Introduction

Otitis media is one of the most common childhood diseases and is the most frequent reason for visits to a physician. At least four-fifths of children will have experienced one or more episodes of otitis media by the age of 3 years. In addition, otitis media affects not only the child, but also the caregiver of the child. It affects hearing and balance, and eventually causes poor language development and poor educational performance. The financial burden associated with otitis media in children 5 years of age and younger is estimated to be $5 billion annually in the United States.

The causes of otitis media include infection and anatomic/physiologic, host, and environmental factors. In general, otitis media is a childhood disease, and anatomic and physiologic changes have great effects on its development. Thus, in vitro or human experimental studies of otitis media are difficult. Several experimental animal models have been introduced to investigate the pathogenesis and treatment of otitis media. However, none are ideal. The aim of this review is to provide a brief overview of the current status of animal models of otitis media with effusion, acute otitis media, and cholesteatoma. This review will assist determination of the most appropriate animal models of otitis media.

Animal Models of Otitis Media with Effusion

There are two categories of animal models of otitis media. One is Eustachian tube ligation or cauterization using a trans-neck or trans-oral approach, and the other is injection of chemical materials through the tympanic membrane. Dysfunction of the Eustachian tube is an important anatomical cause of otitis media with effusion. To simulate this condition, one can block the Eustachian tube by ligation or cauterization. Unfortunately, this is irreversible. The trans-neck approach is an accurate method of blocking the tube. However, it is more time-consuming than the trans-oral approach. Briefly, a rat or other rodent is placed in the supine position after proper intraperitoneal analgesic injection. We pre-
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Table 1. Comparison of various animal models of otitis media

| Models                                | Difficulty | Time  | Reproducibility | Other          |
|---------------------------------------|------------|-------|-----------------|----------------|
| Otitis media with effusion            |            |       |                 |                |
| Ligation or cauterization             |            |       |                 |                |
| Trans-neck approach                   | Difficult  | 30 min| High            |                |
| Trans-oral approach                   | Moderate   | <5 min| Low             |                |
| Injection methods                     | Easy       | <5 min| Moderate        |                |
| Oxgr1 knock-out mouse                 | Easy       |       | High            |                |
| Acute otitis media                    | Easy       | <5 min| Moderate        | High mortality |
| Inoculation via trans-tympanic membrane|           |       |                 |                |
| Cholesteatoma                          |            |       |                 |                |
| External auditory canal ligation      | Moderate   | <10 min| High            | Expensive      |
| Injection methods                     | Easy       | <5 min| Moderate        |                |

fer tiletamine/zolazepam (Zoletil® 50; Virbac Lab, Carros, France)(0.02 mL/100 mg). A longitudinal midline incision is made at the neck, and the platysma muscle is elevated. After identifying the sternocleidomastoid muscle and belly of the digastric muscle, the facial nerve can be found at distal to the bulla. Arteries that are located medially should be controlled. Through meticulous dissection, the orifice of the Eustachian tube can be seen below the facial nerve and belly of the digastric muscle. Bleeding can be controlled with electrical cauterization (Fig. 1). The orifice of the Eustachian tube can be cauterized, the cartilage portion of the Eustachian tube can be ligated using electrical cautery or nylon, or the tube can be blocked using dental material after the bulla has been exposed. After opening the mouth wide using a small blade or mouth gag, an electrical cautery tip is placed on the midline of the soft palate and directed laterally. The orifice of the Eustachian tube is located about 5 mm posterior to the junction of the hard and soft palates (Fig. 2). Severe thermal damage around the Eustachian tube can induce severe bleeding or poor oral intake. Some animals cannot bear this stress. Antibiotics can help to lower the mortality rate. Consistent results are not easy to obtain with this method because of the poor visual field.

