Effect of *Streptococcus pneumoniae* and Influenza A Virus on Middle Ear Antimicrobial Pharmacokinetics in Experimental Otitis Media

Gregg H. Jossart,[1] Daniel M. Canafax,[1,2] Gary R. Erdmann,[1] Michael J. Lovdahl,[1] Henry Q. Russlie,[1] Steven K. Juhn,[1] and G. Scott Giebink[1]

Received January 5, 1994; accepted January 24, 1994

Antimicrobial treatment failures in children with acute otitis media and concomitant viral respiratory tract infection prompted us to study the effects of influenza A virus infection on middle ear antimicrobial drug penetration. Using a chinchilla model of *Streptococcus pneumoniae* we compared middle ear elimination rates in 4 groups of chinchillas: (1) control, (2) influenza A virus inoculation alone intranasally, (3) both influenza A and *S. pneumoniae* inoculation directly into the middle ear, and (4) *S. pneumoniae* inoculation alone into the middle ear. After infection was established, a solution containing amoxicillin, sulfamethoxazole, and trimethoprim was instilled into the middle ear and removed 4 hours later. The rate constant of elimination and half-life were calculated from measured drug concentrations initially and at 4 hours. *S. pneumoniae* infection alone significantly shortened the middle ear elimination half-life compared with the control group: amoxicillin, 2.65 ± 0.73 vs. 6.63 ± 2.55 hr; sulfamethoxazole, 1.75 ± 0.28 vs. 2.74 ± 0.6 hr; and trimethoprim, 1.06 ± 0.14 vs. 1.56 ± 0.34 hr (n = 16 ears, p values all <0.01). The combined influenza virus and *S. pneumoniae* infection significantly lengthened the half-life compared with the *S. pneumoniae* infection alone: amoxicillin, 5.65 ± 6.44 vs. 2.65 ± 0.73 hr; sulfamethoxazole, 2.5 ± 0.85 vs. 1.75 ± 0.28 hr; and trimethoprim, 1.26 ± 0.42 vs. 1.06 ± 0.14 hr (n = 16 ears, p values all <0.01). Influenza virus produced the longest half-lives for all 3 antimicrobials: amoxicillin 25.52 ± 14.96 hr; sulfamethoxazole, 5.46 ± 0.87 hr; and trimethoprim, 2.57 ± 0.75 hr. These effects demonstrate that influenza and *S. pneumoniae* infections alone and together affect middle ear antimicrobial penetration. The decreased penetration of antimicrobials that occurred with the combined viral and bacterial infection vs. the bacteria alone supports the clinical observation that patients with infections caused by both organisms may have decreased middle ear antimicrobial concentrations, producing treatment failures.

KEY WORDS: otitis media; influenza A virus; *Streptococcus pneumoniae;* amoxicillin; sulfamethoxazole; trimethoprim; pharmacokinetics.

Introduction

Otitis media is a principal cause of childhood morbidity. The disease continuum includes symptomatic acute purulent otitis media and less symptomatic chronic otitis media with effusion, which causes fluctuating hearing loss in most patients and permanent hearing loss in some patients. Antibiotic treatment of acute otitis media is complicated by a failure rate of 5% to 10% and by recurrent infection in about 30% of patients (1). It is thought that most cases of antibiotic failure are due to resistant organisms (2) or a concomitant respiratory viral infection (3, 4, 5).

In previous studies using a chinchilla model of acute otitis media, we found that intranasal inoculation of both *Streptococcus pneumoniae* and influenza A virus increased the incidence of otitis media 17-fold (compared with influenza A virus alone) and 3-fold (compared with *S. pneumoniae* alone) (5). Recently, Chonmaitree et al. (3) isolated viral and bacterial organisms from middle ear fluid of 12 patients with acute otitis media, 6 (50%) of whom did not respond to antimicrobial therapy. This compared with only 1 (3%) treatment failure in 30 patients with susceptible bacteria and no virus in their middle ear fluid. Arola et al. (4) studied 22 children with otitis media who were unresponsive to initial antimicrobial therapy; respiratory viruses were isolated from the nasopharynx, middle ear fluid, or both in 68% of these patients.

These studies suggest that drug penetration into the middle ear may be altered by viral infection, possibly as a result of subtherapeutic antimicrobial concentration, which then results in otitis media treatment failure. Therefore, we undertook this study to measure the effects of influenza A and *S. pneumoniae* infections on middle ear drug elimination rate as an indicator of drug penetration rate for amoxicillin, sulfamethoxazole, and trimethoprim, using a chinchilla model of acute otitis media (6).

