

## Experimental models of cholesteatoma: A review

Onur Ismi, Murat Unal

Onur Ismi, Murat Unal, Department of Otorhinolaryngology, Faculty of Medicine, University Hospital of Mersin, 33343-Çiftlikköy, Mersin, Turkey

**Author contributions:** Both authors contributed to conception, design, data collection, analysis and drafting of the manuscript.

**Correspondence to:** Murat Unal, MD, Professor, Department of Otorhinolaryngology, Faculty of Medicine, University Hospital of Mersin, 33343-Çiftlikköy, Yenişehir-Mersin, Turkey. [munal@mersin.edu.tr](mailto:munal@mersin.edu.tr)

Telephone: +90-324-2410000 Fax: +90-324-2410000

Received: July 17, 2014 Revised: August 19, 2014

Accepted: September 16, 2014

Published online: November 28, 2014

DOI: <http://dx.doi.org/10.5319/wjo.v4.i4.23>

### Abstract

Cholesteatoma describes the keratinized, stratified squamous epithelium in the middle ear and mastoid, which has osteoclastic activity and is capable of bone resorption. Its origin is unknown and remains a topic of current investigation. In addition, ongoing studies are investigating new molecules for treatment. This review summarizes the various experimental models of cholesteatoma.

© 2014 Baishideng Publishing Group Inc. All rights reserved.

**Key words:** Animal; Cholesteatoma; Chronic otitis media; Experimental; Murine

**Core tip:** Cholesteatoma is the keratinized, stratified squamous epithelium in the middle ear and mastoid, which has osteoclastic activity and is capable of bone resorption. The mechanism of formation remains unknown, though different theories involving various models of formation have been proposed. This review summarizes the various experimental models of cholesteatoma.

Ismi O, Unal M. Experimental models of cholesteatoma: A review. *World J Otorhinolaryngol* 2014; 4(4): 23-27 Available from: URL: <http://www.wjgnet.com/2218-6247/full/v4/i4/23.htm>

### INTRODUCTION

Cholesteatoma describes the keratinized, stratified squamous epithelium in the middle ear and mastoid, which has osteoclastic activity and is capable of bone resorption. It involves subepithelial connective tissue, called the perimatrix, and is characterized by chronic inflammatory reaction. Resorption of bone occurs in the area neighboring the perimatrix, mediated by osteoclasts, and can lead to hearing loss, vestibular dysfunction, facial paralysis and even lethal intracranial complications<sup>[1]</sup>. The diagnosis for cholesteatoma is based on otoscopic examination, audiologic findings and radiologic examination. The only immediate treatment is surgery, which requires follow-up due to the risk of recurrence (up to 15%)<sup>[2]</sup>.

The pathogenesis of cholesteatoma is unknown, but there are four different theories regarding its genesis<sup>[3]</sup>: (1) metaplasia theory, metaplasia of middle ear epithelium into stratified squamous epithelium; (2) immigration theory, squamous epithelium of the external ear canal migrates to the middle ear through a perforation in the tympanic membrane; (3) hyperplasia theory, basal cell hyperplasia of keratinized epithelium in Shrapnell's membrane due to inflammation; and (4) retraction pocket theory, retraction in the Shrapnell's membrane due to chronic Eustachian dysfunction. Various animal models have been developed over the years to examine the pathogenesis and treatment of cholesteatoma, each indicating a different way of formation. In this review, we summarize and discuss these experimental models and the molecules for prevent or treatment.

