Pharmaceutical Research, Vol. 7, No. 12, 1990

Report

An Experimental Model for Measuring Middle Ear Antimicrobial Drug Penetration in Otitis Media

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Received April 13, 1990; accepted May 30, 1990.

Bacteria are an important cause of acute otitis media and successful treatment depends on achieving inhibitory or bacteriacidal antimicrobial drug concentrations in the middle ear. To evaluate further otitis media treatment success and failure, we developed a chinchilla model to study antimicrobial drug penetration through the middle ear mucosa. Using quantitative histomorphometry, we measured the middle ear space in 10 chinchillas and found a mean ± SD volume of 2.09 ± 0.08 ml and a mean ± SD surface area of 14.41 ± 1.48 cm^2. To measure the apparent rate constant (K_e) of antibiotic elimination from the middle ear, through the middle ear mucosa, an antibiotic solution was inoculated into the middle ear cavity, and samples were aspirated between 1 and 8 hr later. In normal ears, the mean K_e ± SD for amoxicillin was 0.118 ± 0.013 hr^{-1}, that for a trimethoprim 0.461 ± 0.090 hr^{-1}, and that for sulfamethoxazole 0.265 ± 0.062 hr^{-1}. In ears inoculated with type 7F *Streptococcus pneumoniae* to induce acute otitis media, the K_e ± SD increased for all three drugs ($P < 0.05$): amoxicillin to 0.286 ± 0.089 hr^{-1}, trimethoprim to 0.662 ± 0.118 hr^{-1}, and sulfamethoxazole to 0.411 ± 0.056 hr^{-1}. These values demonstrate that amoxicillin had the lowest apparent penetration rate constant of the three antibiotics but the greatest increase from normal to infected mucosa (142%). Trimethoprim had the highest apparent penetration rate constant of the three antibiotics but the smallest increase from normal to infected mucosa (44%), while the sulfamethoxazone apparent penetration rate constant increased from normal to infected mucosa by 55%. The K_e for amoxicillin was the same for inoculation volumes of 0.8 and 1.6 ml ($P = 0.557$) and the same for sampling intervals of 4 and 8 hr ($P = 0.054$). All three antimicrobial drug concentration–time curves were log-linear, as predicted by Fick's first law of diffusion. In conclusion, this model overcomes the technical limitations of previous models and permits investigation of the many factors that can influence antibiotic penetration into the middle ear and reduce otitis media treatment efficacy.

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

INTRODUCTION

The pathogenesis of acute purulent otitis media is closely associated with eustachian tube dysfunction and upper respiratory tract infection with various pathogens, notably *Streptococcus pneumoniae* and nontypable *Haemophilus influenzae* (1). The current treatment of acute purulent otitis media includes antimicrobial drugs in the penicillin, cephalosporin, and sulfonamide classes. Otitis media clinical trials evaluating these antibiotics have shown that several drugs are effective to some degree in most patients (2). However, a significant number of children fail to resolve their middle ear symptoms, fail to clear the causative bacteria from the middle ear, suffer recurrent episodes of otitis media, or develop chronic otitis media with effusion, which may delay speech and language development (3).

For an antimicrobial drug to be effective in treating otitis media, it must leave the circulation and diffuse through the capillary wall and middle ear mucosa into the middle ear cavity. The driving force for middle ear penetration is the blood antibiotic concentration, which, for most of these drugs, declines rapidly in a log-linear fashion. The rate at which a drug penetrates the middle ear mucosa is an important determinant of the concentration achieved in the middle ear effusion and the rate of bacterial inhibition or lysis.