Methods

We used 30 healthy, 1- to 2-year-old chinchillas, weighing between 400 and 600 grams. Procedures and handling were approved by our Research Animal Resources Committee. The chinchillas were anesthetized for all procedures with ketamine (50 mg/kg intramuscularly). Before any procedure, normal middle ear function was confirmed by tympanometry and otoscopy. The chinchillas were divided into three groups of 10 and inoculated with (1) influenza A virus alone on day 0, (2) influenza A virus on day 0 and *S. pneumoniae* on day 3, or (3) *S. pneumoniae* alone on day 0. Influenza A/Alaska/6/77 wild type, clone 3-A (Lot E-111, Flow Labs) was diluted in sterile saline. A total of 10^5 plaque-forming units (0.15 ml) was inoculated into both nares. A phosphate-buffered saline solution (0.1 ml), pH 7.4, containing 40 colony-forming units of log growth-phase type 7F *S. pneumoniae* were inoculated directly into both middle ear, as previously described (6). Nasal washes for virus isolation were done on day 3, as described (5). On day 6, the entire middle ear fluid contents were aspirated and cultured on 5% sheep blood agar. All middle ears were then inoculated with phosphate-buffered saline solution (1.0 ml), pH 7.5, containing 50 μg/ml of amoxicillin, 10 μg/ml of trimethoprim, and 50 μg/ml of sulfamethoxazole. The entire inoculum was aspirated 4 hours later, placed in 3.0 ml bullet

[1] Otitis Media Research Center and the Departments of Otolaryngology, Pediatrics, and Surgery, School of Medicine; Department of Pharmacy Practice, College of Pharmacy, HSUF 7-115, 308 Harvard Street S.E., University of Minnesota, Minneapolis, Minnesota 55455.

[2] To whom correspondence should be addressed.

0724-8741/94/0600-0860$07.00/0

Table I. Middle ear amoxicillin pharmacokinetic characteristics in chinchillas with otitis media (a).

Experimental Conditions	Number of Ears Studied	K (mean ± SD) 1/hr	Half-life (mean ± SD) hr	4 Hr ME Concentration (mean ± SD) μg/ml
Control	10	0.12 ± 0.04 (b)	6.63 ± 2.55	32.3 ± 5.5 (c)
Influenza A alone	16	0.04 ± 0.02	25.52 ± 14.96	39.3 ± 3.5 (c)
Influenza A and *S. Pneumoniae*	16	0.19 ± 0.09 (b)	5.65 ± 6.44	28.6 ± 9.8
S. Pneumoniae alone	16	0.28 ± 0.08	2.65 ± 0.73	18.0 ± 6.5

(a) P values for K and concentration values between groups were <0.01. Symbols: ME = middle ear, K = elimination rate, hr = hour, SD = standard deviation
(b) p = 0.03
(c) p = 0.3

tubes, and stored at −70°C for analysis, usually the next day.

Amoxicillin concentrations were determined in a 75 μl sample and an assay whose lower limit of detection was 0.25 μg/ml (7). Trimethoprim and sulfamethoxazole concentrations were determined using 25 μl samples and an assay whose lower limit of detection was 0.25 μg/ml (8). The rate constant of drug elimination from the middle ear (K) was calculated using $K = -[\ln(C_t/C_o)]/t$, where C_o and C_t are the concentrations of antibiotic in the middle ear initially and at 4 hours (6). As penetration through the middle ear mucosa decreased, so did the K value. Half-life was equal to 0.693/K; as penetration through the middle ear mucosa decreased, half-life (T½) increased. Statistical comparisons were made using the Student's t test for non-paired data.

On day 3, all ears in the influenza alone and the influenza plus *S. pneumoniae* groups had negative pressures by tympanometry. Influenza A virus was isolated from all nasal wash samples. Of the 60 ears studied, 12 were excluded from the K calculations for the following reasons. In the influenza alone group, 2 ears had tympanic membrane perforation secondary to catheter manipulation, and 2 had minimal recovery of the study solution. In the influenza plus *S. pneumoniae* group, 2 ears had tympanic membrane perforation, 1 had a negative bacterial culture, and 1 had minimal recovery of the antibiotic solution. In the *S. pneumoniae* alone group, 4 ears had minimal recovery of the antibiotic solution.

