Estimating Amoxicillin Influx/Efflux in Chinchilla Middle Ear Fluid and Simultaneous Measurement of Antibacterial Effect[▽]

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Understanding the transport process and the factors that control the influx/efflux of antibiotics between plasma and middle ear fluid is essential in optimizing the antimicrobial efficacy in the treatment of acute otitis media. In this study, an experimental chinchilla model with the application of a microdialysis technique was utilized to evaluate amoxicillin middle ear distribution kinetics. Amoxicillin solutions at various doses were instilled into the middle ear with a simultaneous intravenous bolus dose. Unbound amoxicillin levels were monitored by microdialysis in both ears. Serial phlebotomy provided samples for the measurement of unbound amoxicillin concentration in plasma ultrafiltrates. In infected chinchillas, discrete middle ear fluid samples were plated and cultured to characterize *Streptococcus pneumoniae* growth-kill kinetics. Noncompartmental analysis was used to estimate distributional and elimination clearances assuming linear pharmacokinetics. A nonlinear Michaelis-Menten equation was also used to determine the efflux clearance (from middle ear fluid to plasma) in a mammillary compartment model. No difference was observed in amoxicillin pharmacokinetics between control and infected chinchillas. Influx clearance was $(4.6 \pm 2.4) \times 10^{-3}$ ml/min-kg and significantly lower than the efflux clearance estimated as $(19.2 \pm 9.7) \times 10^{-3}$ ml/min-kg ($P < 0.002$). Nonlinear kinetics was observed in the locally dosed ear. The microdialysis procedure did not interfere with the bacterial growth-kill profile, thereby enabling pharmacokinetic and pharmacodynamic evaluation concurrently. In conclusion, the results suggested that the distribution equilibrium of amoxicillin in the middle ear favors efflux to plasma over influx. An active transport mechanism across middle ear mucosal epithelium may be involved in amoxicillin distribution.

Knowledge of antibiotic distribution kinetics between systemic circulation and middle ear fluid (MEF) is essential in optimizing antimicrobial efficacy in the treatment of otitis media (5, 6, 9, 11). Middle ear (ME) infection, or acute otitis media (AOM), is one of the most common childhood illnesses (3, 4). Most antibiotics prescribed for the treatment of pediatric ear infections are dosed orally or by injection. Only one of more than a dozen approved antibiotic preparations is dosed via topical administration directly into the external ear in the form of an otic drop (23).

After systemic administration, the antibiotic agent needs to reach the peripheral tissue space where the infection exists. Multiple factors may be involved in determining the antibiotic permeability into the infected tissue site (2, 18). Systemic pharmacokinetics (PK), i.e., absorption, distribution, metabolism, and excretion, as well as plasma protein binding, are critical factors controlling delivery of the antibiotic to the target tissue. In addition, permeability of the antibiotic through the membrane barrier between blood and tissue extracellular fluid also determines efficiency of delivery to the site.

Antimicrobial efficacy in the treatment of ME infection is directly related to the distribution of antibiotics into the MEF. Following systemic dosing, this distribution can be character-

ized by a balance of influx and efflux clearances across the ME mucosal membrane. Despite abundant preclinical and clinical reports describing the monitoring of antibiotics in the MEF (1, 5–9, 11, 14–16), there is little kinetic information on antibiotic ME distribution as it relates to influx and efflux across the ME mucosal membrane.

An experimental animal model was previously reported which involved the application of microdialysis to continuously measure antibiotic concentrations in plasma and MEF in the awake chinchilla (14). In a crossover study, amoxicillin was dosed as a single intravenous (i.v.) bolus followed by constant-rate i.v. infusion for 10 to 15 h with or without confusion of probenecid. The PK following single-dose i.v. bolus, as well as at steady state during i.v. infusion, were determined. The distribution ratios (MEF/plasma) of amoxicillin based on unbound steady-state concentrations and areas under the concentration-time curves (AUCs) were consistently lower than unity, averaging approximately 0.3. The clearance of amoxicillin into the MEF from plasma (influx, CL_{in}) and that from MEF to plasma (efflux, CL_{out}) were also determined by fitting model parameters to the MEF data using the plasma concentration-time profile as a forcing function (21). The ratio of CL_{in}/CL_{out} was significantly less than unity, indicating a distribution unbalance in favor of efflux. Modeling was based on the assumption that the distribution kinetics across the ME mucosal membrane was linear.