### ANIMALS USED IN EXPERIMENTAL MODELS

Chinchillas, guinea pigs, Mongolian gerbils (*Meriones unguiculatus*) and rats have all been used in experimental models of cholesteatoma. The auditory apparatus of the

**Table 1 Canal ligation technique cholesteatoma formation articles**

Ref.	Year	Result
Park <i>et al</i> <sup>[4]</sup>	2005	Control of inflammation with ofloxacin and maintenance of normal clearance mechanisms can manage early stage cholesteatomas
Choufani <i>et al</i> <sup>[2]</sup>	2007	Animal model cholesteatomas can differ from human cholesteatoma regarding growth regulatory markers
Park <i>et al</i> <sup>[6]</sup>	2001	Phospholipase C-γ1 may play a role in signaling pathways on genesis of cholesteatoma
Park <i>et al</i> <sup>[7]</sup>	2001	Cholesteatoma proliferates at a higher rate than retroauricular and deep meatal skin
Yamamoto-Fukuda <i>et al</i> <sup>[8]</sup>	2010	Tympanic membrane epithelium is the probable source of cholesteatoma epithelium

Chinchilla is similar to humans, and guinea pigs have a similar mastoid and epithelial and subepithelial lining ultrastructure of the middle ear. The osteoclastic and bone resorption characteristics of cholesteatoma in gerbils is similar to humans<sup>[3]</sup>. In the Mongolian gerbils and fat sand rat *Psomomys obesus*, cholesteatoma can form spontaneously, and these species have therefore been widely used in experimental cholesteatoma models<sup>[1]</sup>. Moreover, the incidence and severity of cholesteatoma increases with age in gerbils<sup>[3]</sup>.

## METHODS USED FOR DEVELOPMENT OF CHOLESTEATOMA

### Ligation of external auditory canal

McGinn first popularized the method of retroauricular skin incision and ligation near the bony external auditory canal with 4.0 silk sutures in 1982<sup>[4]</sup>. This method of cholesteatoma formation is very effective and occurs in 100% of ligated Mongolian gerbils. The disadvantage of this method involves the high cost of the animals<sup>[3,5]</sup>. After formation of cholesteatoma, development of cholesteatoma can be staged into five groups: (1) accumulation of keratin debris on the outer surface of tympanic membrane; (2) medial displacement of tympanic membrane without contact with the bulla; (3) cholesteatoma is in contact with the prominence of the cochlea; (4) cholesteatoma fills the bulla; and (5) intracranial extension<sup>[3]</sup>.

By using the external canal ligation method, Park *et al*<sup>[4]</sup> studied the reversibility of cholesteatoma with ofloxacin ear drops and saline irrigation<sup>[4]</sup>. They concluded that cholesteatomas can be managed with conservative mechanisms, such as control of inflammation and maintenance of normal clearance mechanisms at an early stage. Choufani *et al*<sup>[2]</sup> compared levels of differentiation and growth regulatory markers, including retinoic acid receptors, galectins, and macrophage migration inhibitory factors, in ligated gerbils with cholesteatomas in humans. Their immunohistochemical analyses showed that only macrophage migration inhibitory factors were similar, thus they concluded that animal models differ from the clinical presentation. Another study using the canal ligation technique by Park *et al*<sup>[6]</sup> in 2001 indicated that phospholipase C-γ1 plays a role in the formation of cholesteatoma. Further work from this group evaluating proliferative activity markers, including cytokeratin 13/16, proliferating cell nuclear antigen, epidermal growth factor receptor and thrombomodulin, demonstrated that cho-

lesteatoma proliferates at a higher rate than retroauricular and deep meatal skin<sup>[7]</sup>.

In 2010, Yamamoto-Fukuda *et al*<sup>[8]</sup> combined ligation of the ear canal with a new hybridization approach to find the origin of cells in the cholesteatoma. After making a perforation in the tympanic membrane of male gerbils, they performed myringoplasty using female gerbils' tympanic membranes as grafts; they ligated the external auditory canal to form cholesteatoma. After using *in situ* polymerase chain reaction, they found epithelium in the cholesteatoma of female origin in the male gerbil and concluded that tympanic membrane epithelium is the probable source for cholesteatoma<sup>[8]</sup> (Table 1).

