Comparison of Two Otitis Media Models for the Study of Middle Ear Antimicrobial Pharmacokinetics

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Received January 5, 1994; accepted January 24, 1994

We compared two models of acute otitis media that estimate middle ear antimicrobial pharmacokinetics. Using a crossover study design, we compared a systemic drug administration model with a diffusion model we devised that measures the disappearance of antimicrobials from the middle ear. We induced acute otitis media in 14 chinchillas by inoculating *S. pneumoniae* into the middle ear, then administered 3 antimicrobials: amoxicillin, trimethoprim, and sulfamethoxazole. Next we collected middle ear fluid samples to analyze drug concentrations and compare rate constants for the systemic and diffusion models by analysis of variance. We found that amoxicillin K values were not affected by model testing sequence ($p = 0.827$) or model type (systemic versus diffusion, $p = 0.310$), nor were sulfamethoxazole K values: model testing sequence ($p = 0.917$), model type ($p = 0.963$). Trimethoprim K values were also not affected by model testing sequence ($p = 0/760$), but were by model type ($p = 0.0001$). Trimethoprim elimination from the diffusion model was faster ($K = 0.33 \pm 0.17$ versus 0.57 ± 0.09 hr^{-1}) than from the systemic model, although it appears this was caused by sampling before drug distribution into the middle ear was complete. In conclusion, it appears K values derived from either systemic antimicrobial administration or direct middle ear instillation are similar for assessing middle ear anitmicrobial pharmacokinetics, and these models can be used interchangeably to study factors affecting otitis media treatment response.

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

Introduction

The study of antimicrobial dose and concentrations in the blood and middle ear fluid is critical for understanding which factors cause otitis media treatment failures (1, 2). We devised two models of acute otitis media that allow us to study antimicrobial pharmacokinetics and factors that affect antimicrobial diffusion into and out of the middle ear (3, 4). We used a crossover study design to compare middle ear antimicrobial diffusion from the middle ear. The diffusion model was devised to measure the disappearance of antimicrobials from the middle ear. It would thus overcome the technical limitations of systemic drug administration and permit investigation of factors such as protein binding, pH, and viral infections—all of which affect antimicrobial penetration into the middle ear and may play a role in reducing otitis media treatment efficacy.

Materials and Methods

Animals. This study required chinchillas that were 1 to 2 years old, weighed 400 to 650 g, and were in good health. They were housed individually and were given food and water ad lib. All procedures and handling were reviewed and approved by our Research Animal Resources Division and Committee. The chinchillas were anesthetized for procedures with ketamine (50 mg/kg given intramuscularly).

All middle ear procedures were done by inserting a 16 gauge needle through the center of the dorsal bulla. If fluid was added to the middle ear, a 23 gauge needle was placed adjacent to this needle, acting as a pressure vent to prevent rupture of the tympanic membrane during injection. A thin plastic catheter was then passed carefully through the 16 gauge needle to the base of the labyrinthine bulla. The drug solution was then slowly introduced at 1 ml/minute into the middle ear through the catheter. Middle ear fluid samples were aspirated using the same needle-catheter technique.

Microbiology. Purulent otitis media was produced by the direct inoculation of type 7F *Streptococcus pneumoniae* into the cephalad middle ear bulla, as previously described (3). A small bacterial inoculum of between 10 and 50 colony forming units was used to reduce the likelihood of disseminated infection. The presence of otitis media with effusion was determined by otoscopy and tympanometry using a Grason-Stadler Model 1722 Auto Tympamometer (Grason-Stadler, Inc., Littleton, MA) and an algorithm we have validated in this model (3). Middle ear fluid from each animal was aspirated 3 days after bacterial inoculation and one drop (20 μl) was placed on a 5% sheep blood agar plate for culture. All plates were incubated at 37°C under 10% CO_2 and read at 24 and 48 hours. The remaining fluid was placed directly into a plastic centrifuge tube and frozen at −70°C while awaiting analysis, usually within the next 24 hours.