K values from a group of previously studied chinchillas with normal middle ears were used for comparison (6). These 6 control animals were studied 1 day after eustachian tube obstruction, as previously described (9). An additional group of 24 animals was studied to determine plasma clearance of the 3 drugs. These animals were divided into the same 4 groups and plasma samples taken at 1, 2, 3, 4, and 6 hours after the doses.

Results

The results of middle ear elimination studies for the 3 antimicrobials are summarized in Tables I (amoxicillin), II (sulfamethoxazole), and III (trimethoprim) and Figures 1 (amoxicillin), 2 (sulfamethoxazole), and 3 (trimethoprim). *S. pneumoniae* infection alone significantly shortened the middle ear elimination half-life compared with the control group:

Table II. Middle ear sulfamethoxazole pharmacokinetic characteristics in chinchillas with otitis media (a).

Experimental Conditions	Number of Ears Studied	K (mean ± SD) 1/hr	Half-life (mean ± SD) hr	4 Hr ME Concentration (mean ± SD) μg/ml
Control	10	0.27 ± 0.06 (b)	2.74 ± 0.61	20.92 ± 10.42 (c)
Influenza A alone	16	0.13 ± 0.02	5.46 ± 0.87	29.4 ± 2.5 (c)
Influenza A and *S. Pneumoniae*	16	0.30 ± 0.07 (b)	2.50 ± 0.85	13.59 ± 4.06
S. Pneumoniae alone	16	0.41 ± 0.06	1.75 ± 0.28	9.72 ± 2.5

(a) P values for K and concentration values between groups were <0.01. Symbols: ME = middle ear, K = elimination rate, hr = hour, SD = standard deviation
(b) p = 0.24
(c) p = 0.02

Table III. Middle ear trimethoprim pharmacokinetic characteristics in chinchillas with otitis media (a).

Experimental Conditions	Number of Ears Studied	K (mean ± SD) 1/hr	Half-life (mean ± SD) hr	4 Hr ME Concentration (mean ± SD) μg/ml
Control	10	0.46 ± 0.09	1.56 ± 0.34	1.38 ± 0.49 (b)
Influenza A alone	16	0.31 ± 0.17	2.57 ± 0.75	3.06 ± 1.02
Influenza A and *S. Pneumoniae*	16	0.58 ± 0.12 (c)	1.26 ± 0.42	1.06 ± 0.72 (b,d)
S. Pneumoniae alone	16	0.67 ± 0.10 (c)	1.06 ± 0.14	0.69 ± 0.26 (d)

(a) P values for K and concentration values between groups were <0.01. Symbols: ME = middle ear, K = elimination rate, hr = hour, SD = standard deviation
(b) p = 0.24
(c) p = 0.04
(d) p = 0.06

for amoxicillin, 2.65 ± 0.73 vs. 6.63 ± 2.55 hr; for sulfamethoxazole, 1.75 ± 0.28 vs. 2.74 ± 0.6 hr; and for trimethoprim, 1.06 ± 0.14 vs. 1.56 ± 0.34 hr (n = 16 ears, p values all <0.01). The combined influenza virus and *S. pneumoniae* infection significantly lengthened the half-life compared with the *S. pneumoniae* infection alone: amoxicillin, 5.65 ± 6.44 vs. 2.65 ± 0.73 hr; sulfamethoxazole, 2.5 ± 0.85 vs. 1.75 ± 0.28 hr; and trimethoprim, 1.26 ± 0.42 vs. 1.06 ± 0.14 hr (n = 16 ears, p values all <0.01). Influenza virus produced the longest half-lives for all 3 antimicrobials: amoxicillin, 25.52 ± 14.96 hr; sulfamethoxazole, 5.46 ± 0.87 hr; and trimethoprim, 2.57 ± 0.75 hr.

Plasma Clearance. Table IV shows that plasma clearance of the 3 drugs did not change under each experimental condition. An important trend can be seen in the combined virus and bacterial infection group, suggesting an increase in half-life. We measured creatinine levels in all the animals and found increased levels in the combined infection group that could cause an increased half-life of the 3 drugs.

Discussion

Patients with both a pathogenic bacterium and a virus isolated from the middle ear during acute otitis media have more antimicrobial failures, compared with patients who have middle ear bacterial infection alone (3, 4). We used an acute otitis media chinchilla model with decreased middle ear antimicrobial elimination as a marker of decreased middle ear penetration. We demonstrated *S. pneumoniae* infection significantly increased antimicrobial elimination, most likely because of increased vascular permeability (10). Combined influenza and *S. pneumoniae* infection decreased rates, as did viral infection alone, but to a greater extent. Treatment failures in combined influenza and *S. pneumoniae* infection, therefore, might be explained by decreased drug penetration.