### Eustachian tube blocking model with ligation or electrocauterization

Dysfunction of Eustachian tube leads to pars flaccida retraction pocket and subsequent cholesteatoma. This method was popularized by Chloe and Wolfmann in 1986. With this technique, cholesteatoma formation occurred in three quarters of animals<sup>[3,5]</sup>. Eustachian tube blockage can be achieved surgically *via* trans-neck or trans-oral approaches. In the trans-neck approach, the Eustachian tube can be seen below the facial nerve and digastric muscle. In the trans-oral approach, the Eustachian tube orifice is found 5 mm posterior to the junction of hard and soft palates<sup>[5]</sup>.

In 2001, Kim *et al*<sup>[9]</sup> investigated cytokeratins in the cholesteatoma using unilateral electrocauterization of the Eustachian tube and formation of retraction pocket cholesteatoma, which was staged as follows: stage 1: mild retraction of tympanic membrane with or without middle ear effusion; stage 2: retraction pocket surrounds ossicular chain; stage 3: deep retraction pocket with bone erosion and keratin formation; stage 4: total adhesion of the tympanic membrane. The expression of cytokeratin 13/16 with advancing stage of cholesteatoma suggested that the epithelium of retraction pocket cholesteatoma increasingly proliferates with stage. Wilmoth *et al*<sup>[10]</sup> used bilateral Eustachian tube obstruction to study matrix metalloproteinases and tumor necrosis factor alpha in the atelectatic tympanic membranes. Elevation of these markers with progression of retraction pocket stage indicated the possible role in cholesteatoma formation.

In 2009, von Unge *et al*<sup>[11]</sup> used repeated pressure loads to the tympanic membrane and Eustachian tube of gerbils with simulated habitual sniffing to form retraction pocket cholesteatoma similar to the Eustachian tube

**Table 2** Articles using eustachian tube blocking model for cholesteatoma formation

Ref.	Year	Result
Kim <i>et al</i> <sup>[9]</sup>	2001	Cytokeratin expression increases with advancing stage of retraction pocket cholesteatoma
Wilmoth <i>et al</i> <sup>[10]</sup>	2003	Matrix metalloproteinases and tumor necrosis factor alpha may have a role in retraction pocket cholesteatoma
Tinling <i>et al</i> <sup>[12]</sup>	2006	Basal cell keratinocytes' cell division rate is much more in cholesteatoma (with combination of canal ligation)

**Table 3** Articles using chemical reagent injection technique for cholesteatoma formation

Ref.	Year	Result
White <i>et al</i> <sup>[14]</sup>	1995	Hyaluronic acid doesn't inhibit cholesteatoma formation in experimental model
Kayhan <i>et al</i> <sup>[16]</sup>	2006	Prednisolone may inhibit cholesteatoma formation
Antunes <i>et al</i> <sup>[17]</sup>	2008	Trans-retinoic acid may inhibit cholesteatoma formation
Melo <i>et al</i> <sup>[18]</sup>	2013	Mitomycin-C may inhibit cholesteatoma formation
Massuda <i>et al</i> <sup>[13]</sup>	2005	Latex biomembrane is as effective as propylene glycol injection in cholesteatoma formation in experimental model
Kim <i>et al</i> <sup>[19]</sup> (With combination of canal ligation and Eustachian tube blocking)	2002	Expression of different types of cytokeratins increases according to cholesteatoma formation way

blocking model. To simulate sniffing, a vacuum was used to produce negative pressure in the chamber. They found that with a Moire interferogram, the gerbil tympanic membrane retains its stiffness after 7 to 12 d of repeated pressure loading, resulting in retraction pocket formation, but no cholesteatoma formation<sup>[11]</sup>.

A combination of ear canal ligation and Eustachian tube obstruction can be used to form cholesteatoma. Tinling *et al*<sup>[12]</sup> compared gerbils after ear canal ligation, Eustachian tube obstruction or both and found that the rate of cell division of basal cell keratinocytes in the tympanic membrane and external auditory canal of gerbils with cholesteatomas was seven times higher than controls. However, there were no differences among the methods used for cholesteatoma formation (Table 2).