Study Protocol and Pharmacokinetic Model. We studied 2 pharmacokinetic models in each animal and used a crossover study design to control for between-animal variability. We compared the middle ear elimination rate constants between the models. The rate constant K_s was calculated from middle ear concentration versus time values after systemic drug administration. K_{s1} is the rate of appearance in the middle ear and K_{s2} is the rate of disappearance. The second model also measures a rate constant (K_d), which represents the diffusion of antimicrobials from the middle ear.

We divided 14 chinchillas into 2 groups of 7 each. One group was given systemic drug administration with middle ear fluid sampling 3 days after bacterial inoculation, followed the next day by direct middle ear instillation and middle ear fluid sampling. The other group was treated the same except the opposite sequence was used: diffusion model then systemic model evaluation.

The systemic model required each chinchilla to be given 50 mg/kg of amoxicillin, prepared fresh each day in a solution of 0.25 gm/ml diluted in phosphate-buffered saline solution, pH 7.5; a dose of 10 mg/kg of trimethoprim; and a dose

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0724-8741/94/0600-0855$07.00/0

Table I. Middle ear tympanometry, otoscopy, and culture results in 14 chinchillas with purulent otitis media.

Chinchilla #	Weight (gm)	Day 0 Pressure (daPa)	Day 0 Otoscopy (a)	Day 4 Pressure (daPa)	Day 4 Otoscopy (a)	Day 4 Volume (μl)	Culture	Day 5 Pressure (daPa)	Day 5 Otoscopy (a)	Day 5 Volume (μl)
Group 1 systemic/diffusion										
938	575	−50	0	−70	1	0	Strep	−35	1	0
939	582	25	1	−205	5	100	Strep	−75	4	150
940	490	10	1	−355	4	0	Strep	−410	2	0
941	556	90	1							
942	429	25	1	−40	5	0	Strep	−140	4	0
943	674	25	1	65	1	50	Strep	−180	3	200
944	478	10	1	10	2	200	Strep	−160	3	0
Group 2 diffusion/systemic										
945	579	10	0	15	1	0	Strep	−185	3	70
947	656	30	0	110	0	100	Strep	−194	4	120
964	593	35	0	100	1	50	Strep	−140	5	110
965	619	5	0	−410	5	300	Strep	180	3	200
966	586	45	1							
967	516	10	0	115	5	0	Strep	150	3	0
968	552	45	1	65	5	300	Strep	−145	3	190
		25	1	80	5	350	Strep	−130	3	140

(a) Otoscopy: 0 = gray/translucent, 1 = gray/opaque, 2 = red/translucent, 3 = red/opaque, 4 = yellow translucent, 4 = yellow/opaque, 6 = perforation

of 50 mg/kg of sulfamethoxazole. These antimicrobials were injected intramuscularly into the anterior aspect of the thigh muscle after a small area of fur had been removed. Following antimicrobial injection, 1 ml of phosphate-buffered saline solution at pH 7.5 was instilled into each ear. The diffusion model required each chinchilla to receive 1 ml of a solution containing 50 μg/ml of amoxicillin, 10 μg/ml of trimethoprim, and 50 μg/ml of sulfamethoxazole. This solution was instilled directly into the middle ear using the needle-catheter technique (3).

Ear fluid samples (150 μl) were aspirated through the dorsal bulla of both ears at 1, 2, 3, 4, and 6 hr after drug delivery. The pharmacokinetic data reported is for the right ear only (left ear data was collected and the results were similar for both ears). All samples were immediately stored at −70°C and analyzed the next day using high-performance liquid chromatography (HPLC). Amoxicillin concentrations were determined in a 75 μl sample with a lower limit of detection of 0.25 μg/ml (5). Trimethoprim and sulfamethoxazole concentrations were determined using 25 μl samples by an assay with a lower limit of detection equal to 0.25 μg/ml (6).