In the current study, a novel experimental approach was developed by assuming that both right and left ME bullae were identical morphologically (12, 13) and kinetically. PK studies using simultaneous i.v. and intrabulla (intra-ME) dosing with

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multiple sampling sites were conducted. The purpose was to compare the distribution kinetic parameters with those obtained from the previous study which showed consistently lower-than-unity distribution ratios of MEF to plasma. In addition, the potential nonlinear characteristics in the ME distribution kinetics were explored by intra-ME administration of amoxicillin over a broad dose range.

Another goal of the present study was to evaluate the feasibility of exploring the antimicrobial efficacy using bacterial count in the MEF as a pharmacodynamic (PD) marker by combining microdialysis, direct sampling, and culture of the infected MEF. Integration of PK and PD in the same experiment is extremely challenging using traditional sampling techniques because of the small ME space. The very limited volume of MEF limits the number of samples and hence the quality of the data. Microdialysis, which does not involve fluid removal for sampling, was used to obviate this limitation.

MATERIALS AND METHODS

Chemicals and reagents. Amoxicillin and cefadroxil sodium were purchased from Sigma (St. Louis, MO). Solvents were of high-pressure liquid chromatography (HPLC) grade, and all other chemicals were analytical reagent grade.

Animal preparation. Male chinchillas (*Chinchilla laniger*) (body weight, 400 to 600 g) were obtained from a local breeder (Dan Moulton, Rochester, MN). These animals were not immunosuppressed, and only those with healthy sanitary status were studied, e.g., chinchillas with eye infections were excluded. They were acclimatized individually in metal wire cages for 7 days at room temperature and were nourished ad libitum with water and feed. All animal procedures were approved by the Institutional Animal Care and Use Committee at the University of Minnesota.

Chinchillas were randomly assigned to two groups; noninfected healthy controls (NCs) and infected animals (AOM). The Eustachian tube was surgically blocked in NC chinchillas to reduce the MEF turnover. Fifty colony-forming units of *Streptococcus pneumoniae* WT3 was inoculated in both ME bullae of the AOM chinchillas as a 100- μ l suspension in phosphate-buffered saline (PBS). The successful induction of an ear infection, or sterility for NC animals, was validated using criteria previously reported, based on tympanometric and microbiologic measurements (14).

Surgeries were performed to cannulate the femoral artery and jugular vein for i.v. dosing and blood sample withdrawal following procedures described elsewhere (14). All surgeries were performed with the chinchilla sedated by anesthetics (ketamine, intramuscularly, 5 to 10 mg/kg of body weight, with or without pentobarbital, intraperitoneally, 10 mg/kg). A microdialysis probe (CMA/20; 0.5 $\times$ 10 mm; CMA/Microdialysis, North Chelmsford, MA) was implanted into each ME via the top of the hypotympanic bulla through a tiny hole drilled manually with a 15-gauge needle. The 24-mm total length of the probe (14-mm shaft plus 10-mm membrane tip) fits well into the chinchilla ME space that spans approximately 22 mm from top to bottom. A sterile PE-10 catheter was implanted into the ME space, next to the probe via the same access point for direct MEF sampling and local dosing. A small plastic crown was cemented around the probe wings and catheter to secure them on top of the skull. The chinchilla was allowed to completely recover from anesthesia after surgeries. The PK experiments were conducted on conscious freely moving chinchillas (14, 26).

Dosing and sampling design. (i) **Dosing.** Two doses of amoxicillin were given simultaneously to the animal. An i.v. bolus of 40 mg/kg was administered (except in one chinchilla where a 15-mg/kg i.v. bolus was given) and a local intra-ME dose of amoxicillin was given directly into a randomly chosen (either left or right) ear. Intra-ME doses, ranging from 13 to 235 μ g/kg, were prepared in 1 ml of PBS and instilled locally into the ME bulla via the sterile indwelling catheter. The contralateral ear, which was not dosed, received 1 ml of blank PBS solution as artificial MEF. Naturally occurring MEF, if any, was aspirated from the ear before drug solution or blank PBS was added.

(ii) **Sampling.** Two online sampling loops (5 μ l) were fitted into a 10-port valve (Valco Instruments, Houston, TX) controlled by a digital sequence programmer model DVSP2 (VICI, Houston, TX) set to switch every 9 min. While one loop collected microdialysate, the alternate loop injected microdialysate sample onto the HPLC. A perfusion flow rate of 0.4 μ l/min was used for all in vivo microdi-

FIG. 1. Simultaneous i.v. bolus and local intra-ME dose plus multiple sampling sites—a schematic diagram of study design and experiment setup.

alysis studies. Microdialysis recovery was measured by simultaneous retrodialysis (25) using cefadroxil as the calibrator.