When the soft palate is opened with a wide incision, the pharyngeal orifice of the Eustachian tube can be identified under microscopic visualization. This approach provides a better visual field. Trichloroacetic acid (50%) can be used instead of electrical cautery. Injection of chemical materials through the tympanic membrane is easier than the above-described methods. A spinal needle can be used. Histamine solution (0.1 mL) has been

![Fig. 1. Trans-neck approach to ligation or cauterization of the Eustachian tube for otitis media with effusion. A: Midline incision. B: Elevation of platysma. C: SCM and belly of digastric muscle. D: Exposure of bulla and Eustachian tube orifice. SCM: sternocleidomastoid muscle.](image)
shown to induce otitis media with effusion in half of rats 24 h after injection. Spontaneous otitis media with effusion was recently demonstrated in a mouse model of a specific genetic mutation in a G protein-coupled receptor encoded by the Oxgr1 gene.

Animal Models of Acute Otitis Media

Injection of bacteria through the tympanic membrane is the most common way of inducing acute otitis media in animals (Fig. 3). Common causes of infections include Streptococcus pneumoniae, Haemophilus influenzae, and Moraxella catarrhalis. Methicillin-resistant Staphylococcus aureus and Pseudomonas aeruginosa are also commonly used in experimental studies. An insufficient inoculum does not induce acute otitis media, and an excess can result in meningitis or sepsis. Thus, bacteria should be diluted to the appropriate levels.

Systemic reactivation of otitis media with effusion in a rat model was studied using peptidoglycan-polysaccharide. Genetic predisposition has been demonstrated in animal models of middle ear inflammation.

Animal Models of Cholesteatoma

Four theories of cholesteatoma development exist: metaplasia, immigration, hyperplasia, and retraction pocket formation. The retraction pocket theory is the most widely used. Animal models of cholesteatoma are usually based on one of these theories.

Several categories of animal models of cholesteatoma are available. One involves a surgical method, such as external auditory canal ligation, Eustachian tube blocking, or autologous dermal implantation. The other involves the use of chemical materials in conjunction with various delivery methods. The external auditory canal ligation model is a relatively simple and well-documented method; however, Mongolian gerbils are required. Mongolian gerbils are useful because cholesteatoma can be easily induced by ligation, and spontaneous cholesteatoma formation is known to occur. Cholesteatoma is induced in 100% of ligated ears; this is a very high success rate.
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in terms of experimental studies of cholesteatoma. Unfortunately, Mongolian gerbils are very expensive in Korea.

Eustachian tube blocking was shown to induce cholesteatoma formation in three-quarters of Mongolian gerbils within 16 weeks.\(^8\) Cholesteatoma can be induced by removing the pars flaccida of the tympanic membrane and transplanting a tympanic membrane to the defect of the original tympanic membrane.\(^9,10\)

Chemical reagents are usually delivered by injection. Reagents can be injected through the tympanic membrane or bulla. Materials that can induce cholesteatoma include talcum powder, dimethylbenzanthracene, propylene glycol, and latex.\(^11,12\) Propylene glycol (concentration, 90%) results in induction of cholesteatoma in most rats (Fig. 4).

Conclusions

Several animal models of otitis media are available. Unfortunately, none are ideal. Researchers who study otitis media should be familiar with the strengths and weak points of each model when selecting the model most appropriate for their purposes. Further physiological animal models of otitis media should be developed.