To measure the rate of antibiotic penetration we have previously utilized a model where an antibiotic is given systemically and blood and middle ear effusion concentrations are measured (4). Although this technique can effectively

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0724-8741/90/1200-1242$06.00/0

measure the rate of middle ear antibiotic appearance and disappearance, the effusion volumes are small and rarely more than a few samples can be obtained. The dynamic process of changing drug concentrations in both the blood and the middle ear effusion requires multiple samples for the most accurate determination of the drug penetration and elimination rates and tissue transit time. Therefore, we modified the chinchilla models of acute otitis media to study further the rate of antibiotic penetration through the middle ear membrane by measuring the apparent penetration rate constant. This new model measures the rate at which an antimicrobial drug leaves the middle ear effusion.

MATERIALS AND METHODS

Animals. Experiments were performed with healthy 1- to 2-year-old chinchillas weighing between 400 and 600 g. All procedures and handling were approved by our Research Animal Resources Committee. Chinchillas were anesthetized for all procedures with ketamine hydrochloride, 50 mg/kg, given intramuscularly.

Antibiotics. Amoxicillin, trimethoprim, and sulfamethoxazole were studied, and two solutions were used. An amoxicillin solution was made by dissolving 50 μg of amoxicillin into each milliliter of sterile saline. To increase the yield of experimental information from each chinchilla and decrease the number of animals required, an amoxicillin, trimethoprim, and sulfamethoxazole solution was made by dissolving 50 μg of amoxicillin, 10 μg of trimethoprim and 50 μg of sulfamethoxazole into each milliliter of sterile 0.01 *M* phosphate buffer solution at pH 7.5.

Middle Ear Volume. The middle ear volumes were measured using the intact tympanic bulla specimens from 10 healthy chinchillas that weighed a mean ± SD of 507.5 ± 68.3 g. To obtain these specimens, both tympanic bulla were removed and the soft tissue was cleaned away. The lateral wall of the external canal was then retracted and the tympanic membrane and pharyngeal orifice of the eustachian tube were covered with a thick layer of glue. The dry bulla was placed in melted paraffin at 65°C for a few minutes to cover the surface of the bulla and keep water from flowing out during the measurement process. A 2-mm hole was then made in the wall of the dorsal bulla. Water was injected through this hole with a tuberculin syringe, and exact volume measurements to 0.01 ml were taken. The hole was then covered with melted paraffin, and this procedure was repeated for the labyrinthine and mastoid bulla (Fig. 1). Additional holes were made to evacuate air bubbles in the bulla.

Middle Ear Surface Area. Ten bulla specimens were obtained from six adult chinchillas with an average ± SD weight of 541.83 ± 29 g. The chinchillas were sacrificed, and the bullae were isolated and cleaned of the soft tissue. Fixation began immediately by placing each bulla in 10% formalin at room temperature for one week. The bullae were then decalcified in 50% trichloracetic acid solution, dehydrated in a series of alcohol solutions, and embedded in graded celoidin. The bulla were sectioned serially to a thickness of 0.04 mm and every fifth section was stained with hematoxylin and eosin. The entire cross section was photographed at low power and magnified 10 times. Surface areas from these pictures were calculated by planimetry, using an IBM PCAT and a Summagraphics bit pad system. Measurements included the circumference of the mucosa on the walls of all bullae, the septa, and the three ossicles. The total measured circumference of each section was divided by 10 (photographic magnification factor) and multiplied by 0.2 mm (5 times the 0.04 thickness of every fifth section). Total surface area of the bulla was the sum of the surface areas of all the sections.

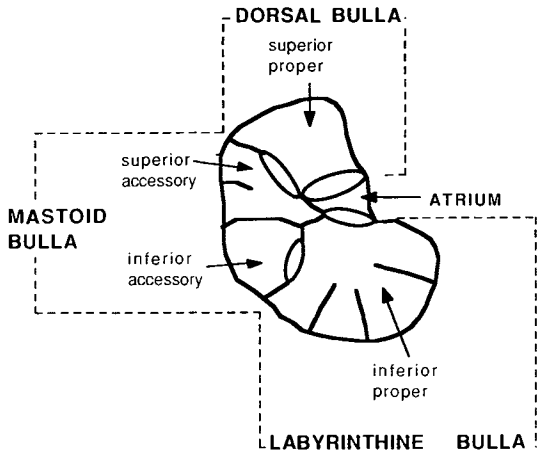

Fig. 1. This schematic drawing shows the sagittal section of a chinchilla tympanic bulla and was adapted from Hanamure and Lim (5).

