Pharmaceutical Research, Vol. 6, No. 4, 1989

Research Article

Experimental Animal Models for Studying Antimicrobial Pharmacokinetics in Otitis Media

Daniel M. Canafax,[1,2,7] Naobumi Nonomura,[1,6] Gary R. Erdmann,[1,2] Chap T. Le,[1,5] Steven K. Juhn,[1,3] and G. Scott Giebink[1,3,4]

Received August 23, 1988; accepted November 15, 1988

Antimicrobial treatment of otitis media, especially drug dosing considerations, is largely empiric, with few reported pharmacologic studies of drug distribution into the middle ear. A chinchilla animal model of serous and purulent otitis media has been used for some time to investigate mechanisms of disease pathogenesis. This model was adapted to investigate the penetration of amoxicillin, trimethoprim, and sulfamethoxazole into middle ear effusion. Purulent otitis media was produced by direct middle ear inoculation with type 7F *Streptococcus pneumoniae*. Serous otitis media was produced by eustachian tube obstruction using silastic sponge or Coeflex cement, but the Coeflex caused an undesirable local inflammatory response. The three antibiotics were administered to chinchillas with serous and purulent middle ear effusion. Plasma and ear fluid drug concentrations were measured by liquid chromatography and demonstrated the value of this model in assessing antibiotic penetration.

KEY WORDS: otitis media; pharmacokinetics; amoxicillin; trimethoprim; sulfamethoxazole.

INTRODUCTION

Bacteria contribute substantially to the pathogenesis of acute and chronic otitis media. Although antimicrobial agents are commonly used to treat otitis media, controversies abound in clinical practice over drug selection, treatment duration, and treatment failure (1). To enhance our understanding of otitis media treatment and improve treatment efficacy, we adapted an animal model of otitis media to study antibiotic pharmacokinetics. The animal model employed chinchillas and has been extensively used to study otitis media pathophysiology (2). A critical step in using the model for studying antibiotic pharmacokinetics was the development of procedures which allowed serial studies of antibiotic concentrations in plasma and middle ear fluid. This report describes the adaptation of the chinchilla model of serous and purulent otitis media to antibiotic pharmacokinetic studies. The adaptation permitted analysis of drug concentrations on serial samples of very small volumes of ear fluid.

MATERIALS AND METHODS

Chinchillas used were 1 to 2 years of age, weighed 400 to 800 g, were in good health, were housed individually, and were given food and water *ad lib*. All procedures and handling were approved by the University of Minnesota Research Animal Resources Division. Animals were anesthetized for all procedures with ketamine, 20 mg/kg intramuscularly.

Serous otitis media was produced by surgically exposing the eustachian tubes in each animal through the soft palate and inserting either three 1- to 2-mm silastic sponges (Dow Corning, Midland, Mich.) or approximately 0.1 ml of Coeflex paste (Coe Lab, Chicago) to obstruct the tube. Purulent otitis media was produced by direct inoculation of type 7F *Streptococcus pneumoniae* into the lower portion of each bulla as previously described (3). Log-phase bacteria were used, and 0.1 ml of phosphate-buffered saline solution, pH 7.4, containing about 400 colony forming units/ml was inoculated.

Otoscopy and tympanometry were performed at 2- to 5-day intervals after surgery. Tympanic membrane color and opacity were scored as previously described (4), and tympanogram configurations were classified into 10 types (Table I).

Histologic analysis was performed on temporal bones fixed in 10% formalin with phosphate-buffered saline, decalcified with 10% trichloracetic acid, and embedded in JB-4

[1] University of Minnesota Otitis Media Research Center, University of Minnesota, Minneapolis, Minnesota 55455.
[2] Department of Pharmacy Practice, College of Pharmacy, University of Minnesota, Minneapolis, Minnesota 55455.
[3] Department of Pediatrics, School of Medicine, University of Minnesota, Minneapolis, Minnesota 55455.
[4] Department of Otolaryngology, School of Medicine, University of Minnesota, Minneapolis, Minnesota 55455.
[5] Biometry Division, School of Public Health, University of Minnesota, Minneapolis, Minnesota 55455.
[6] Department of Otolaryngology, Niigata University School of Medicine, Niigata, Japan.
[7] To whom correspondence should be addressed at Department of Pharmacy Practice, HSUF 7-107, College of Pharmacy, University of Minnesota, Minneapolis, Minnesota 55455.