REFERENCES

1) Rovers MM. The burden of otitis media. Vaccine 2008;26 Suppl 7: G2-4.
2) Teede DW, Klein JO, Rosner B. Epidemiology of otitis media during the first seven years of life in children in greater Boston: a prospective, cohort study. J Infect Dis 1989;160:83-94.
3) Shellel P, Takata G, Chan LS, Mangione-Smith R, Corley PM, Mor- phew T, et al. Diagnosis, natural history, and late effects of otitis media with effusion. Evid Rep Technol Assess (Summ) 2002;1:5.
4) Casselbrant ML, Mandel EM. Acute Otitis Media and Otitis Media with Effusion. In: Flint PW, Haughey BH, Lund VJ, Niparko JK, Richardson MA, Robbins KT, editors. Cummings Otolaryngology-Head and Neck Surgery. 5th ed. Philadelphia, PA: Mosby; 2010. p.2761-77.
5) Capra AM, Lieu TA, Black SB, Shinefield HR, Martin KE, Klein JO. Costs of otitis media in a managed care population. Pediatr Infect Dis J 2000;19:354-5.
6) Proud GO. Eustachian tube function and middle ear pressures as they influence susceptibility to disease. Laryngoscope 1972;82: 1643-6.
7) Yamamoto-Fukuda T, Takahashi H, Koji T. Animal models of mid- dle ear cholesteatoma. J Biomed Biotechnol 2011;2011:394241.
8) Preciado D, Kuo E, Ashktorab S, Manes P, Rose M. Cigarette smoke activates NFkB-mediated Tnf-α release from mouse middle ear cells. Laryngoscope 2010;120:2508-15.
9) DiFranza JR, Aline CA, Weitzman M. Prenatal and postnatal envi- ronmental tobacco smoke exposure and children’s health. Pediatrics 2004;113(4 Suppl):1007-15.
10) Proud GO, Odo H. Effects of Eustachian tube ligation. Ann Otol Rhinol Laryngol 1970;79:30-2.
11) Pilcher OR, Swarts JD, Magnuson K, Alper CM, Doyle WJ, Hedba PA. A rat model of otitis media with effusion caused by eustachian tube obstruction with and without Streptococcus pneumoniae infec- tion: methods and disease course. Otolaryngol Head Neck Surg 2002: 126:490-8.
12) Aynali G, Yariktaş M, Yasan H, Karahan N, Baspınar S, Tür M, et al. The effects of methylprednisolone, montelukast and indometha- cine in experimental otitis media with effusion. Int J Pediatr Otolar- naryngol 2011;75:15-9.
13) Russell JD, Gies JS. Persistent otitis media with effusion: a new exper- imental model. Laryngoscope 1998;108(8 Pt 1):1181-4.
14) Huang Q, Zhang Z, Zheng Y, Zheng Q, Shen S, Xu Y, et al. Hypoxia-inducible factor and vascular endothelial growth factor pathway for the study of hypoxia in a new model of otitis media with effusion. Audiol Neurootol 2012;17:349-56.
15) Kerschner JE, Hong W, Taylor SR, Kerschner JA, Khampang P, Wrege KC, et al. A novel model of spontaneous otitis media with ef- fusion (OME) in the Oxgr1 knock-out mouse. Int J Pediatr Otorhinolaryngol 2013;77:79-84.
16) Giebink GS. Otitis media: the chinchilla model. Microb Drug Resist 1999;5:57-72.
17) Jewett BS, Prazma JP, Hunter SE, Rose AS, Clark JM, Sartor BR, et al. Systemic reactivation of otitis media with effusion in a rat model. Otolaryngol Head Neck Surg 1999;121:7-12.
18) Clark JM, Brinson G, Newman MK, Jewett BS, Sartor BR, Prazma J, et al. An animal model for the study of genetic predisposition in the pathogenesis of middle ear inflammation. Laryngoscope 2000;110: 1511-5.
19) Kim HJ, Chole RA. Experimental models of aural cholesteatomas in Mongolian gerbils. Ann Otol Rhinol Laryngol 1998;107:129-34.
20) Wolfman DE, Chole RA. Experimental retraction pocket cholestea- toma. Ann Otol Rhinol Laryngol 1986;95(6 Pt 1):639-44.
21) Yamamoto-Fukuda T, Hishikawa Y, Shibata Y, Kobayashi T, Taka- hashi H, Koji T. Pathogenesis of middle ear cholesteatoma: a new model of experimentally induced cholesteatoma in Mongolian ger- bils. Am J Pathol 2010;176:2602-6.
22) Massuda ET, Oliveira JA. A new experimental model of acquired cholesteatoma. Laryngoscope 2005;115:481-5.
23) White SJ, Wright CG, Robinson KS, Meyerhoff WL. Effect of topi- cal hyaluronic acid on experimental cholesteatoma. Am J Otolaryn- gol 1995;16:312-8.