Blood samples were withdrawn via the femoral artery cannula, predose, immediately following dosing (time zero), and at 10 to 12 time points up to 180 min after dosing. Blood samples (200 to 300 μ l) were centrifuged to obtain plasma that was immediately frozen and stored at -60°C until off-line analysis.

The MEF samples for PD measurement were withdrawn via a sterile indwelling catheter implanted in each ear. For the dosed ear, the same catheter had been used for dosing amoxicillin locally, whereas for the nondosed ear the catheter had been used for instilling blank MEF. The MEF samples were drawn at 1 h predose and at 1, 2, 5, 8, and 10 h after dosing. At least 30 but no more than 100 μ l of MEF was withdrawn immediately, transferred to a plastic centrifuge tube (Chromtech, Inc., Apple Valley, MN), and stored frozen at -60°C for 3 to 5 h before plating and culture of the sample.

The dosing and sampling setup is illustrated in Fig. 1.

Sample analysis. (i) **Online PK.** Amoxicillin concentrations in microdialysates were determined by a sensitive online HPLC assay coupled with microdialysis sampling. A Supelco LC-8-DB column (150 $\times$ 2.1 mm, 5 μ m; Supelco, Bellefonte, PA) and a 2-cm guard column (Supelguard) were used at 40°C . The mobile phase consisted of 5.3% acetonitrile, 0.3% triethylamine, and 94.4% (vol/vol) 30 mM monobasic sodium phosphate buffer (pH 2.8) at a flow rate of 0.25 ml/min (Shimadzu LC-10AD; Shimadzu, Columbia, MD). The column effluent was monitored at 230 nm UV (Shimadzu SPD-6A). With a 3.6- μ l injection volume, the limit of quantitation of amoxicillin in the dialysate was 0.1 μ g/ml. The chromatograms were recorded and analyzed on a Hewlett-Packard HP 3390 integrator (Hewlett-Packard, Wilmington, DE).

(ii) **Off-line PK.** To determine the unbound amoxicillin concentration in chinchilla plasma taken by direct sampling, 150 μ l of chinchilla plasma was passed through a Centrifree (Millipore, Bedford, MA) ultrafiltration unit (1,500 $\times$ g, 15 min, 37°C). The ultrafiltrate was then processed with solid-phase extraction through an Analytichem Bond Elut (Chromtech, Inc., Apple Valley, MN) C₁₈ column before being injected onto the HPLC.

(iii) **Microbiology.** Type 3 *Streptococcus pneumoniae* (strain WT3, kindly provided by G. S. Giebink of the Otitis Media Research Center, University of Minnesota) frozen suspension was thawed, grown to mid-log phase, and then diluted into 0.01 M PBS to 500 CFU/ml to produce the inoculum. An inoculum size of 100 μ l was applied. The detection threshold was 25 CFU/ml with assay variability comparable to that in a previous study (22).

The MEF samples were immediately processed to measure the bacterial count. Briefly, a 10- μ l aliquot of MEF underwent 10-, 1,000-, and 100,000-fold serial dilution with sterile PBS. Another 20- μ l aliquot of original MEF and 20 μ l of the diluted suspension were placed on a 5% sheep blood agar plate for culture. All plates were incubated at 37°C under 10% CO_2 , and quantitative reading was done after 24 and 48 h of culture.

Data analysis. (i) **Microdialysis calibration.** The recovery (R_s) of the solute of interest (here, amoxicillin) was determined to be equal to the loss (L_c) of the calibrator (cefadroxil) measured by retrodialysis (from the perfusate to the dialyzed medium) as previously reported (10, 25)

$$R_s = L_c = 1 - \frac{C_{cc}}{C_{ic}} \quad (1)$$

FIG. 2. Four-compartment model for CL_{in} and CL_{out} determination in the study with simultaneous systemic (D_{iv}) and local intra-ME dosing (D_m).

where C_{ec} and C_{ic} are calibrator concentrations in the dialysate and perfusate, respectively. The solute concentration in the probed medium was then calculated as the concentration in the dialysate divided by L_c .