We modified the traditional chinchilla model of otitis media that uses systemic antimicrobial administration (6). This new model uses antimicrobial diffusion out of the middle ear. It is not limited by small middle ear fluid volumes produced by either eustachian tube obstruction alone or acute middle ear bacterial infection (11). The diffusion model assumes that the rate of antibiotic elimination *from* middle ear fluid equals the rate of antibiotic penetration through the middle ear mucosa *into* the middle ear—passive middle ear diffusion occurs in both directions. This assumption is rea-

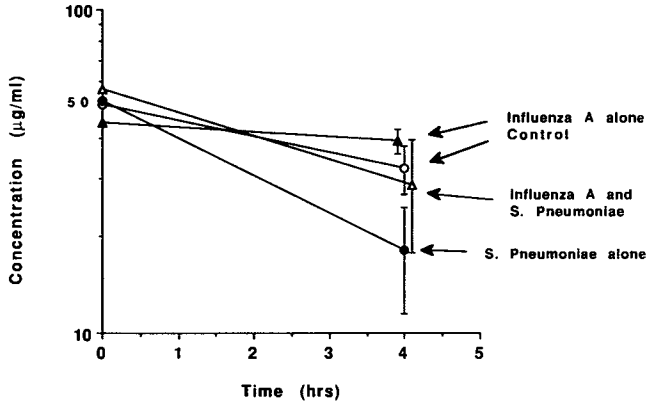

Figure 1. Shown are the effects of 3 middle ear mucosal conditions (influenza A alone, *S. pneumoniae* alone, and both influenza A and *S. pneumoniae*) on **amoxicillin** middle ear elimination rates, compared with control middle ear elimination rates.

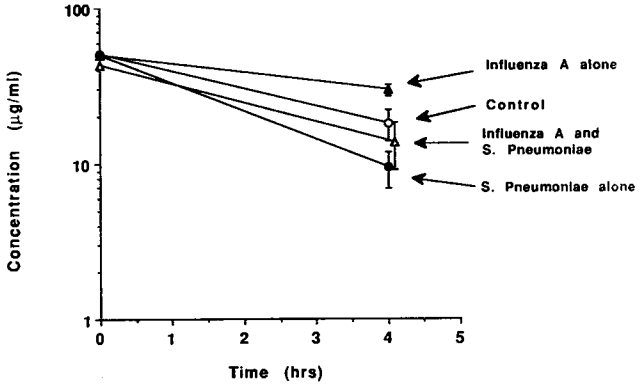

Figure 2. Shown are the effects of 3 middle ear mucosal conditions (influenza A alone, *S. pneumoniae* alone, and both influenza A and *S. pneumoniae*) on **sulfamethoxazole** middle ear elimination rates, compared with control middle ear elimination rates.

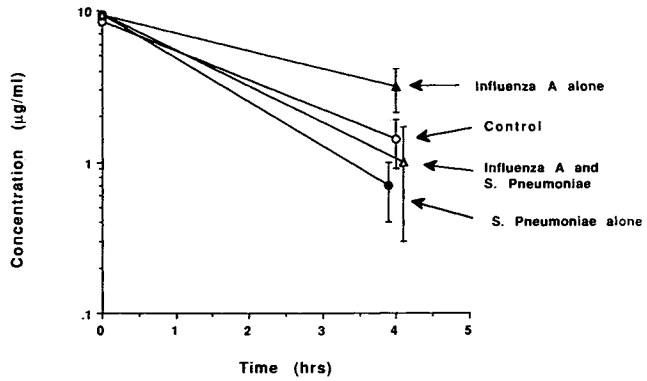

Figure 3. Shown are the effects of 3 middle ear mucosal conditions (influenza A alone, *S. pneumoniae* alone, and both influenza A and *S. pneumoniae*) on **trimethoprim** middle ear elimination rates, compared with control middle ear elimination rates.

sonable, since active antibiotic transport into the middle ear has not been demonstrated. Therefore, as the rate constant for antibiotic elimination from the middle ear decreases, the penetration rate also decreases. Conversely, as the disappearance half-life increases, the penetration rate decreases. Using a crossover study design, we compared the systemic drug administration model with the diffusion model; it appears K values derived from either are similar (12).