Antibiotic Inoculation and Sampling Technique. After ketamine anesthesia, a 16-gauge needle was placed through the center of the dorsal bulla and a 23-gauge needle was placed adjacent to this needle to act as a pressure vent to prevent rupture of the tympanic membrane during injection of the antibiotic solution. A thin plastic catheter was carefully passed through the 16-gauge needle into the base of the labyrinthine bulla, depicted in Fig. 1, via the atrium (5). The antibiotic solution was then slowly inoculated at 1 ml/min into the middle ear through the thin catheter. The catheter and both needles were removed. Samples were aspirated using the same needle-catheter technique as for inoculation of the antibiotics at either time 0 and 4 hr (two-sample method) or at times 0, 1, 2, 3, and 4 hr.

Normal Model. Eustachian tube obstruction was performed on 17 chinchillas using the Silastic sponge (Dow Corning, Midland, MI) as previously described (6) and studied 1 day later. Eight ears were aspirated 4 hr after inoculating 0.8 ml of amoxicillin 50 μg/ml,and eight ears were aspirated 4 hr after inoculating 1.6 ml of this amoxicillin solution. Ten ears were aspirated once 8 hr after inoculating 0.8 ml of the amoxicillin solution, and 10 ears were aspirated once 4 hr after inoculating 0.8 ml of the three-drug solution. Eight ears were aspirated at 1, 2, 3, and 4 hr (0.10 ml each hour) after inoculating 1.0 ml of the three-drug solution.

POM Model. Purulent otitis medium was induced with type 7F *Streptococcus pneumoniae*. A phosphate-buffered solution (0.1 ml), pH 7.4, containing 40 colony-forming units of bacteria was inoculated into the middle ear through the thin catheter, as previously described (7). The bacteria in this solution were in log growth, determined by previous studies of optical density, and serial dilution studies were done on blood agar plates to assure that the solution contained 400 colony-forming units.

Fifteen chinchillas were studied 3 days after bacterial inoculation. Any purulent effusion present at this time of study was aspirated before inoculating the antibiotic solution and cultured on 5% sheep blood agar. All these cultures were positive for type 7F *Streptococcus pneumoniae*. One chinchilla developed ataxia and was excluded from the study. Ten ears were aspirated 4 hr after inoculating 0.8 ml of the three-drug solution. Eighteen ears were aspirated 1, 2, 3, and 4 hr (0.10 ml each hour) after inoculating 1.0 ml of the drug solution.

Antibiotic Concentration Analysis. High-performance liquid chromatography was used to analyze concentrations of amoxicillin, trimethoprim, and sulfamethoxazole (8). Amoxicillin concentrations were determined in 75 μl of material and the limit of assay sensitivity was 0.25 μg/ml. Trimethoprim and sulfamethoxazole concentrations were determined in 25 μl of material and the limit of assay sensitivity was also 0.25 μg/ml.

Calculations. Under steady-state conditions where a lipid membrane separates two well-stirred solutions that have different concentrations, the net rate of diffusion across this membrane is proportional to the surface area of the membrane and the concentration gradient across the membrane. This relationship has been expressed as Fick's first law of diffusion:

$$J = -PS(C_b - C_e) \quad (1)$$

where J is the net rate of diffusion in units of amount per time (i.e., flux), P is the membrane permeability coefficient in units of length per time and is proportional to the lipid solubility of the solute, S is the surface area of the middle ear in contact with the solute, C_b is the concentration of solute in the blood, and C_e is the concentration of solute in the middle ear (9). In our model, the antibiotic concentration in the blood is so small, it is considered negligible. Assuming that the volume (V) of solution in the middle ear remains constant, J can be expressed as $V(dC_e/dt)$. Substitution and rearrangement yield

$$dC_e/C_e = -(PS/V)dt = (K_e)dt \quad (2)$$

where PS/V is equal to K_e, the rate constant of elimination. Integration of this relationship gives us

$$K_e = -[\ln (C_t/C_o)]/t \quad (3)$$

where C_o and C_t are the concentrations of solute in the middle ear initially and at some time (t), respectively. This equation was used to calculate K_e (the apparent penetration rate constant) for our studies with two concentration–time points.