0724-8741/89/0400-0279$06.00/0

(Polysciences, Warrington, Pa.). Sections were to cut to a 4-μm thickness and stained with hematoxylin, eosin, and toluidine blue.

Antibiotics were given intramuscularly, and five 0.5-ml blood samples were taken by cardiac puncture serially at 0.5, 1, 2, 4, and 5 hr after the amoxicillin dose and at 1, 2, 5, 8, and 12 hr after the trimethoprim/sulfamethoxazole dose. Blood samples were placed in EDTA-containing tubes and immediately centrifuged, and the plasma was separated and frozen at −70°C until analysis.

Middle ear effusion was sampled in POM at day 5 and in SOM between 2 and 4 weeks after eustachian tube obstruction (ETO) by placing a 16-gauge needle into the epitympanic bulla of the anesthetized animal and aspirating fluid by passing a thin catheter through the needle into the base of the bulla (3). Care was taken not to perforate the tympanic membrane. Four serial samples of approximately 50–100 μl each were taken between 2 and 12 hr after antibiotic injection.

Antibiotic Concentration Analysis

Methods to analyze trimethoprim, sulfamethoxazole, and amoxicillin concentrations in microliter volumes of plasma and effusion were developed using high-performance liquid chromatography. Trimethoprim and sulfamethoxazole were analyzed in 25 μl of effusion and 50 μl of plasma. Middle ear effusion samples were adjusted to pH 6.2 and extracted with 25% ethyl acetate:75% dichloromethane (v/v) containing cimetidine as the internal standard. The plasma samples were adjusted to pH 7.4 and extracted in the same manner as middle ear effusion. The organic layer was collected, evaporated to dryness under nitrogen, and reconstituted in methanol. Trimethoprim and sulfamethoxazole were separated on a cyanopropylsilane column using 16% acetonitrile:84% NaH_2PO_4 (v/v), 40 m*M*, pH 4.8, at a flow rate of 1.8 ml/min. The internal standard and trimethoprim were monitored at 230 nm; sulfamethoxazole was detected at 250 nm. The sensitivity of trimethoprim and sulfamethoxazole was 0.1 and 0.5 μg/ml, respectively, in both plasma and middle ear effusion.

Amoxicillin was determined in 75 μl of plasma and middle ear effusion. An aliquot of the sample was transferred to a glass tube containing 25 μl of hydroflumethiazide, 50 μg/ml (internal standard). The proteins were precipitated by adding 25 μl of 10% perchloric acid (w/v) and the excess perchlorate was removed by adding 25 μl of 0.9 *M* KCl. The samples were centrifuged and the supernatant was collected and adjusted to pH 3 by adding 25 μl of NaH_2PO_4, 0.8 *M*, pH 10.4. A 6-μl volume was injected onto the column. Chromatography was performed using an octylsilane column with a mobile phase consisting of 6% methanol:0.5% acetonitrile:93.5% (v/v) NaH_2PO_4, 10 m*M*, pH 2.8. The flow rate was 1.4 ml/min and the column effluent was monitored at 230 nm. The limit of sensitivity was 0.25 μg/ml for both plasma and middle ear effusion.

RESULTS

Eustachian Tube Obstruction Modeling

Bilateral eustachian tube obstruction (ETO) with silastic sponge was performed on 19 animals (38 ears), a second silastic sponge obstruction was attempted on 13 of these animals (26 ears), and a third attempt on six animals (12 ears).

Middle ear pressure data for all of the 72 silastic sponge-obstruction attempts are shown in Fig. 1. Preoperative middle ear pressures were slightly positive, as we have previously documented in chinchillas during ketamine anesthesia (5).