Data Analysis. Plots were constructed for the plasma concentration versus time data. Inspection of the plots suggested the post-absorptive/penetration phase for amoxicillin and trimethoprim in the systemic model started at about 2 hours after the dose. Therefore, calculation of K_{s2} values only included concentrations after this time. The sulfamethoxazole concentrations were still increasing at the last sampling time; therefore, this negative slope or the actual penetration rate was used to calculate K_{s1}. Since the diffusion model has no absorption phase, values of K_d were calculated from all the date points. The Wagner-Nelson regression analysis was used for these calculations (7). The K_s values were calculated from the linear portion of a log-linear plot of the concentration-time data.

Statistical comparisons of mean data were performed using analysis of variance; post hoc testing, using Student's t test.

Results

Of the 14 chinchillas that began the study, 2 died before

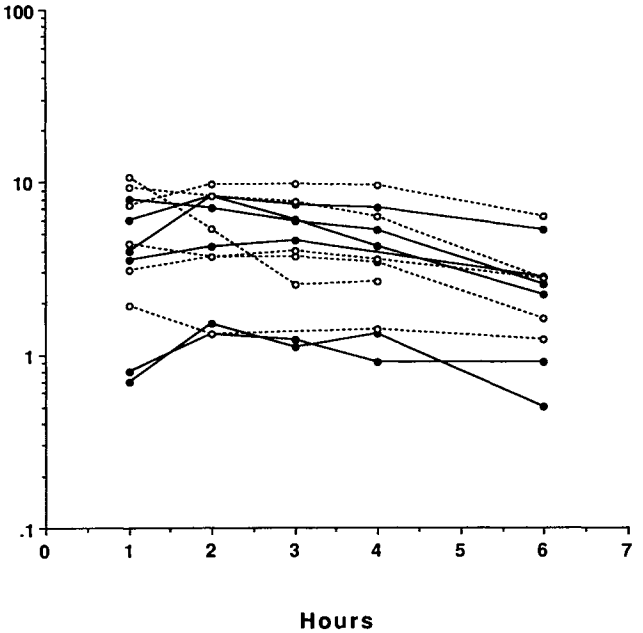

Figure 1. Amoxicillin concentration-time data after systemic administration are shown for individual chinchilla right ears with acute otitis media. The closed circles, solid lines are for sequence 1: systemic then diffusion: The open circles, dotted lines are for sequence 2: diffusion then systemic.

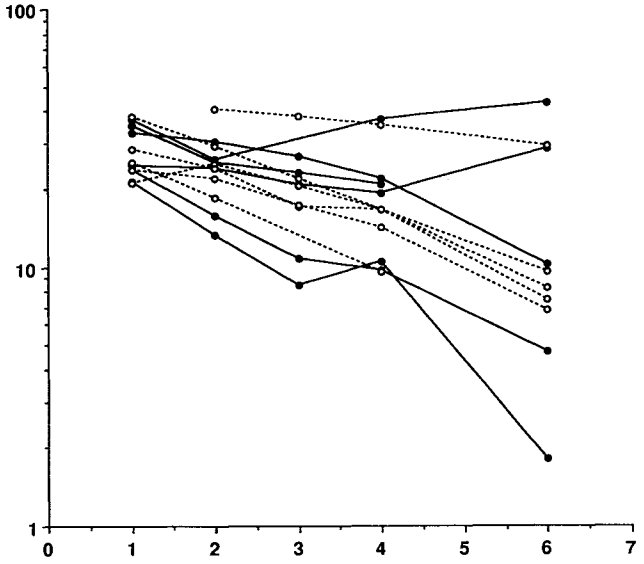

Figure 2. Amoxicillin concentration-time data after local administration (diffusion model) are shown for individual chinchilla right ears with acute otitis media. The closed circles, solid lines are for sequence 1: systemic then diffusion. The open circles, dotted lines are for sequence 2: diffusion then systemic.

