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Calcium deposition and expression of bone modelling markers in the tympanic membrane following acute otitis media

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Summary

Background and objectives In accordance with clinical findings, myringosclerosis develops after otitis media (OM) and paracentesis in an experimental setting. The pathogenesis of this phenomenon of calcification is poorly understood. As the calcification process and the sclerotic plaques of the drum mimics features of bone tissue, this study explores tympanic membrane calcium deposition in association with the expression of three bone modelling markers: osteopontin (OPN), osteoprotegerin (OPG) and osteonectin (ON). OPN is secreted by osteoblasts and is found at calcification sites, e.g. during pathological calcification in chronic OM. The cytokine OPG is an inhibitor of bone resorption and consequently bone remodelling. ON is a calcium binding glycoprotein necessary for the maintenance of bone mass and remodelling. It is found in bone matrix and synthesized by osteoblasts.
Method A rat model of acute otitis media (AOM) caused by non-typeable *Haemophilus influenzae* was used. Four days following middle ear inoculation, a myringotomy was performed in six animals. Another group of ten animals was inoculated only. The drum was dissected in two animals from each group on day 4, 7, 14 and 28 post-inoculation, and the expression of OPN, OPG and ON was determined by immunohistochemistry. von Kossa staining determined the deposition of calcium and immune staining for CD68 identified macrophages.
Results Calcium depositions were initially accumulated in the cytoplasm of macrophages and dispersed in the connective tissue layers of the pars flaccida and tensa. Late accumulation occurred in the lamina propria of pars tensa, more extensively in myringotomized ears. OPN expression was found early in inflammatory cells including

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especially macrophages and late in pars tensa fibrocytes. OPG expression was initially located to inflammatory cells and late to pars tensa fibrocytes and the inner basal membrane of pars flaccida. Some ears displayed a marked pars flaccida expression of ON in the connective tissue matrix on early days and at the inner basal membrane on later days. The latter cases were from myringotomized ears. Otherwise, no apparent differences of marker expression occurred between myringotomized and non-myringotomized animals.

Conclusion We conclude that osteopontin, osteoprotegerin and osteonectin are expressed by different cell types in the tympanic membrane during calcification in association with AOM, with or without myringotomy. These molecules may accordingly play a role in the pathogenesis of myringosclerosis, in which macrophages and fibrocytes appear as potential major players.

1. Introduction

Myringosclerosis is described as a pathological condition affecting the lamina propria of the tympanic membrane (TM), characterized by a hyalinization and calcification of the intermediate connective tissue layer. Clinically, myringosclerotic lesions are seen as whitish, bone-like sclerotic plaques in the TM. In accordance with clinical findings, myringosclerosis develops after otitis media (OM) and paracentesis in an experimental setting [1,2]. The pathogenesis of this phenomenon of calcification is still inadequately understood.

Calcification of immature bone matrix is a central process in the formation of new bone, which occurs during or following a number of infectious or inflammatory diseases, e.g. gingivitis, arthritis, chronic OM and experimental acute otitis media (AOM) [3]. This calcification process mimics the calcification of a hyalinized lamina propria during development of myringosclerosis. Due to the similarities of appearance and pre-formed matrix calcification between the development of myringosclerosis and the formation new bone, this paper explores TM calcification in relation to the expression of bone modelling markers during and after AOM, in search of new information on the pathogenesis of myringosclerosis. From several known bone morphogenetic proteins and bone modelling markers, examination of the expression of osteopontin (OPN), osteoprotegerin (OPG) and osteonectin (ON) was chosen, as the effects of these markers appear as potentially relevant in the pathogenesis of myringosclerosis, as indicated below. Further, these three markers are among the most important and best described regulators of bone modelling.

OPN is a major acidic phosphorylated glycoprotein of bone [4,5]. It acts as a regulator of bone formation [6,7] and is involved in pathological calcification, such as calcification of atherosclerotic plaques [8,9]. OPN is secreted by osteoblasts and found at calcification sites, e.g. during pathological calcification in chronic otitis media [10,11]. OPN is present in the normal inner ear [12], whereas, no reports exist on OPN expression in the normal middle ear.

OPG is a member of the tumor necrosis factor ligand and receptor superfamily [13,14], and is one of the primary regulators of bone metabolism [15]. OPG is produced by osteoblasts/stromal cells and regulates bone turnover by inhibition of the differentiation, survival, and fusion of osteoclastic precursor cells, by suppression of osteoclast activation, and by promotion of osteoclast apoptosis [16]. Thus, OPG is an inhibitor of bone resorption and consequently bone remodelling. Apparently, OPG is produced constitutively at high levels in the cochlea, which may explain the extremely slow turnover of peri-labyrinthine bone tissue structures [17]. A role of OPG in bone modelling associated with cholesteatoma has been implicated [18,19], whereas, no reports exist on OPN expression in the normal middle ear.

ON (also known as SPARC—secreted protein acidic and rich in cysteine) is a calcium binding extracellular matrix glycoprotein that regulates cell interaction with the extracellular millieu during development and in response to injury [20]. Osteonectin is synthesized by osteoblasts and found in bone matrix. It regulates bone matrix assembly and is accordingly expressed in areas of active bone remodelling [20]. It is further necessary for normal osteoblast formation, maturation, and survival [21], and essential for the maintenance of bone mass, normal remodelling, and bone quality [22]. ON may play a role in the developing cochlea [23], whereas, no reports exist on ON expression in the middle ear.

2. Materials and methods

2.1. Experimental animals and operating procedures

A well-established rat model of acute otitis media (AOM) caused by *Haemophilus influenzae* was