Eighteen of the 19 silastic sponge-obstructed chinchillas survived at least 1 week after the initial surgery; one died of peritonitis on day 3. Of the 18 surviving animals, 19 (53%) of the 36 ears showed a profound fall in middle ear pressure to levels below −100 mm H_2O between 6 and 10 days after surgery, consistent with tubal obstruction. However, pressure returned toward normal in 13 of the 19 negative pressure ears between 2 and 6 weeks after surgery.

A second silastic sponge obstruction was attempted on these 13 ears and on 13 ears that failed to respond to the initial surgery; 22 of these 26 ears (85%), including 11 of 13 that failed the initial surgery, developed profound negative pressure during the week after the second surgery. However, pressure again returned toward normal in 8 of the 22 negative-pressure ears followed to 6 weeks after surgery.

A third silastic sponge obstruction was attempted on these 8 ears and on 2 ears that failed the second surgery, and 5 of the 10 ears (50%) developed profound negative pressure during the week after the third surgery; neither of the two ears that failed to respond to the second procedure developed negative pressure with the third procedure.

Bilateral ETO with Coeflex was performed on 13 animals (25 ears) as reported by Jung *et al.* (6). Middle ear pressure data for all of the 25 Coeflex-obstruction attempts are shown in Fig. 2. All 13 of the Coeflex-obstructed chinchillas survived at least 1 week after surgery; five animals died during the subsequent 3 weeks—one of sepsis on day 9 post-ETO, two of pulmonary edema (days 20 and 24), one of labyrinthitis (day 16), and one of pneumonia (day 7). Twenty-one of the 25 operated ears (84%) showed a profound fall in middle ear pressure to levels below −100 mm

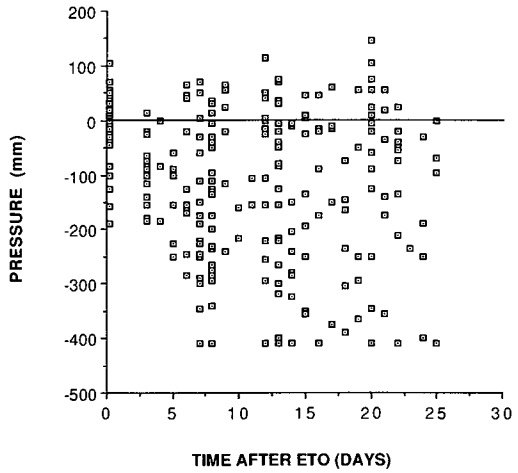

Fig. 1. A scatter plot of middle ear pressures is shown for 72 animals with bilateral silastic sponge eustachian tube obstruction.

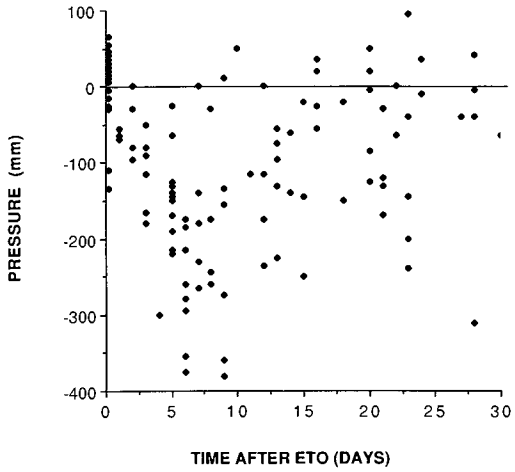

Fig. 2. Middle ear pressures are shown for 13 animals with bilateral Coeflex obstructed eustachian tubes.

H_2O between 6 and 10 days after surgery. However, pressure returned toward normal in 9 of 13 negative-pressure ears between 2 and 4 weeks after surgery.

Middle ear aspiration was performed on 35 of the silastic-obstructed ears between day 7 and day 81 after surgery. Effusion was obtained from 18 ears (51%), and all were sterile. Coeflex-obstructed ears were aspirated between 6 and 50 days postsurgery and 13 ears (57%) had sterile effusion, 2 ears (9%) had culture positive effusion, and 9 ears (37%) had no effusion (tapped on days 6, 6, 8, 12, 24, 24, 29 and 29). The majority of effusion volumes ranged between 0 and 500 μl (Fig. 3). Similar effusion volumes (0 to 800 μl) were found in 11 animals (22 ears) at 5 days after inducing POM.