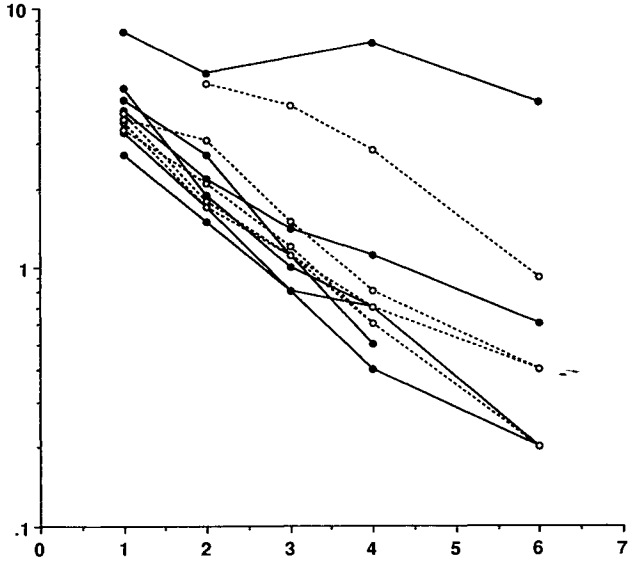

Figure 4. Trimethoprim concentration-time data after local administration (diffusion model) are shown for individual chinchilla right ears with acute otitis media. The closed circles, solid lines are for sequence 1: systemic then diffusion. The open circles, dotted lines are for sequence 2: diffusion then systemic.

completing the protocol; post-mortem cultures indicated the cause of death to be *S. pneumoniae* sepsis. All the inoculated middle ears were culture positive for *S. pneumoniae* 3 days after bacterial instillation. **Table I** lists the middle ear characteristics, fluid volumes, and culture results. Concentration-time plots for amoxicillin, trimethoprim, and sulfamethoxazole in both the systemic and diffusion models are shown in **Figures 1 through 6.** The calculated pharmacokinetic parameters from these plots are shown in **Table II.**

Concentration-time plots for amoxicillin in both models

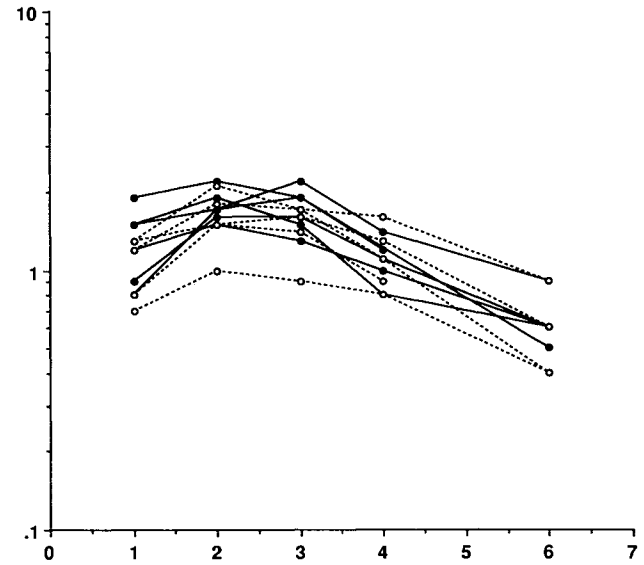

Figure 3. Trimethoprim concentration-time data after systemic administration are shown for individual chinchilla right ears with acute otitis media. The closed circles, solid lines are for sequence 1: systemic then diffusion. The open circles, dotted lines are for sequence 2: diffusion then systemic.