The Wagner Nelson semilog regression analysis was used to calculate K_e for the studies with multiple concentration–time data (10). Statistical comparisons were done using Student's t test for nonpaired data.

RESULTS

Total Surface Area/Volume Study. The mean ± SD volume of the 10 chinchilla bullae was 2.09 ± 0.08 ml, with a range of 1.88–2.17 ml. The mean ± SD surface area of the 10 chinchilla bullae was 14.41 ± 1.48 cm^2, with a range of 12.52–16.55 cm^2. The mean surface area-to-volume ratio of the chinchilla bullae is 6.89 cm^2/ml. During injection of the antibiotic solutions, maximum filling volume of approximately 1.6 ml was noted. We sacrificed a chinchilla and removed the soft tissue from the bulla. We then injected methylene blue dye into the bulla and using transbullar illumination observed the fluid meniscus rising in the large chambers. Air trapped in the inferior accessory chamber approximated the difference between total volume we were able to instill (1.6 ml) and actual measured maximum filling volume (2.09 ml).

Normal Middle Ear and POM Studies. Table I shows the apparent K_e values for amoxicillin in the normal model. There was no difference in K_e between a volume of 0.8 and one of 1.6 ml ($P = 0.557$). There was no difference in K_e between 4 and 8 hr ($P = 0.054$). There was no difference in K_e for amoxicillin between the amoxicillin solution and the three-antibiotic solution ($P = 0.975$).

Table II shows the K_e values for amoxicillin, trimethoprim, and sulfamethoxazole in the normal model and in the POM model. Comparing the data from normal mucosa to the infected mucosa, increases of 142% for amoxicillin, 44% for trimethoprim, and 55% for sulfamethoxazole were seen.

Figure 2 shows the first-order elimination profiles of individual ears for amoxicillin in the normal and POM models. The mean K_e ± SD for amoxicillin in the normal model was 0.068 ± 0.01 hr^{-1}, with a mean correlation coefficient of 0.87 and $n = 7$. The mean K_e ± SD for amoxicillin in the POM model was 0.211 ± 0.12 hr^{-1}, with an average correlation coefficient of 0.97 and $n = 12$. The lowest correlation coefficient accepted was 0.87 and three ears were excluded because of correlation coefficients less than 0.87. In addition, three tympanic membranes perforated and could not be evaluated.

Figure 3 shows the first-order elimination profiles of individual ears for trimethoprim in the normal model and the POM model. The mean K_e ± SD for trimethoprim in the normal model was 0.304 ± 0.065 hr^{-1}, with an average correlation coefficient of 0.98 and $n = 8$. The mean K_e ± SD for trimethoprim in the POM model was 0.543 ± 0.19 hr^{-1}, with an average correlation coefficient of 0.99 and $n = 8$. The lowest correlation coefficient accepted was 0.97.