Pressures less than or equal to -100 mm H_2O were commonly associated with effusion, and pressures greater than -100 mm H_2O were rarely associated with effusion (Table I). Tympanogram configurations of the As, C1s, C2, C2d, C2s, and B types were almost always associated with

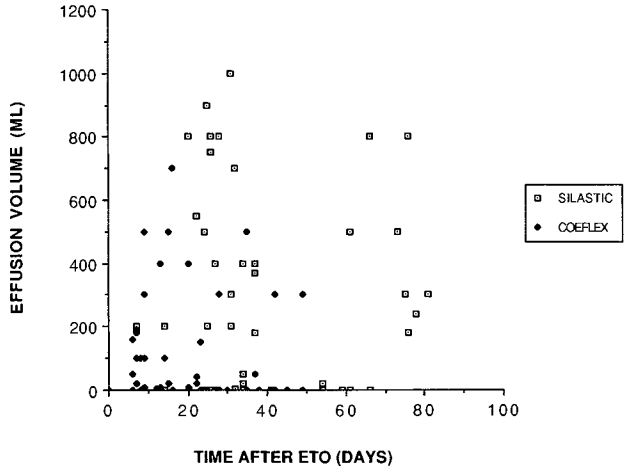

Fig. 3. A scatter plot of middle ear effusion volumes is shown at various times after obstruction for animals with both silastic and Coeflex methods.

Table I. Relationship Between Middle Ear Pressure and the Presence of Middle Ear Effusion

Middle ear pressure (mm H_2O)	Number (%) of ears with middle ear effusion	
	Present	Absent
-10 to $+95$	5 (17)	24
-100 to -11	15 (38)	24
-200 to -101	31 (61)	20
-400 to -201	49 (82)	11
<-400	21 (88)	3
Total	121 (60)	82

effusion, whereas types A, Ad, C1, and C1d configurations were not often associated with effusion (Table II). Tympanic membranes which scored 0–3 (i.e., gray or red) were infrequently associated with effusion, and membranes scoring 4 and 5 (i.e., yellow) were often associated with effusion (Table III).

Combining information from otoscopy and tympanometry resulted in an algorithm which yielded four predictive levels for effusion (Table IV). Ears with type A, Ad, C1, or C1d tympanogram configurations and tympanic membrane scores less than 4 were defined as a negative algorithm result, and ears with an As, C1s, C2, C2d, C2s, or B configuration regardless of the membrane score were defined as a positive algorithm result; other ears were unclassified. The diagnostic algorithm yielded 88% classified ears, for which the algorithm had a sensitivity of 97.5% (157 of 161) and a specificity of 74.2% (46 of 62); of the 12% unclassified ears, the probability of effusion was 42%.

Roentgenographic Detection of Effusion

The ability to detect middle ear effusion by roentgenography was examined in Coeflex-injected animals using a posterior–anterior projection with the animals lying prone on the film cassette. The roentgenogram did not accurately reflect the volume of Coeflex injected into the eustachian tube, but in several cases the roentgenogram showed Coeflex which had extravasated into the tissues surrounding the tubal lumen. Of the 213 ears examined by this technique, only 4 contained effusion on aspiration; the roentgenogram suggested that effusion was "possible" in 2 and "absent" in 2. All 19 ears without effusion had roentgenographic scores of "absent effusion." Thus, the sensitivity of the roentgenogram was only 50% (2 of 4), while the specificity was 100% (19 of 19).