(ii) **PK analysis.** A mammillary four-compartment model (Fig. 2) was used for PK data analysis. The directions in and out are referenced to MEF. Differential equations were derived based on the model

$$\frac{dA_c}{dt} = CL_{out} \times (C_L + C_R) - (2 CL_{in} + CL_p) \times C_p - k_{12} \times A_c + k_{21} \times A_p \quad (2)$$

$$\frac{dA_p}{dt} = k_{12} \times A_c - k_{21} \times A_p \quad (3)$$

$$\frac{dA_L}{dt} = CL_{in} \times C_p - CL_{out} \times C_L \quad (4)$$

$$\frac{dA_R}{dt} = CL_{in} \times C_p - CL_{out} \times C_R \quad (5)$$

where A_c , A_p , A_L , and A_R represent the amount of amoxicillin in central, peripheral, left ear, and right ear compartments, respectively. C_p , C_L , and C_R are unbound drug concentrations in plasma and in MEF of left and right ears, respectively. CL_p , CL_{in} , and CL_{out} represent unbound plasma clearance and unbound influx and efflux clearances, respectively; k_{12} and k_{21} are the transfer rate constants between central and peripheral compartments. The PK parameters of interest, i.e., clearances (CL_p , CL_{in} , and CL_{out}), can be obtained, using noncompartmental analysis, as follows (26):

$$CL_{out} = \frac{D_m}{AUL - AUR} \quad (6)$$

$$CL_{in} = CL_{out} \times \frac{AUR}{AUC} \quad (7)$$

$$CL_p = \frac{D_{iv} + D_m}{AUC} \quad (8)$$

where D_m and D_{iv} are simultaneous doses given directly to the left ear and i.v., respectively, and AUC, AUL, and AUR represent areas under the curve in plasma, left ear (in this case, the locally dosed ear), and right ear (the nondosed ear), respectively. AUCs (expressed in terms of unbound concentrations of amoxicillin in plasma and MEF) were determined using the trapezoidal rule, with a correction to infinite time. The model assumes linear kinetics and equivalent distribution parameters for the left and right ear (26).

Nonlinear regression analysis of the amoxicillin data from each animal using a nonlinear efflux (MEF to plasma) rate was also performed. The concentration-time data from plasma and both MEFs (dosed and nondosed) were analyzed by fitting the parameters of the mammillary compartment model shown in Fig. 2 to all three data sets simultaneously (21). In this analysis, the influx from plasma to MEF was assumed to be linear, while the efflux was assumed to be nonlinear. Therefore, CL_{in} was equal to transfer rate constant from plasma to MEF (k_{in}) multiplied by central volume of distribution (V_{cu}) (defined as amount in central compartment divided by the unbound plasma concentration). In contrast, CL_{out} was expressed in terms of C_{MEF} and the Michaelis-Menten parameters, V_{max} and K_{mu} .

$$CL_{in} = k_{in} \times V_{cu} \quad (9)$$

FIG. 3. Amoxicillin plasma concentration-time profiles from 10 chinchillas after i.v. bolus of 40 mg/kg (except for NC031, for which i.v. bolus dose was 15 mg/kg).

$$CL_{out} = \frac{V_{max}}{K_{mu} + C_{MEF}} \quad (10)$$

The fitted parameters (CL_{in} , V_{max} , and K_{mu}) and $CL_{out,max}$ were compared to the values calculated based on the linear model, obtained by noncompartmental analysis.

(iii) **PD analysis.** Graphical examination of the growth-kill profile of WT3 *S. pneumoniae* in MEF was performed in an exploratory manner.

(iv) **Statistical analysis.** Concentration-time data and fitted or calculated parameters, expressed as means $\pm$ standard deviations, were based on $n \geq 3$ unless noted. Differences were considered significant when P was <0.05 using Student's t test. Nonparametric analysis was also explored.

RESULTS

Amoxicillin concentration-time profiles in chinchilla plasma and MEF. The amoxicillin plasma concentration-time data following an i.v. bolus dose obeyed a two-compartment model and exhibited a rapid elimination phase. The plasma levels fell below the detection limit within 2 to 3 h after dosing with a terminal half-life averaging approximately 0.3 h (Fig. 3). As in the previous study (14) no significant difference in amoxicillin distribution kinetics was observed between infected (AOM) and NC animals ($P > 0.05$). The amoxicillin concen-

FIG. 4. Amoxicillin MEF concentration-time profiles in the dosed ears from 10 chinchillas after i.v. bolus of 40 mg/kg (except for NC031, 15 mg/kg) and simultaneous local intra-ME doses of 13 to 235 μ g/kg. The local ME dose (D_m ; unit, μ g/kg) for each chinchilla is shown in parentheses after the chinchilla identification number in the symbol key.