Figure 4 shows the first-order elimination profiles of individual ears for sulfamethoxazole in the normal model and the POM model. The mean K_e ± SD for sulfamethoxazole in the normal model was 0.186 ± 0.03 hr^{-1}, with a mean

Table I. The Mean ± SD Middle Ear Mucosal Apparent Penetration Rate Constants (K_e) for Amoxicillin, When Comparing Volume Instilled or Dwell Time, for Normal Chinchilla Ears 1–2 Days After Eustachian Tube Obstruction

Solution	N	Volume (ml)	Time (hr)	K_e (hr^{-1})
Amoxicillin	8	0.8	4	0.118 ± 0.013
Amoxicillin	8	1.6	4	0.115 ± 0.008
Amoxicillin	10	0.8	8	0.151 ± 0.043
Amoxicillin, trimethoprim, and sulfamethoxazole instilled together	10	0.8	4	0.118 ± 0.042

Table II. The Mean ± SD Antibiotic K_e Values Comparing (1) Normal Ears 1–2 Days After Eustachian Tube Obstruction or Bacterial Infection 3 Days After Bacterial Inoculation and (2) Between the Antibiotics[a]

Antibiotic	N	K_e (normal) (hr^{-1})	N	K_e (infected) (hr^{-1})
Two samples				
Amoxicillin	8	0.118 ± 0.013	10	0.286 ± 0.089
Trimethoprim	10	0.461 ± 0.090	10	0.662 ± 0.118
Sulfamethoxazole	10	0.265 ± 0.062	10	0.411 ± 0.056
Five samples				
Amoxicillin	7	0.068 ± 0.011	12	0.211 ± 0.12
Trimethoprim	8	0.304 ± 0.065	8	0.543 ± 0.19
Sulfamethoxazole	8	0.186 ± 0.030	8	0.336 ± 0.11

[a] Data from the two sampling methods for measuring K_e (two samples and five samples) are shown.

correlation coefficient of 0.98 and $n = 8$. The mean K_e ± SD for sulfamethoxazole in the POM model was 0.336 ± .11 hr^{-1}, with a mean correlation coefficient of 0.97 and $n = 13$. The lowest correlation coefficient accepted was 0.91. Two ears were excluded with correlation coefficients less than 0.91 and three tympanic membranes perforated and could not be evaluated.

DISCUSSION

The etiology of childhood otitis media is multifactorial, with bacteria being a major contributor to the pathogenesis of this disease (1). The ability to effectively treat episodes of acute otitis media depends on the bacterial susceptibility to the antibiotic given and the ability of the antibiotic to reach the site of infection. It is difficult to quantitate the penetration of antibiotics into the human middle ear since tympanosyntesis causes discomfort to the patient and could be performed only once.

To overcome the technical difficulties that patients present to the study of otitis media, we have used a chinchilla model to evaluate middle ear antibiotic penetration and bacterial killing (4). This older experimental model has produced useful information about the treatment of otitis media but has the limitation of producing only small effusion volumes when using either eustachian tube obstruction or acute bacterial infection. Our experience has shown that this does not preclude, but does limit, the ability to quantitate antibiotic appearance in and disappearance from middle ear effusion (4). Therefore, we have further adapted this model to remove this limitation by instilling the antibiotic solution directly into the middle ear space.

Several models involving artificially created extravascular chambers have been designed to study the penetration of antibiotics from the intravascular to the extravascular space (9,11,12). These models have revealed factors that in-

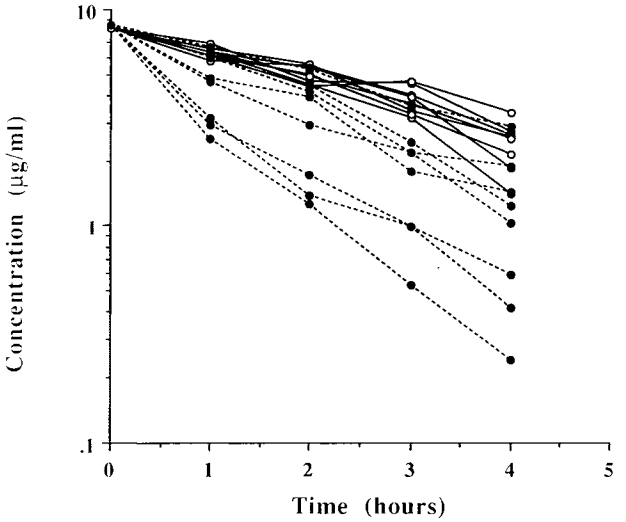

Fig. 3. Elimination profiles of normal (open circles, solid lines) or infected (filled circles, dashed lines) individual chinchilla middle ears are shown for trimethoprim.