Histopathology

A histological study was performed to compare eustachian tube and labyrinthine bulla histopathology caused by Coeflex and silastic sponge. Two chinchillas had Coeflex injected into one eustachian tube and silastic sponge in the contralateral tube (Fig. 4), two animals had unilateral silastic sponge implants, and two animals had unilateral Coeflex implants. All animals were sacrificed 7 days after surgery and the temporal bones were examined microscopically and re-

Table II. Tympanogram Configurations and Relationship to the Presence of Middle Ear Effusion

Configuration	Pressure (mm H_2O)	Compliance	Number (%) of ears with middle ear effusion	
			Present	Absent
A	−10 to +50	0.4 to 2.1	1 (5)	21
As	−10 to +50	<0.4	4 (100)	0
Ad	−10 to +50	>2.1	0 (0)	3
C1	−100 to −11	0.4 to 2.1	11 (44)	14
C1s	−100 to −11	<0.4	4 (100)	0
C1d	−100 to −11	>2.1	0 (0)	10
C2	−400 to −101	0.4 to 2.1	50 (75)	17
C2s	−400 to −101	<0.4	31 (89)	4
Csd	−400 to −101	>2.1	2 (15)	11
B	< −400	<0.4	21 (88)	3
Total			122 (60)	83
A, Ad, C1, C1d			12 (20)	48
As, C1s, C2, C2d, C2s, B			110 (76)	35

vealed marked destruction of tubal epithelium adjacent to the Coeflex and silastic sponges. However, the quantity of polymorphonuclear (PMN) leukocytes infiltrating the tissues surrounding the implants was appreciably greater with Coeflex than with silastic sponges. Ciliated epithelial cells near the tympanic orifice of the tubes were equally preserved with both types of implant.

Subepithelial space thickening due to edema fluid in the labyrinthine bulla was more severe in bullae obstructed with Coeflex than with silastic sponge. Significant osteoneogenesis, reflecting chronic inflammation, was also observed in the labyrinthine bulla on the side of Coeflex obstruction but not with silastic sponge obstruction.

The tissue reaction to Coeflex was measured by implanting both paste and solidified Coeflex in the subcutaneous tissues of the back of one chinchilla. Tissue biopsy was performed 4 days after implanting the material and was processed with routine hematoxylin and eosin stains. Microscopic examination revealed an acute inflammatory reaction with many PMN and mononuclear leukocytes and fibroblasts surrounding both types of Coeflex, but the reaction was more intense surrounding the Coeflex paste.

Table III. Relationship Between the Tympanic Membrane Score and the Presence of Middle Ear Effusion

Tympanic membrane score (description)	Number (%) of ears with middle ear effusion	
	Present	Absent
0 (gray/translucent)	0 (0)	8
1 (gray/opaque)	7 (20)	28
2 (red/translucent)	4 (50)	4
3 (red/opaque)	11 (44)	14
4 (yellow/translucent)	50 (76)	16
5 (yellow/opaque)	49 (91)	5
Total	121 (62)	75

Antimicrobial Pharmacokinetic and Penetration Studies

Amoxicillin plasma and middle ear concentrations were measured in chinchillas with purulent otitis media over time, after a single 100-mg/kg im dose. Rapid decline in plasma amoxicillin concentrations was observed at a half-life of 0.9 hr after a maximum value of 82 μg/ml was achieved in a representative animal (Fig. 5).

The effusion concentrations lagged behind the plasma values, as expected, and reached a maximum concentration of 31 μg/ml. The half-life of amoxicillin from the effusion was 1.6 hr, which was slower than the elimination rate from plasma. The areas under the curves (AUC) for plasma and effusion were 103 and 86 μg · hr/ml, respectively, suggesting relatively similar exposure for the two body compartments.

Amoxicillin concentrations in plasma and middle ear effusion from animals with serous otitis media showed the same plasma concentration behavior as in purulent otitis media. However, in this model of eustachian tube obstruction the maximum effusion concentration achieved was 3.1 μg/ml, which is 10-fold less than that seen in the infected animals as shown by a representative animal (Fig. 6). In addition, the rate of amoxicillin elimination from the middle ear was slower in serous otitis media than in purulent otitis media (half-life, 9.2 vs 1.6 hr). Also, the AUC in serous effusion was about half that of purulent effusion value (41 vs 86 μg · hr/ml).