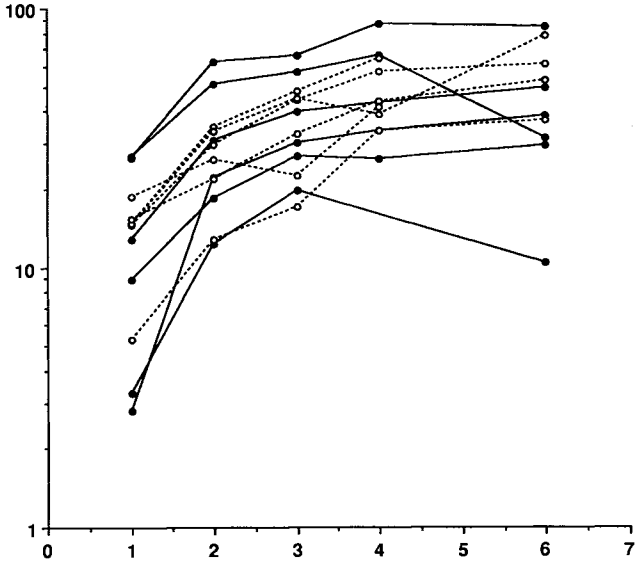

Figure 5. Sulfamethoxazole concentration-time data after systemic administration are shown for individual chinchilla right ears with acute otitis media. The closed circles, solid lines are for sequence 1: systemic then diffusion. The open circles, dotted lines are for sequence 2: diffusion then systemic.

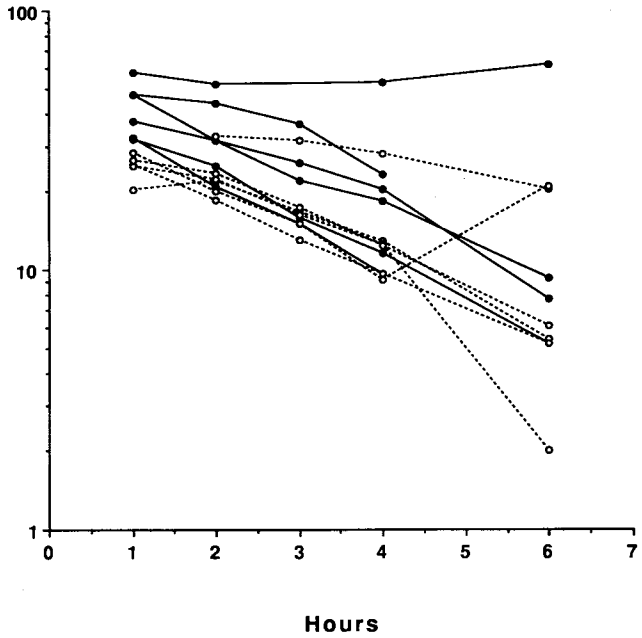

Figure 6. Sulfamethoxazole concentration-time data after local administration (diffusion model) are shown for individual chinchilla right ears with acute otitis media. The closed circles, solid lines are for sequence 1: systemic then diffusion. The open circles, dotted lines are for sequence 2: diffusion then systemic.

are shown in **Figures 1 and 2.** Using analysis of variance, estimated amoxicillin K values were not affected by model testing sequence ($p = 0.827$) or model type (systemic versus diffusion, $p = 0.310$). Trimethoprim K values were not affected by model testing sequence ($p = 0.760$) but were affected by model type ($p = 0.0001$): elimination from the diffusion model was faster ($0.33 \pm 0.17\ hr^{-1}$) than from the systemic model ($0.57 \pm 0.09\ hr^{-1}$). Comparison of the trimethoprim concentration-time plots **(Figures 3 and 4)** suggests this difference was likely produced by the sampling methodology and not an actual difference in the 2 models since the systemic samples were collected before trimethoprim had completed penetration into the middle ear. Sulfamethoxazole concentration-time plots in both models are shown in **Figures 5 and 6.** Sulfamethoxazole K values were not affected by model sequence ($p = 0.917$) or model type ($p = 0.963$).