How can these effects be related to the clinical situation, such as a child with acute otitis media? The true "control" condition is the middle ear with a bacterial infection alone. Not all patients with pure bacteria-induced acute otitis media respond to treatment; therefore, our finding that elimination of antimicrobials from the middle ear suggests that effective concentrations may not always be maintained.

When a virus is present with bacteria, antimicrobial elimination is decreased, compared with bacteria alone. We are assuming that penetration into and elimination from the middle ear are the same (12). This suggests a virus may decrease the ability of an antimicrobial to enter the middle ear and kill the bacteria. When the elimination of an antimicrobial is measured in the presence of a viral infection alone, elimination is further decreased compared with bacteria alone. This supports the notion that viral infection can affect middle ear antimicrobial penetration.

The use of an uninfected "control" group is for comparison of the 3 experimental conditions with a near "normal" middle ear. However, the middle ear condition is of interest only when bacteria are present, since this is the reason for administering antimicrobials.

It is possible that changes in plasma clearance of the 3 antimicrobials could affect middle ear drug concentrations. We did not see a statistically significant change, but did note an important trend toward increased clearance in the combined viral and bacterial group, potentially altering the concentration gradient between the plasma and middle ear and thus producing a longer half-life of antimicrobial elimination from the ear. The cause of this may have been decreased renal function, which we confirmed with creatinine levels. Since this effect was not seen in either group infected with virus alone or bacteria alone, it is of little importance here.

The differences we found in the antimicrobial penetration rate between chinchillas infected with influenza virus alone vs. *S. pneumoniae* alone suggest that these organisms produce different middle ear mucosal inflammatory changes. In chinchillas intranasally inoculated with influenza A virus alone, we previously demonstrated epithelial damage, goblet cell metaplasia, increased eustachian tube secretory activity, and accumulation of cellular and mucous debris in the tubal lumen (13). A parallel study showed that intranasal influenza A virus inoculation caused middle ear polymorphonuclear and mononuclear leukocyte infiltration, epithelial metaplasia, vascular congestion, fibrous tissue, and subepithelial thickening throughout the middle ear mucoperiosteum (14). Still another study found that influenza A virus inoculated directly into the middle ear caused inflammatory cell infiltration into the epithelial and subepithelial layers and damage to the ciliary apparatus (15).

Upper respiratory infection with various viruses is strongly associated with acute otitis media in children (16). These infections probably cause eustachian tube obstruction and middle ear histopathologic changes. The mucosal histopathologic changes may affect antimicrobial penetration into the middle ear, decreasing the amount of drug available for bactericidal activity. In contrast, the increased penetra-

Table IV. Amoxicillin, trimethoprim, and sulfamethoxazole plasma half-life values in chinchillas with acute otitis media (a).

Experimental Conditions	Number of Animals Studied	Amoxicillin (mean ± SD) hr	Trimethoprim (mean ± SD) hr	Sulfamethoxazole (mean ± SD) hr	Creatinine Concentration (mean ± SD) µg/ml
Control	6	1.6 ± 0.5 (b)	1.9 ± 0.58	6.8 ± 0.74	0.7 ± 0.2 (c)
Influenza A alone	5	1.5 ± 0.5	1.9 ± 0.8	6.1 ± 1.2	1.0 ± 0.3
Influenza A and *S. Pneumoniae*	4	4.8 ± 2.7 (b)	2.8 ± 1.1	8.4 ± 2.4	1.2 ± 0.2 (c)
S. Pneumoniae alone	6	1.6 ± 0.3	1.9 ± 0.7	6.6 ± 1.1	0.8 ± 0.2

(a) P values for half-life values between groups were <0.01. Symbols: ME = middle ear, K = elimination rate, hr = hour, SD = standard deviation.
(b) p = 0.03
(c) p = 0.04

tion seen in chinchillas infected with *S. pneumoniae* is most likely due to the effects of bacterial cell surface components and host inflammatory mediators released by the presence of bacteria (17).

Decreased middle ear antimicrobial drug penetration is probably not the only cause of otitis media treatment failure. Other factors such as the patient's immune response, middle ear fluid composition, and concomitant therapy may play an important role as well. Further research is needed to better define the factors that affect penetration. This will enhance our understanding of the role of middle ear drug penetration in otitis media antimicrobial treatment failures and further elucidate the many mechanisms that cause treatment failures.