### Chemical reagent injection

Chemical substance injection to the middle ear or bulla of animals is another widely used method for cholesteatoma formation. Materials that can induce cholesteatoma formation are talcum powder, dimethylbenzanthracene, latex, and propylene glycol<sup>[5,13-17]</sup>, which is most widely used. Experimental usage of propylene glycol to induce cholesteatoma began after it was observed that the application of topical eye drops containing propylene glycol (Cortisporin) to the middle ear of chinchillas resulted in epithelial migration and formation of cholesteatomatous chronic otitis media. Cholesteatoma formation percentage increases to 100% with an increase in the application dose to 90% concentration of propylene glycol<sup>[3]</sup>. Application of propylene glycol can be with intratympanic injection or by the trans mastoid way through a small hole in the bulla<sup>[14-18]</sup>. With this method, different chemicals are used to inhibit the formation of cholesteatomas. 5-fluorouracyl, trans-retinoic acid, mitomycin-c and systemic prednisolone inhibit cholesteatoma in experimental models, whereas hyaluronic acid and cyclophosphamide have no significant effect<sup>[3,14,16-18]</sup>.

In 2005, Massuda *et al*<sup>[13]</sup> reported on a different ap-

plication of propylene glycol in rats. After forming a posterosuperior perforation in the tympanic membrane, they used a latex biomembrane with 50% propylene glycol or introduced a natural latex biomembrane into the orifice of the tympanic membrane with one end in the middle ear and the other in the external auditory canal. Cholesteatoma occurred in 80% of ears with the propylene glycol, and 90% of ears using the latter method. They concluded that both methods are effective in experimental cholesteatoma formation<sup>[13]</sup>. Application of propylene glycol can also be combined with canal ligation and Eustachian blockage technique. Kim *et al*<sup>[19]</sup> studied proliferation and migration states of experimental cholesteatomas using canal ligation, Eustachian blockage (retraction pocket) or propylene glycol in Mongolian gerbils. Expression of cytokeratin 13/16 was mostly persistent in the group receiving the retraction pocket, whereas cytokeratins 5/6 and 1/10 were mostly expressed in the group with canal ligation. They stated that there was a complex alteration in the epidermal maturation pathway in the pathogenesis of cholesteatoma. Studies using chemical substance injection for cholesteatoma formation are summarized in Table 3.

### Skin graft transfer to middle ear of animal

Another method used to form cholesteatoma is full thickness skin graft transfer. After skin graft implantation with superimposed infection, cholesteatoma formed with 89.3% success, but bone resorption was not observed<sup>[3]</sup>. However, Si *et al*<sup>[20]</sup> formed cholesteatoma by autologous skin graft implantation and pseudomonas injection to the middle ears of mice with 92% success. All cholesteatoma-forming mice had hearing loss measured by auditory-evoked brainstem responses and there was bone resorption demonstrated by computed tomography.

### Dermal implant transfer to non-temporal bone

To form cholesteatoma and show the bone resorption pattern, dermal implants in mice can be transferred to

the calvarium or femoral bone. Sudhoff *et al*<sup>[1]</sup> transferred dermal implants consisting of skin and underlying cartilage to calvaria that resulted in localized inflammation and bone resorption. The authors therefore concluded that this method of cholesteatoma bone resorption would be a useful device in a genetically well-defined animals, such as mice. Chole *et al*<sup>[21]</sup> implanted keratin (from human volunteer fingernail filings, and mouse hair and nails) and polymethylmethacrylate to mice calvaria to form a bone resorption model similar to cholesteatoma. They showed a chronic inflammatory response with angiogenesis, mononuclear cell recruitment and osteoclastic bone resorption in calvaria that was similar to cholesteatoma. A similar model was used by Jung *et al*<sup>[22]</sup> who studied nitric oxide synthase levels after implantation of keratin to calvaria of rats. They found that levels of nitric oxide synthase, especially type II, were upregulated in response to keratin. In 2005, Magalhaes *et al*<sup>[23]</sup> formed cholesteatoma after implantation of full thickness skin graft to femoral bones of rat, concluding that a trapped keratinized epithelium (skin) causes epithelial cyst formation to expel the foreign tissue.