Discussion

The chinchilla model provides well-controlled conditions for studying otitis media. All the practical requirements for measuring antimicrobial pharmacokinetics are met by this model: the disease can be reliably produced with reasonably homogeneous pathology among animals, middle ear fluid can be sampled repetitively, and the baseline pathology and histochemistry has been described in untreated animals, providing a reference for future studies **(8).**

We have used the chinchilla model to evaluate middle ear antibiotic pharmacokinetics and bacterial killing that requires systemic antimicrobial administration **(3).** A limitation of this model, however, is its small middle ear fluid volumes, which make it hard to access the effects of various factors on antibiotic penetration. In our experience, these small volumes do not preclude, but do limit, the ability to quantitate antibiotic appearance in and disappearance from middle ear fluid. To remove this limitation, we have instilled the antibi-

Table II. Middle ear pharmacokinetic characteristics: systemic vs. diffusion otitis media models.

Experimental Conditions by Sequence	Number of Right Ears Studied	K (a) (mean ± SD) 1/hr	Half-life (mean ± SD) hr	R-squared (mean ± SD)
I. Amoxicillin				
Systemic/Diffusion				
systemic	5	0.22 ± 0.09	3.74 ± 1.99	0.96 ± 0.04
diffusion	5	0.29 ± 0.20	3.57 ± 2.41	0.97 ± 0.02
Diffusion/Systemic				
systemic	6	0.21 ± 0.15	4.49 ± 2.27	0.95 ± 0.04
diffusion	5	0.26 ± 0.02	2.64 ± 0.16	0.98 ± 0.01
II. Trimethoprim				
Systemic/Diffusion				
systemic	6	0.29 ± 0.04 (b)	2.44 ± 0.31	0.97 ± 0.02
diffusion	5	0.55 ± 0.14 (b)	1.33 ± 0.37	0.99 ± 0.01
Diffusion/Systemic				
systemic	6	0.33 ± 0.17 (b)	2.53 ± 1.01	0.95 ± 0.03
diffusion	6	0.57 ± 0.09 (b)	1.34 ± 0.21	0.98 ± 0.02
III. Sulfamethoxazole				
Systemic/Diffusion				
systemic	6	0.35 ± 0.28	2.81 ± 1.56	0.90 ± 0.06
diffusion	6	0.33 ± 0.07	2.21 ± 0.51	0.98 ± 0.02
Diffusion/Systemic				
systemic	6	0.32 ± 0.09	2.28 ± 0.52	0.94 ± 0.04
diffusion	6	0.34 ± 0.15	2.56 ± 1.61	0.97 ± 0.02

(a) Symbol: K = elimination rate constant
(b) $p = <0.01$

otic solution directly into the middle ear space and measured diffusion of the drug from the middle ear (4).

To measure the apparent rate constant of antibiotic elimination from the middle ear fluid through the middle ear mucosa in the diffusion model, we inoculated an antibiotic solution into the middle ear cavity, and then aspirated samples for analysis. We found amoxicillin, trimethoprim, and sulfamethoxazole drug concentration-time curves are log-linear after middle ear installation, as predicted by Fick's first law of diffusion. This suggests simple diffusion is the rate-limiting factor for removal; it is likely that penetration into the middle ear is by simple diffusion also.

In developing the diffusion pharmacokinetic model, we postulated that factors such as middle ear dwell time and sampling technique might influence the rate a drug transverses the middle ear membrane, independent of the other factors mentioned. We found that inoculation volumes of 0.8 versus 1.6 ml and sampling intervals of 4 versus 8 hours did not affect the estimation of K (4).

This study was conducted to show the similarities and differences between systemic drug administration and local drug instillation on estimating an elimination rate constant. Our results suggest that both models give similar K values and can be used to test the effects of other factors on middle ear antimicrobial pharmacokinetic behavior. This model can be used to explain clinical observations: for example, in the diffusion model, influenza and *S. pneumoniae* infections affect middle ear antimicrobial penetration, producing middle ear drug concentrations that may be inadequate to treat otitis media (10).

Acknowledgment

Supported by grant number PO1-DC00133 from the National Institute on Deafness and Other Communication Disorders

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