Trimethoprim and sulfamethoxazole plasma concentrations after a 1‰-mg/kg dose demonstrated an elimination half-life of 1.2 and 2.5 hr, respectively in a representative animal (Figs. 7 and 8). The rate of middle ear effusion penetration in serous otitis media was faster for trimethoprim than sulfamethoxazole, as was the rate of removal from the effusion (i.e., trimethoprim half-life was 2.2 hr, and sulfamethoxazole 6.1 hr).

DISCUSSION

The goals of acute otitis media treatment are to provide

Table IV. An Algorithm for Middle Ear Effusion Derived from the Results of Otoscopy and Tympanometry

Tympanogram configuration	Tympanic membrane score	Number (%) of ears with middle ear effusion	
		Present	Absent
A, Ad, C1, C1d	0–3	4 (8)	46
	4–5	10 (42)	14
As, C1s, C2, C2d, C2s, B	0–3	18 (69)	8
	4–5	89 (92)	8

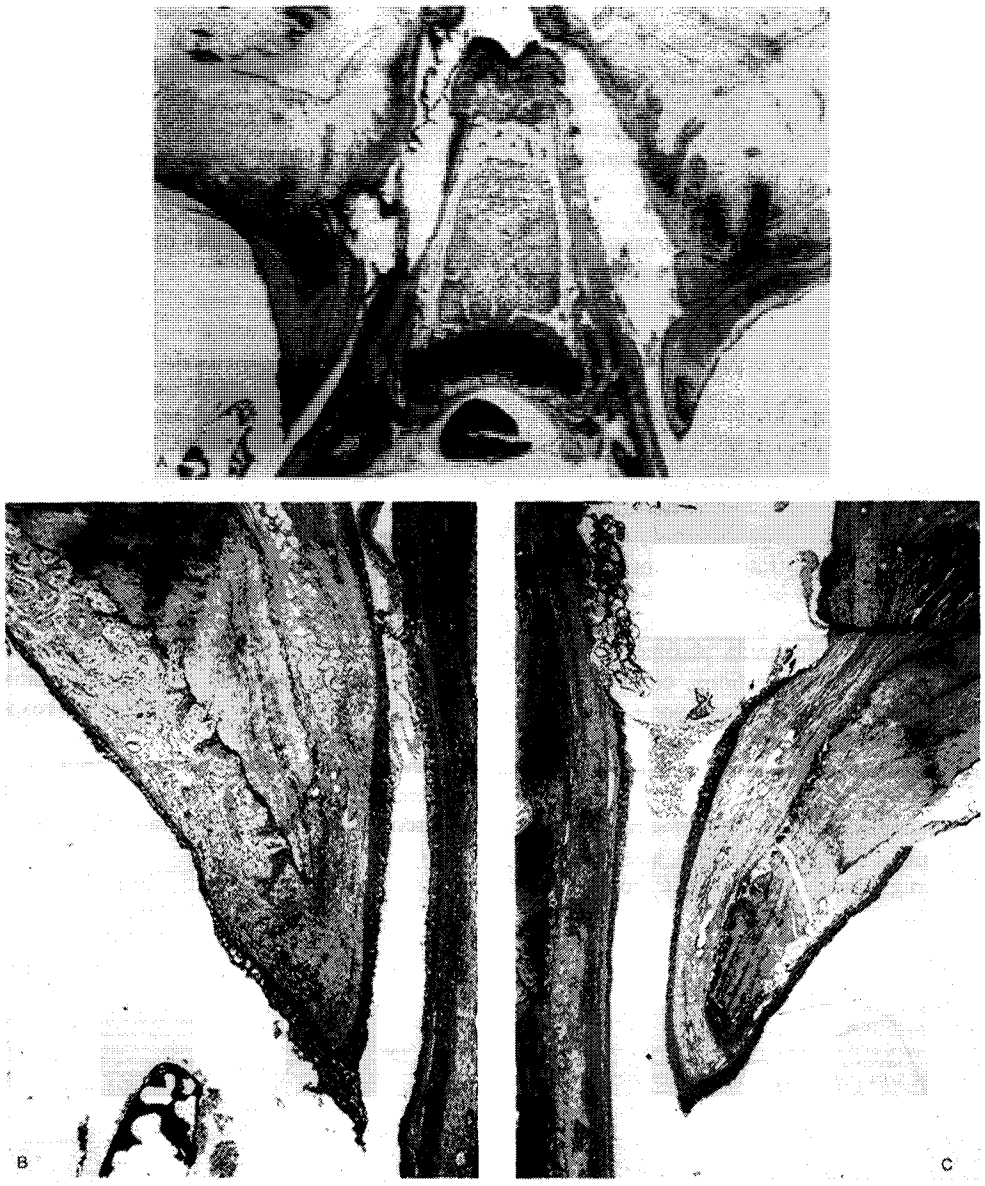

Fig. 4. Shown is a histologic comparison of Coeflex (left) and silastic sponge (right) eustachian tube obstruction techniques at 7 days after the operation. A is oriented with the nasopharynx at the top. B and C (Coeflex and silastic sponge, respectively), show the inflammatory response to Coeflex with neutrophil infiltration, mucosal thickening, and new bone formation. (A) 2.5× and (B,C) 10×; reduced 45% for magnification.

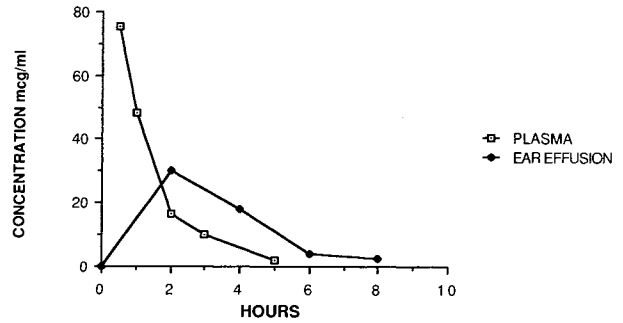

Fig. 5. Amoxicillin concentrations in plasma and *S. pneumoniae*-induced purulent middle ear effusion are shown from a representative chinchilla given 100 mg/kg im.

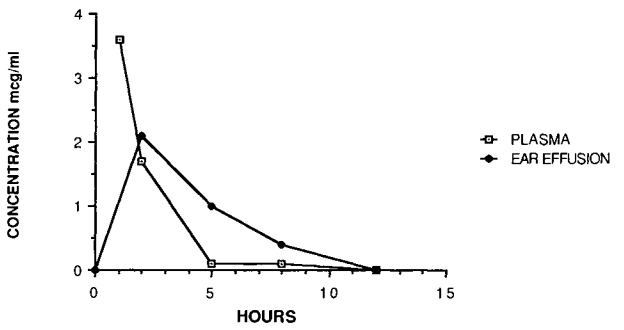