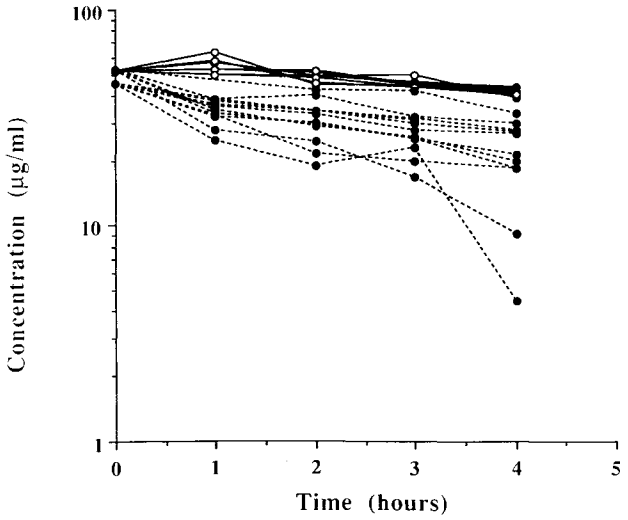

Fig. 2. Amoxicillin concentration versus time data are shown for individual chinchilla ears that are normal (open circles, solid lines) or infected (filled circles, dashed lines) to produce otitis media.

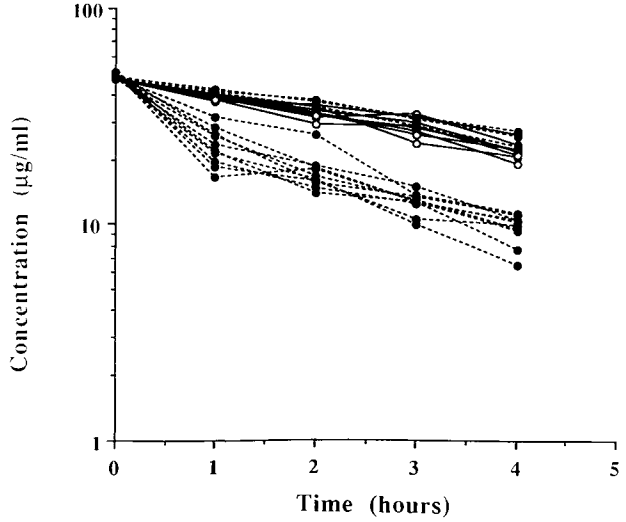

Fig. 4. Elimination profiles of normal (open circles, solid lines) or infected (filled circles, dashed lines) individual chinchilla middle ears are shown for sulfamethoxazole.

fluence the penetration of an antibiotic including, surface area-to-volume ratio, molecular weight, lipid solubility, pH, and protein binding. The model presented herein can be used to study the role each of these factors play in middle ear antibiotic penetration.

The rate constant at which the antibiotics were eliminated from the middle ear (apparent K_e) was used in this model as a measure of antibiotic penetration through the middle ear mucosal membrane. This assumes that these drugs passively diffuse into the ear and this rate is equal to the exit rate. This is reasonable since there is not evidence, as yet, of active antibiotic transport processes in the middle ear mucosa. Therefore, as the K_e for antibiotic elimination from the middle ear increases, so does the extent of antibiotic penetration.

The rate a drug enters and leaves a tissue space, such as the middle ear, only partially determines exposure to the drug. Another important factor is the systemic rate of elimination for the drug. The combination of these factors, called tissue transit time, may be the best way to quantitate middle ear antibiotic exposure. This will require additional model design incorporating the effects of those factors found to significantly influence antibiotic penetration.