### Bone marrow samples of mice for osteoclastogenic activity and cell cultures

Bone marrow samples are not used for forming cholesteatoma, but for determining bone resorption pathways. Nason *et al*<sup>[24]</sup> collected osteoclastic cell precursors from bone marrow of mice that showed transformation to bone-resorbing osteoclasts with lipopolysaccharide from *Pseudomonas aeruginosa*<sup>[24]</sup>. As the most cultured organism in infected cholesteatomas, *P. aeruginosa* may thus have a role in osteoclastic activity of cholesteatomas.

## CONCLUSION

Experimental animal models are crucial for understanding the pathogenesis of cholesteatoma and for identifying new molecules for prevention or treatment. The use of differing models will ensure that all aspects of cholesteatoma formation are explored.

## REFERENCES

- 1 Sudhoff H, Liebehenz Y, Aschenbrenner J, Jung J, Hildmann H, Dazert S. A murine model of cholesteatoma-induced bone resorption using autologous dermal implantation. *Laryngoscope* 2003; **113**: 1022-1026 [PMID: 12782815]
- 2 Choufani G, Roper N, Delbrouck C, Hassid S, Gabius HJ. Animal model for cholesteatoma induced in the gerbil: will the profiles of differentiation/growth-regulatory markers be similar to the clinical situation? *Laryngoscope* 2007; **117**: 706-711 [PMID: 17415142 DOI: 10.1097/mlg.0b013e318031d09d]
- 3 Yamamoto-Fukuda T, Takahashi H, Koji T. Animal models of middle ear cholesteatoma. *J Biomed Biotechnol* 2011; **2011**: 394241 [PMID: 21541229 DOI: 10.1155/2011/394241]
- 4 Park K, Choung YH, Chun YM, Lee JS, Hong SP. Reversibility of experimental cholesteatoma epithelium using Mongolian gerbils. *Acta Otolaryngol* 2005; **125**: 540-546 [PMID: 16092548 DOI: 10.1080/00016480510029400]
- 5 Park MK, Lee BD. Development of animal models of otitis media. *Korean J Audiol* 2013; **17**: 9-12 [PMID: 24653896 DOI: 10.787/kja.2013.17.1.9]
- 6 Park K, Chun YM, Lee DH. Expression of phospholipase C-gamma1 in experimental cholesteatoma using Mongolian gerbils. *Acta Otolaryngol* 2001; **121**: 477-480 [PMID: 11508507]
- 7 Park K, Park HJ, Chun YM. Immunohistochemical study on proliferative activity of experimental cholesteatoma. *Eur Arch Otorhinolaryngol* 2001; **258**: 101-105 [PMID: 11374247]
- 8 Yamamoto-Fukuda T, Hishikawa Y, Shibata Y, Kobayashi T, Takahashi H, Koji T. Pathogenesis of middle ear cholesteatoma: a new model of experimentally induced cholesteatoma in Mongolian gerbils. *Am J Pathol* 2010; **176**: 2602-2606 [PMID: 20413684 DOI: 10.2353/ajpath.2010.091182]
- 9 Kim HJ, Tinling SP, Chole RA. Expression patterns of cytokeratins in retraction pocket cholesteatomas. *Laryngoscope* 2001; **111**: 1032-1036 [PMID: 11404616]
- 10 Wilmoth JG, Schultz GS, Antonelli PJ. Matrix metalloproteinases in a gerbil cholesteatoma model. *Otolaryngol Head Neck Surg* 2003; **129**: 402-407 [PMID: 14574296 DOI: 10.1016/S0194-5998(03)01317-2]
- 11 von Unge M, Dircks JJ. Functional effects of repeated pressure loads upon the tympanic membrane: mechanical stiffness measurements after simulated habitual sniffing. *Eur Arch Otorhinolaryngol* 2009; **266**: 1219-1224 [PMID: 19130069 DOI: 10.1007/s00405-008-0906-3]