Fig. 7. Trimethoprim concentrations in plasma and serous middle ear effusion are shown for a representative animal given 10 mg/kg im.

symptomatic relief, resolve middle ear fluid, and minimize recurrence, complications, and sequelae. The use of antimicrobial agents to treat this disease partially achieve these goals since bacteria play an etiologic role in otitis media pathogenesis (7). The current approach to antibiotic selection for treating acute otitis media is based on the antimicrobial susceptibility of *Streptococcus pneumoniae, Hemophilus influenzae,* and *Branhamella catarrhalis* strains cultured from the general population. Antibiotic doses are based on body weight alone and do not account for differences in each patient's drug clearance, extent of middle ear penetration, and ability of the antibiotic to kill organisms in the middle ear.

We report here a chinchilla model of serous and purulent otitis media that allows for serial sampling of blood and middle ear effusion for studying antibiotic pharmacokinetic characteristics and for biochemical, microbiologic, and histopathologic events that occur during the treatment of acute otitis media. This report demonstrates that antibiotic drug concentrations can be measured in the small volume of effusion available after serial sampling from each animal. The chromatographic analysis of chinchilla plasma and middle ear effusion for antibiotic concentrations, complicated by the relatively small sample volumes and some interfering substances, was critical to achieving this result. In addition, ETO is a delicate operation that requires care to assure success.