Acknowledgment

We thank Moses Quartey, Carol Liebeler, Naomi Johnson, and Jeff Steury for technical assistance, and the Virology Laboratory of the Department of Laboratory Medicine and Pathology, University of Minnesota, for performing viral isolation. Supported by grant number PO1-DC00133 from the National Institute on Deafness and Other Communication Disorders and the Viking Children's Fund.

References

1. G. S. Giebink, D. M. Canafax, J. Kempthorne. Antimicrobial treatment of acute otitis media. *J. Pediatr.* 119(3):495–500 (1991).
2. D. W. Teele, S. I. Pelton, J. O. Klein. Bacteriology of acute otitis media unresponsive to initial antimicrobial therapy. *J. Pediatr.* 98:537–539 (1981).
3. T. Chonmaitree, M. J. Owen, V. M. Howie. Respiratory viruses interfere with bacteriologic response to antibiotics in children with acute otitis media. *J. Infect. Dis.* 162:546–549 (1990).
4. M. Arola, T. Ziegler, O. Ruuskanen. Respiratory virus infection as a cause of prolonged symptoms in acute otitis media. *J. Pediatr.* 116:697–701 (1990).
5. G. S. Giebink, I. K. Berzins, S. C. Marker, G. Schiffman. Experimental otitis media after nasal inoculation of Streptococcus pneumoniae and influenza A virus in chinchillas. *Infect. Immun.* 30:445–450 (1980).
6. G. H. Jossart, G. R. Erdmann, D. G. Levitt, P. Kucera, C. T. Le, S. K. Juhn, G. S. Giebink, D. M. Canafax. An experimental model for measuring middle ear antimicrobial drug penetration in otitis media. *Pharmaceutical Research.* 7(12):1242–1247 (1990).
7. G. R. Erdmann, C. Walker, G. S. Giebink, D. M. Canafax. The analysis of amoxicillin concentrations by high-performance liquid chromatography in experimental middle ear effusion. *J. Liq. Chromatogr.* 13(16):3339–3350 (1990).
8. G. R. Erdmann, D. M. Canafax, G. S. Giebink. High-performance liquid chromatographic analysis of trimethoprim and sulfamethoxazole in microliter volumes of chinchilla middle ear effusion and serum. *J Chromatogr.* 433:187–195 (1988).
9. G. S. Giebink, W. L. Meyerhoff, and E. I. Cantekin. In *Experimental Models in Antimicrobial Chemotherapy, Vol. 1,* Academic Press, London, 1986, pp. 213–36.
10. N. Nanomura, G. S. Giebink, S. K. Juhn, T. Harada, D. Aeppli. Pathophysiology of Streptococcus pneumoniae otitis media: Kinetics of the middle ear biochemical and cytologic host response. *Ann. Otol. Rhinol. Laryngol.* 100:236–243 (1991).
11. D. M. Canafax, N. Nonomura, G. R. Erdmann, C. T. Le, S. K. Juhn, G. S. Giebink. Experimental animal models for studying antimicrobial pharmacokinetics in otitis media. *Pharmaceutical Research* 6:279–285 (1989).
12. D. M. Canafax, M. Lovdahl, H. Russile, G. R. Erdmann, C. T. Le, G. S. Giebink. Comparison of two otitis media models for the study of middle ear antimicrobial pharmacokinetics. *Pharmaceutical Research.* 11(6):855–859 (1994).
13. G. S. Giebink, M. L. Ripley, P. F. Wright. Eustachian tube histopathology during experimental influenza A virus infection in the chinchilla. *Ann Otol Rhinol Laryngol.* 96:199–206 (1987).
14. M. L. Ripley-Petzoldt, M. Solfelt, P. F. Wright, M. M. Paparella, G. S. Giebink. Middle ear and eustachian tube histopathology during experimental influenza A virus infection. Recent Advances in Otitis Media. D. J. Lim (ed), Philadelphia, PA: BC Decker Inc, (1988).
15. D. J. Lim, O. Yoshihiro, D. Myung-Hyun, T. F. DeMaria. Ultrastructural pathology of the tubotympanum following experimental influenza A virus induced otitis media. Recent Advances in Otitis Media. D. J. Lim (ed), Philadelphia, PA: BC Decker Inc, (1988).
16. F. W. Henderson, A. M. Collier, M. A. Sanyal, et al. A longitudinal study of respiratory viruses and bacteria in the etiology of acute otitis media with effusion. *N. Engl. J. Med.* 306:1377–1383 (1982).
17. D. J. Lim. Recent advances in otitis media: Report of the fourth research conference. *Ann Otol Rhinol Laryngol.* 136:23–32 (1988).