articles from national and international journals accessed from Google Scholar, Elsevier, Science Direct, and Pubmed using the keywords antimicrobial, cerumen, and ear. We used 13 relevant articles in this literature review.

Discussion

Cerumen

There are two types of cerumen: the wet type which can be found in Caucasians, Africans and brown or dark skin. The dry type is most commonly found in Asia, Native Americans and people who have a grey skin tone. These genetically determined differences in earwax are even used to track human migration patterns. The genetic background is a single nucleotide change in the "ATP-binding cassette C11" gene. The wet type is dominant, while the dry type is recessive (Matthias S, 2009) [7].

Cerumen is made up of over 40 different substances, not only consisting of waxy substances and oils but also dead skin cells and keratin, which is a highly fibrous protein substance found in the outermost layer of the skin. Cerumen also consists of a mixture of secretion of a viscous substance from the sebaceous glands and secretion of a less viscously substance from modified ceruminous glands (Badwe Ravi, 2013) [11].

Earwax consists of squalene (6.4%), cholesterol ester (9.6%), wax ester (9.3%), triacylglycerol (3.0%), fatty acids (22.7%), cholesterol (20.9%), cholesterol (20.6%), ceramides (18.6%), cholesterol sulfate (2.0%), and some unknown polar components (7.5%) (Matthias S, 2009) [7]. The fat fraction consisted of 52% of the dry weight of cerumen and consisted of squalene (6.4%), cholesterol esters (9.6%), wax esters (9.3%), triacylglycerols (3.0%), fatty acids (22.7%), cholesterol (20.9%), ceramides (18.6%), cholesterol sulfate (2.0%), and some unknown polar components (7.5%). In addition to the extractable lipids, the residue contains an additional 0.9% lipid which can be released after saponification. This covalently bonded lipid consists of two unusual ceramides (63.4%), w-hydroxy acids (27.7%) and non-hydroxy fatty acids (8.8%). The composition of this bound lipid resembles that recently found in the human stratum corneum, which is thought to consist of a lipid envelope on the outer surface of the cytocyte. Free and covalently bound lipids can be significant determinants of cerumen properties. Desquamation of corneocytes and associated lipids from the epidermal layer of the ear canal can make a major contribution to cerumen (Jeffrey TB et al, 1990) [6].

There is some evidence of genetic polymorphisms in cerumen phenotypes. Current evidence classifies cerumen into two phenotypes: wet and dry. Wet cerumen, which is light or dark brown and sticky, is characterized by relatively high concentration of lipid granules and pigments. Dry cerumen, which is grey or brown in colour and brittle, tends to exhibit lower levels of this component. For example, dry cerumen contains about 20% lipid, compared to about 50% in wet cerumen. Moreover, both forms show some other biochemical differences. Both forms are related to race and controlled by two autosomal alleles (Gupta S et al., 2012) [5].

Cerumen acts to lubricate the external auditory canal and traps dust with small particles and other insects, thereby preventing them from reaching and damaging the eardrum. Cerumen also protects from infecting bacteria such as bacteria and fungi, as well as antimicrobial activity caused by fatty acids, lysosomes, and the acidity of cerumen. (Adegbiji WA et al., 2014) [1] Freshly collected cerumen (dry form) suspended at a concentration of 3% in glycerol-sodium bicarbonate buffer exhibits bactericidal activity against several strains of bacteria tested. This suspension reduced the viability of Haemophilus influenza, Escherichia coli K-12, and Serratia marcescens by more than 99%, while the viability of two isolates of Pseudomonas aeruginosa, E. coli K-1, Streptococcus, and two isolates of Staphylococcus aureus of human origin were reduced by 30 to 80%. The results support the hypothesis that cerumen functions to kill certain foreign organisms which enter the ear canal (Chai TJ et al., 1980) [2]. Cerumen has the main function of moisturizing, cleaning, lubricating and protecting the skin of the human ear canal, while acting as an antibacterial to maintain an acidic environment in the ear canal and a barrier against foreign substances such as water, insects and dust. In addition, it can provide important information about individuals including race, ethnicity, gender, disease, food eaten and exposure to environmental pollutants around them (Engy S, 2017) [4].