- 12 Tinling SP, Chole RA. Gerbilline cholesteatoma development Part III. Increased proliferation index of basal keratinocytes of the tympanic membrane and external ear canal. *Otolaryngol Head Neck Surg* 2006; **135**: 116-123 [PMID: 16815195 DOI: 10.1016/j.otohns.2005.12.025]
- 13 Massuda ET, Oliveira JA. A new experimental model of acquired cholesteatoma. *Laryngoscope* 2005; **115**: 481-485 [PMID: 15744162 DOI: 10.1097/01.mlg.0000157826.15756.67]
- 14 White SJ, Wright CG, Robinson KS, Meyerhoff WL. Effect of topical hyaluronic acid on experimental cholesteatoma. *Am J Otolaryngol* 1995; **16**: 312-318 [PMID: 7503374]
- 15 Kayhan FT, Algün Z. The effect of naproxen sodium on experimental otitis media. *Kulak Burun Bogaz Ihtis Derg* 2008; **18**: 14-18 [PMID: 18443397]
- 16 Kayhan FT, Algün Z. The effect of systemic prednisolone on propylene glycol-induced otitis media in guinea pig. *Kulak Burun Bogaz Ihtis Derg* 2006; **16**: 214-220 [PMID: 17124441]
- 17 Antunes ML, Fukuda Y, Penido Nde O, Ferreira R. Effect of trans-retinoic acid in the inhibition of cholesteatoma in guinea pigs. *Braz J Otorhinolaryngol* 2008; **74**: 53-60 [PMID: 18392502]
- 18 Melo AA, Caldas Neto SS, Leão FS, Campos AJ. Effect of intratympanic mitomycin C on the development of cholesteatoma and otitis media in rats. *J Laryngol Otol* 2013; **127**: 359-363 [PMID: 23406694]
- 19 Kim HJ, Tinling SP, Chole RA. Increased proliferation and migration of epithelium in advancing experimental cholesteatomas. *Otol Neurotol* 2002; **23**: 840-844 [PMID: 12438843]
- 20 Si Y, Chen YB, Chen QX, Liu Y, Jiang HL, Zhang ZG, Huang X. Autologous meatal skin graft implantation and intratympanic injection of *Pseudomonas aeruginosa*: a new experimental mouse model of acquired middle ear cholesteatoma. *ORL J Otorhinolaryngol Relat Spec* 2013; **75**: 274-281 [PMID: 24030443]
- 21 Chole RA, Hughes RM, Faddis BT. Keratin particle-induced osteolysis: a mouse model of inflammatory bone remodeling related to cholesteatoma. *J Assoc Res Otolaryngol* 2001; **2**: 65-71 [PMID: 11545151 DOI: 10.1007/s101620010041]
- 22 Jung JY, Pashia ME, Nishimoto SY, Faddis BT, Chole RA. A possible role for nitric oxide in osteoclastogenesis associated with cholesteatoma. *Otol Neurotol* 2004; **25**: 661-668 [PMID: 15353992]
- 23 Magalhaes SL, Reforme OM, Guzmán RL, Fukuda Y, Barbosa F. Growth of cholesteatoma by implantation of epithelial tissue along the femoral bone of rats. *Braz J Otorhinolaryngol*

- 2005; **71**: 188-191 [PMID: 16446916]  
24 **Nason R**, Jung JY, Chole RA. Lipopolysaccharide-induced osteoclastogenesis from mononuclear precursors: a

mechanism for osteolysis in chronic otitis. *J Assoc Res Otolaryngol* 2009; **10**: 151-160 [PMID: 19145462 DOI: 10.1007/s10162-008-0153-8]

**P- Reviewer:** Kojima H, Luers JC, Vlastos IM, Vlastarakos PV  
**S- Editor:** Gong XM **L- Editor:** A **E- Editor:** Wu HL







Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>

