Expression of heme oxygenase 2 in middle ear cholesteatoma

Έκφραση της αιμοξυγενάσης 2 στο χολοστεάτωμα του μέσου ωτός

ABSTRACT

Introduction: Heme oxygenase 2 (HO2) is a constitutively expressed enzyme, localized in different tissues acting as an endogenous protector. The study aims to disclose the eventual presence of HO2 in middle ear cholesteatoma (MECh) as a hyperproliferative tissue with significant clinical complications.

Material and methods: Five human cholesteatoma tissue samples and the corresponding meatal skin were harvested during surgery and submitted further to immunohistochemistry with avidin-biotin affinity method using polyclonal monospecific anti-human HO2 antiserum.

Results: In MECh tissue samples HO2-positive reaction was detected mainly in the suprabasal layers of the hyperproliferative cholesteatoma matrix. Stronger positive staining was observed in MECh samples obtained from the clinical cases associated with extensive bone destruction. The meatal skin samples displayed weaker HO2-reactivity in comparison with the corresponding cholesteatoma samples.

Conclusions: The presence of possible endogenous antioxidant system in MECh favors the autonomous pattern of development of cholesteatoma, supporting the notion that the MECh is rather well defined pathological tissue than simple form of chronic suppurative otitis media.

Key words: Heme oxygenase 2, cholesteatoma, middle ear, immunohistochemistry.
in the middle ear cleft. Paradoxically, regardless these unfavorable conditions the cholesteatoma tissue grows undisturbed and leads to extensive bone destructive lesions in middle ear. Consequently, there is a putative “protective” system of MECh. The potential adaptive effect of neo-angiogenesis, accompanying cholesteatoma development, has been already demonstrated.4,5 In this study we are looking for the presence of another possible component this feasible system – heme oxygenase 2.

MATERIAL & METHODS
We studied 5 MECh and the paired external auditory canal epithelium from deep meatal skin, harvested during surgery upon written consent. Tissue samples were further processed in neutral buffered formalin and subsequently dehydrated in graded ethanols, cleared in xylene and embedded in paraffin blocks. 5 μm thick tissue sections were submitted to blocking of endogenous peroxidase with 3% hydrogen peroxide in methanol for 5 minutes at room temperature (RT). Nonspecific staining was blocked with 10% inactive normal goat serum in PBS for 1 hour at RT. Next samples were incubated with polyclonal monospecific goat anti-human HO2 antiserum (StressGen Biotechnologies corp.) in dilution 1:800 at 4°C, overnight (ON). The first antibody was detected with marked with biotin secondary anti-goat IgG (Binding Site) in concentration 1:1000, incubated ON at 4°C. Next, tissue samples were incubated with Streptavidine-peroxidase (Binding Site) in concentration of 1:1000 for 1 hour at RT and the peroxidase activity was visualized with diaminobenzidine solution (DAB, Sigma). As a negative control replacement of the primary antibody with 10% goat serum in PBS has been used.

RESULTS
At magnification x 400, strongly positive HO2-reactivity was detected predominantly in the suprabasal layers of cholesteatoma samples (Fig. 1A). The matching negative controls show lack of specific staining (Fig. 1B).

The intensity of HO2 positive reaction in meatal skin epithelium was weaker in comparison with the paired cholesteatoma samples (Fig. 2A). HO2-staining is stronger in the suprabasal layers. Elongation and enlargement of the cellular nuclei in the basal layers of deep meatal skin epithelium adjacent to the tympanic membrane were established. The following results probably are consistent with the increased proliferative capacity of the keratinocytes in the basal layers, resulting in relatively stronger HO2 specific immunoreactivity.

A papillary proliferation from the basic layers of the cholesteatoma matrix into the underlying perimatrix granulation tissue was detected in MECh samples, clinically associated with extensive bone destructive lesions. Stronger HO2-reactivity and comparatively wider area with specific immunohistochemical reaction (Fig. 3A) was observed in MECh samples with “aggressive” behavior in comparison with cases associated with limited destructive alterations (Fig. 3B). The established differences in staining patterns could be connected with the different rate of proliferation and differentiation of the cholesteatoma epithelium.

DISCUSSION
In humans heme oxygenase is a ubiquitous enzyme which exists in 2 isoforms - HO1 (inducible) and HO2 (constitutive), characterized by different tissue localization.6 HO system regulates catabolism of the heme ring to ferrous iron, carbon monoxide (CO) and biliverdin, in the presence of NADPH cytochrome P450 reductase (CPR).7 Subsequently, biliverdin is reduced to bilirubin (Bilrb) by biliverdin reductase.6 Bilrb is assumed to be the most abundant endogenous antioxidant in mammalian tissues,9 decreasing the risk for coronary diseases and acts as a cytoprotector in the nervous system.10 Potential vasodilatory effect of CO has been hypothesized in glomus cells and cerebral vascular smooth muscle cells during hypoxia.11-13 Furthermore, heme oxygenase has been hypothesized to be an important factor in regulation of angiogenesis.14,15
According to the Wittmaack's invagination theory, the formation of retraction pocket of tympanic membrane is a prerequisite for middle ear cholesteatoma formation. From the modern perspective, a combination between preliminary developed retraction pocket and further proliferation of the basal cells from cholesteatoma matrix into the underlying perimatrix granulation tissue, gives a plausible explanation of pathogenesis of MECh.16 Due to dysfunction of Eustachian tube a negative pressure is formed in the middle ear cavity. In the presence of hypoxia different cytokines has been released from cholesteatoma tissue stimulating neo-angiogenesis as an adaptive cell response.17 The hypoxia-induced synthesis of hepatocyte growth factor (HGF) in in-vitro condition has been hypothesized to be functionally related with activity of another angiogenic factor – fibroblast growth factor-2 (FGF-2), released by cell culture derived from external auditory canal keratinocytes.18

In favor of the concept that hypoxia triggers cholesteatoma development witnesses the over expression of hypoxia-inducible factor 1 alpha (HIF-1α) and Von Hippel Lindau protein (VHL) in MECh in comparison with middle ear mucosa and normal skin.19 On the other hand, the HIF-1 α activity is presumably associated with another hypoxia-related factor in middle ear cholesteatoma - inducible Nitric Oxide Synthase (iNOS).20 The biological effects of nitric oxide (NO) in middle ear cholesteatoma are related to vasodilatation and bone destruction.21 We assume that NO released during the chronic inflammation in middle ear, could promote vasodilatation in the mucosal microcirculatory network, providing per diapedezem a blood as a substrate for heme oxygenase 2 in cholesteatoma keratinocytes. The presence of Bilrb antioxidant system in cholesteatoma tissue could be associated with neutralization of free radicals released during the bone destruction and inflammation in middle ear cavity. The CO produced by heme catabolism could further stimulate vasodilatation, while the heme oxygenase induces neo-angiogenesis in the perimatrix granulation tissue.

CONCLUSION
The presence of HO2 specific reactivity in middle ear cholesteatoma epithelium could be associated with anti-oxidative properties, as well as putative modulating effects of heme oxygenase enzymes on the proliferation and differentiation, providing adaptive regulatory outcome on the growth and clinical behavior of MECh tissue.

REFERENCES