Penetration was measured in models of serous and purulent effusion for amoxicillin, trimethoprim, and sulfamethoxazole. All three drugs disappeared rapidly from plasma, much like the rapid clearance seen in children given these agents. Interestingly, the rates of accumulation in middle ear effusion differed among the three drugs as did the elimination rates from the effusion. Moreover, the extent of middle ear effusion amoxicillin penetration was less in serous than purulent otitis media. This observation may conflict with current thinking that serous effusion is formed primarily by transudative processes.

The observations with amoxicillin that penetration into purulent effusion is greater than into serous effusion and that elimination from purulent effusion is faster than from serous effusion suggest that middle ear membranes are more permeable during infection. Since middle ear amoxicillin concentrations were consistently less than plasma values, middle ear fluid during infection is not a perfect transudate, and some membrane barrier persists.

Based on these preliminary antibiotic concentration results, it is tempting to speculate that clinical responses to antimicrobial therapy will be different for each drug, and these differences may explain some treatment failures. These animal models will permit the future study of various antibiotic doses used clinically to treat otitis media and the assessment of middle ear microbiologic response. The modeling of plasma and effusion concentrations in animals to predict effusion concentrations in animals and, eventually, in humans will improve the understanding of treatment response.

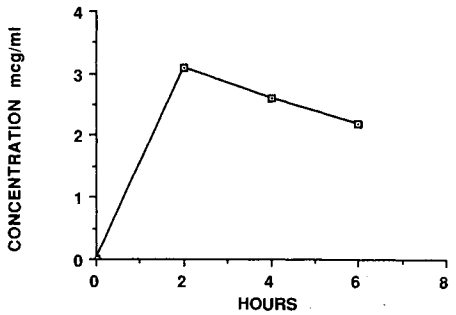

Fig. 6. Amoxicillin concentrations in serous middle ear effusion are shown for a single chinchilla after a 100-mg/kg im dose.

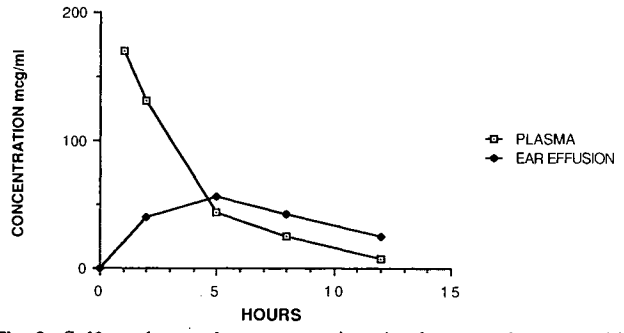

Fig. 8. Sulfamethoxazole concentrations in plasma and serous middle ear effusion are shown for a representative animal given 50 mg/kg im.

ACKNOWLEDGMENTS

This work was supported in part by Grant NS-14538 from the National Institute of Neurological and Communicative Disorders and Stroke.

We would like to thank Michelle Hulse and Jozette Bley for performing some of the surgical procedures and JoAnn Knox for preparing the manuscript.

REFERENCES

1. G. S. Giebink and D. M. Canafax. In S. C. Aronoff, W. T. Hughes, S. Kohl, N. T. Speck, and E. R. Wald (eds.), *Advances in Pediatric Infectious Diseases,* Year Book Medical, Chicago, Vol. 3, 1988.
2. 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.
3. G. S. Giebink. *Rev. Infect. Dis.* 3:342–352 (1981).
4. G. S. Giebink. *Infect. Immunol.* 41:913–920 (1983).
5. G. S. Giebink, R. A. Heller, and E. R. Harford. *Ann. Otol. Rhinol. Laryngol.* 91:20–24 (1982).
6. T. T. K. Jung, S.-J. Hwang, D. Pool, *et al.* In D. J. Lim (ed.), *Recent Advances in Otitis Media,* B. C. Decker, Philadelphia, 1988.
7. M. L. Ripley-Petzoldt and G. S. Giebink. *J. Infect. Dis.* 157:245–255 (1988).