In developing this model, we postulated that factors such as middle ear dwell time, type and constituents of the effusion, and sampling technique might influence the apparent K_e independent of the mucosal membrane and the factors mentioned above. Therefore, we first evaluated the effects of these factors on K_e. K_e was not changed by the length of time an antibiotic was allowed to dwell in the middle ear, however, variability in K_e increased the longer a study was allowed to progress. This appears to be the result of between-animal variability and the method of K_e calculation.

Our technique of taking multiple effusion samples produced a mean K_e for both the normal and the POM models that was less than the mean apparent K_e for the two sample approach. We are not sure if this difference is significant or why it occurred, but this difference may be secondary to the prolonged ketamine anesthesia required to take multiple samples, which could reduce solution mixing in the middle ear effusion, decreasing the concentrations at the membrane. This difference could have been produced by a concentration shift at time 0 from the presence of small amounts middle ear fluid when using the two sample method. The effect of this would be less for the five-point method since apparent K_e is based on more observed concentrations.

Using an *in vitro* model, Van Etta *et al.* found that the surface area (SA)-to-volume (V) ratio of an extravascular space was an important determinant of the drug concentration behavior in the space. They studied compartments with a SA/V of 3, 7, 10, and 100 and found that the kinetic behavior was similar for the compartments with a SA/V between 3 and 10, but the large SA/V (100) closely mimicked the intravascular space (11).

The chinchilla middle ear SA/V of 6.93 is in the lower range of these compartment values, and therefore, we would not expect to see a significant change in K_e with small changes in volume. Experimentally, the amoxicillin K_e was not changed by increasing the inoculum volume from 0.8 ml to 1.6 ml. This suggests that as the volume was increased, there was a proportional increase in surface area contact by the antibiotic solution and thus the SA/V remained relatively constant. Viewing this in the clinical situation, this indicates that the concentration of an antibiotic achieved in the middle ear effusion is the same for any given effusion volume, although this assumes that the effusion is homogeneous and mixed, an issue that needs further study.

Table III. The Molecular Weights, pK_a Values, Octanol/Water Coefficients, and Percentage Protein Binding in Chinchilla Plasma, Serous, and Purulent Middle Ear Effusion for the Three Antibiotics Studied

	Amoxicillin	Trimethoprim	Sulfamethoxazole
MW	387.4	290.3	253.3
pK_a	2.4, 7.4, 9.6	6.6	5.6
Octanol/water	0.87	0.91	0.88
Protein binding			
Human			
Plasma	20%	40–70%	65%
Chinchilla			
Plasma	35%	45%	50%
Normal	35%	40%	55%
Infected	45%	25%	35%

The lipid solubility, measured by the octanol/water partition coefficients (13), and molecular weights, shown in Table III, were similar for the three antibiotics studied, yet differences in apparent K_e were still found. This suggests that the model is a very sensitive measure of differences in apparent penetration rate constants.

Trimethoprim penetrated the mucosal membrane at the fastest rate in both the normal and the infected models. This increase in the trimethoprim apparent penetration rate constant from normal to infected mucosa was the smallest of the three antibiotics. The amoxicillin apparent penetration rate constant was the slowest rate in both the normal and the infected models. The apparent penetration rate constant of amoxicillin did increase from the normal to the infected model and this difference was the greatest of the three antibiotics studied.

Before the comparison between rate and extent of penetration for these and other antibiotics can be translated into recommendations for clinical management of patients with otitis media, other factors, such as, pH, mean transit time, and protein binding, must be evaluated. In addition, this model lends itself to quantitating bacterial killing by the various antibiotics used to treat acute otitis media and this type of antimicrobial efficacy study will need to be performed to help bridge the gap between experimentation and patient therapy.

ACKNOWLEDGMENTS

This work was supported in part by Grant NIDCD 8P50-DC00133-11 from the National Institutes of Health, a Minnesota Medical Foundation Student Research Grant, the Minnesota Medical Foundation George H. & Lilian K. Williams Scholarship in Otolaryngology, and the Park Nicollet Medical Foundation Student Research Grant.

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