FIG. 5. Amoxicillin MEF concentration-time profiles in the non-dosed ears from 10 chinchillas after i.v. bolus of 40 mg/kg (except for NC031, 15 mg/kg) and simultaneous local doses of 13 to 235 $\mu\text{g/kg}$ into the opposite ear. Numbers in parentheses are as explained in the legend to Fig. 4.

tration-time profiles in the MEF, corresponding to a wide range of intra-ME dose levels—e.g., low (13 to 19 $\mu\text{g/kg}$), medium (66 to 96 $\mu\text{g/kg}$), and high (164 to 235 $\mu\text{g/kg}$)—appeared downward concave on a logarithmic y axis. This suggested nonlinear efflux kinetics from MEF to plasma. The peak concentrations in the dosed ear were observed very soon after dosing and ranged from 8.8 to 110.6 $\mu\text{g/ml}$ (Fig. 4). In contrast, amoxicillin concentrations in the nondosed ear peaked around 30 to 60 min after dosing at levels from 2 to 27 $\mu\text{g/ml}$ (Fig. 5).

Influx and efflux clearances between plasma and MEF. The plasma unbound clearance (CL_p) of amoxicillin as well as the influx (CL_{in}) and efflux (CL_{out}) clearances were first estimated noncompartmentally using equations 6, 7, and 8. As shown in Table 1, the calculated systemic clearance of amoxicillin was 22.1 ± 6.7 ml/min-kg, which was comparable to estimates obtained previously (14) (15 to 20 ml/min-kg). Noncompartmental estimates of the unbound clearances in the present study, CL_{in} and CL_{out} , were $(2.8 \pm 1.3) \times 10^{-3}$ and $(6.9 \pm 2.7) \times 10^{-3}$ ml/min-kg, respectively. The $\text{CL}_{in}/\text{CL}_{out}$ ratio was 0.44 ± 0.15 . CL_{in} is statistically different from CL_{out} ($P < 0.002$).

In the compartmental modeling, based on simultaneous analysis of plasma and MEF data, a nonlinear Michaelis-Menten equation was applied to the unbound efflux clearance,

FIG. 6. Representative chinchilla (AOM025). Simultaneous analysis of three data sets (plasma and left and right MEF) with a mammillary compartment model incorporating Michaelis-Menten relationship for CL_{out} . Symbols represent data points: $\square$, plasma; $\triangle$, dosed ear; $\circ$, nondosed ear. Lines represent the best fit.

CL_{out} . The resulting fitted curve was illustrated by Fig. 6. Model parameters for seven animals were successfully fitted while convergence was not obtained with the data from the other three chinchillas. Parameters are summarized in Table 2. CL_{in} and CL_{out} were $(4.6 \pm 2.4) \times 10^{-3}$ and $(19.2 \pm 9.7) \times 10^{-3}$ ml/min-kg, respectively. The nonlinear PK parameters, V_{max} and the unbound Michaelis constant K_{mu} , averaged 0.48 $\mu\text{g/min-kg}$ and 29.3 $\mu\text{g/ml}$, respectively. Because of the dose dependency of CL_{out} in this compartmental analysis, $\text{CL}_{out,max}$ represents the maximum unbound intrinsic efflux clearance from MEF calculated as the quotient of V_{max}/K_{mu} . As with the noncompartmental analysis results, the difference between influx and efflux clearances was statistically significant ($P = 0.006$) with a $\text{CL}_{in}/\text{CL}_{out}$ mean ratio of 0.25.

Antimicrobial efficacy of amoxicillin in chinchilla MEF. Microbiologic evaluation based on sampling and culture of *S. pneumoniae*-infected MEF was performed for five chinchillas. A slight contamination of staphylococcus was visible in only one animal (AOM024); all other cultures showed no sign of contamination. The bacterial growth-kill curves in the non-dosed and locally dosed ME are illustrated by Fig. 7.