Human Antimicrobial Proteins in Cerumen

Antimicrobial peptide (AMP)

In general, surfaces such as the skin or oral mucosa are protected by several factors including the innate and adaptive immune systems, against invading bacteria and fungi. The antimicrobial mucosal barrier in the human airway has been the subject of several studies and is well-investigated. AMP has a broad but not identical spectrum of antimicrobial activity against bacteria, viruses, or fungi. Synergistic and additive effects of different AMPs have been reported. In addition to their antimicrobial characteristics, AMPs play a major role in inflammation, immune activation, and wound healing. Several well-described AMPs were subject to this study and will therefore be described further (Schwaab *et al*., 2011) [8].

Human ß-Defensins (hBD)

Human ß-Defensins (hBD) are named according to their beta-sheet structure which is stabilized by intramolecular disulfide bonds. Human ß-Defensin 1 (hBD1) was first isolated from hemofiltrates and expressed in epithelial cells of the urinary tract, respiratory tract, and keratinocytes. Human ß-Defensin has a strong antimicrobial effect on gram-negative bacteria. Human ß-Defensin 2 (hBD2) was induced by *Escherichia coli, Pseudomonas aeruginosa, Helicobacter pylori, Lipoprotienacid*, Candida-Necrosis-Factor-α, and Interleucine 1-ß. Peptides have strong antimicrobial effect on *Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans*, while relatively weak on *Staphylococcus aureus*. Human ß-Defensin 3 (hBD3) can be induced by tumour necrosis factor and contact with *Pseudomonas aeruginosa* or *Staphylococcus aureus*, e.g. in keratinocytes. HBD3 has antimicrobial activity against *Staphylococcus aureus*, including *Methicillin*

Staphylococcus aureus (MRSA), *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Escherichia coli, Enterococcus faecium*, *Candida albicus influenza*, and *Candida albicans* (Schwaab *et al*., 2011) [8].
Human ß-defensin-1 (hBD-1) and human ß-defensin-2 (hBD-2) are antimicrobial peptides present in cerumen. It consists of epithelial keratin and exfoliated glandular secretions and may provide the first line of defence against microbes in the skin of the external auditory canal (EAC) (Yong JY *et al*., 2008) [13].

Human LL-37 (LL-37)
LL-37 is a long 37-amino acid C-terminus and the antimicrobial active component of the human antimicrobial peptide Cathelicidin 18 (hCAP-18). The compound is expressed in leukocytes (monocytes, neutrophils, T-cells, B-cells), epithelial cells (skin, digestive tract and respiratory tract) and is secreted into wound surface fluids and airways. LL-37 alone and in combination with other AMPs is bactericidal against a broad spectrum of Gram-positive and Gram-negative bacteria. Apart from antimicrobial activity, LL-37 plays a role in angiogenesis, cancer development, neutralization of bacterial lipopolysaccharide and is chemotactic for human monocytes, neutrophils and CD4 T lymphocytes (Schwaab *et al*., 2011) [8].

Human Secretory Leucoprotease Inhibitor (hSLPI)
hSLPI is an 11.7-kDa heavy protein expressed in macrophages, neutrophils, and epithelial cells. The antimicrobial activity against Gram-negative and Gram-positive bacteria lies in the N-terminal domain of the protein. hSLPI inhibits human immunodeficiency virus (HIV) infection by blocking viral DNA synthesis (Schwaab *et al*., 2011) [8].

Human Bactericidal / Permeability-Increasing Protein (BPI)
Human bactericidal/permeability-increasing protein (BPI) is a 55 kD heavy single-chain cationic protein that can be divided by proteolysis into two fragments with antibiotic and endotoxin-trapping functions in the N-terminal fragment. BPI is mainly found in neutrophil granules but also detected in dermal fibroblasts and excretory lacrimal gland ducts. BPI has strong antimicrobial potential and selectivity against Gram-negative bacteria. Von der Mohlen *et al*. could suggest that BPI has a significant protective effect on meningococcal sepsis. Several phase I and II studies have addressed the pharmacokinetics and possible indications of recombinant fragments of BPI, but have not led to an approved clinical application (Schwaab *et al*., 2011) [8].

Human Lactoferrin (Lfc)
Human lactoferrin (Lfc) can be found in tears, saliva, milk, neutrophil granulocytes, salivary glands and nasal mucosa. Its antimicrobial spectrum includes *Streptococcus mutans, Vibrio cholerae, Escherichia coli, Actinobacillus actinomycetemcomitans*, *Legionella pneumophila, Enterobacteriaceae, Candida albicans* and *Pseudomonas aeruginosa* (Schwaab *et al*., 2011) [8].

Human Neutrophil Peptides 1-3 (HNP1-3)
Human Neutrophil Peptides 1-3 (HNP1-3) belong to the α-Defensin family and were originally isolated from the azurophilic granules of neutrophil granulocytes. Apart from the broad spectrum of antimicrobial activity, HNP1-3 plays a role in the inflammatory regulation and immunological processes that affect complement activation, cytotoxicity, chemotaxis of immature dendritic cells, T cells and monocytes, enhancement of immune responses and wound repair (Schwaab *et al*., 2011) [8].

External Auditory Canal Microbiology
In the initial study, Staphylococcus was found to be the predominant organism (69% Senturia and 56% Perry), followed by coryneform (diphtheroids) being second most common organism isolated. Although taxonomy has changed since the 40s and 50s, and coryneform was not identified at the genus or species level, the results of these previous studies are consistent with more recent literature (David WS et al., 2001).
In David WS's study, one hundred and sixty-four subjects were bred. Seventeen canals and 16 cerumen specimens showed no growth. One hundred and forty-eight cerumen specimens yielded 314 organisms, including 23 fungi. One hundred and forty-seven canal specimens yielded 310 organisms, including seven fungi. Of the 291 bacteria isolated from cerumen, 99% were Gram-positive. Of the 302 bacteria isolated from the canals, 96% were Gram-positive. Staphylococci are 63% of cerumen bacteria and tract bacteria. Coryneforms represented 22% of bacteria in the cerumen and 19% in the canals. Turicella otitidis is the primary coryneform form isolated from the canal and cerumen. Streptococcilike bacteria are 10% of cerumen with 7% of canals (David WS et al., 2001).
The Naqi study showed that the proportion of pathogenic bacteria was significantly (p-value = 0.000) higher in the case of acute otitis externa patients (44% vs 8.57%) compared to normal healthy patients (Table 1). There was a significant (p-value <0.05) gram-negative bacteria was higher in cases of otitis externa compared to the healthy control group. The most common microorganisms found in the patient group were Staphylococcus aureus (24.6%), Bacillus (21.2%) and Pseudomonas (9.0%). Meanwhile, Staphylococcus epidermidis (41.5%), Diphtheroids (24.7%) and Streptococcus (19.5%) were microorganisms commonly found in the control group (Naqi, 2016) [9].
In the study by Vaghela, 115 ear discharge samples were examined for the presence of microorganisms. Of the 115, 93 (80.86%) samples were positive for microorganism growth and 22 (19.13%) samples were sterile. Of the

93% positive samples, 61 (65.59%) samples were pure bacterial growth, 8 (8.60%) samples showed pure fungal growth and 24 (25.80%) showed mixed growth of both antifungal bacteria (Vaghela MM, 2016) [12].

Conclusion

The peptides and proteins contained in cerumen exhibit broad-spectrum activity against various microorganisms.

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