All five chinchillas provided consistently positive bacterial culture in the nondosed ear for all the samples taken. No significant trend in growth was evident. The *S. pneumoniae* levels in the nondosed ear MEF remained at an average of 10^6 CFU/ml, indicating that a stagnant phase of bacterial growth was achieved. In contrast, a rapid and evident killing effect was

TABLE 1. Summary of amoxicillin PK parameters from noncompartmental analysis

Animal	D_{iv} ($\mu\text{g/kg}$)	D_m ($\mu\text{g/kg}$)	CL_p (ml/min-kg)	CL_{in} (ml/min-kg)	CL_{out} (ml/min-kg)	$\text{CL}_{in}/\text{CL}_{out}$
NC023	40,000	233	14.11	0.0130 ^a	0.0093 ^a	1.40 ^a
NC031	15,000	164	26.19	0.0016	0.0030	0.53
NC037	40,000	215	35.71	0.0026	0.0056	0.46
NC038	40,000	66	23.17	0.0032	0.0093	0.34
NC039	40,000	13	19.28	0.0024	0.0092	0.26
NC043	40,000	19	15.57	0.0019	0.0098	0.19
AOM020	40,000	170	14.49	0.0018	0.0043	0.42
AOM025	40,000	235	24.15	0.0055	0.0086	0.64
AOM028	40,000	96	21.53	0.0041	0.0085	0.48
AOM029	40,000	19	26.79	0.0022	0.0035	0.63
Mean			22.10	0.0028	0.0069	0.44
SD			6.68	0.0013	0.0027	0.15

^a NC023 was excluded from the mean and standard deviation calculation as it was considered to be an outlier.

TABLE 2. Summary of amoxicillin PK parameters from compartmental analysis incorporating Michaelis-Menten fitting on the efflux clearance

Animal	D_m ($\mu\text{g/kg}$)	$V_{\max}$ ($\mu\text{g/min-kg}$)	K_{mu} ($\mu\text{g/ml}$)	CL_{in} (ml/min-kg)	$\text{CL}_{\text{out,max}}^a$ (ml/min-kg)	$\text{CL}_{\text{in}}/\text{CL}_{\text{out}}$
NC031	164	0.23	17.8	0.0053	0.0128	0.41
NC037	215	0.36	9.3	0.0090	0.0394	0.23
NC038	66	0.62	54.9	0.0047	0.0113	0.42
NC039	13	0.08	4.5	0.0025	0.0186	0.13
NC043	19	0.42	34.0	0.0020	0.0123	0.16
AOM025	235	0.89	48.6	0.0033	0.0184	0.18
AOM028	96	0.78	35.9	0.0053	0.0217	0.24
Mean		0.48	29.3	0.0046	0.0192	0.25
SD		0.29	19.3	0.0024	0.0097	0.12

$$^a \text{CL}_{\text{out,max}} = V_{\max}/K_{\text{mu}}$$

observed in MEF that received a local dose of amoxicillin. At minimum, a 2- to 3-log-unit drop in bacterial count was observed within 10 h postdose. In some instances *S. pneumoniae* was completely eradicated from the MEF, leading to a negative culture result.

Due to the limited sample size, no formal PD analysis was conducted. In an exploratory manner the PK-PD relationship was examined by overlaying the amoxicillin concentration measured by microdialysis and different levels of the MIC for the resistant (MIC = 8 $\mu\text{g/ml}$), intermediately resistant (MIC = 1 $\mu\text{g/ml}$), and susceptible (MIC $\leq$ 0.1 $\mu\text{g/ml}$) serotypes of *S. pneumoniae*. For all doses, amoxicillin concentrations in the MEF were maintained above the MIC corresponding to the intermediate resistant strains, to which the *S. pneumoniae* WT3 strain belongs. There was no apparent graphic evidence for a dose-dependent effect or amoxicillin concentration-effect relationship, although a slight trend was suggested by the fact that the highest amoxicillin local concentration (chinchilla AOM024) also had the fastest-declining killing curve.

DISCUSSION

Methodology. By incorporating microdialysis sampling for the analysis of antibiotic levels in MEF over time, the study of antimicrobial PK/PD in the MEF was possible in spite of the limited MEF volume available. Microdialysis allows monitoring of bacteriologically relevant free antibiotic levels. The methodology also permits these measurements while reserving MEF volume for bacterial kill-rate analysis.

PK. The influx and efflux clearances and their ratios evaluated following combined i.v. and local intra-ME dosing were comparable to those obtained previously where doses were given only as an i.v. bolus or i.v. infusion. The $\text{CL}_{\text{in}}/\text{CL}_{\text{out}}$ ratio of 0.44 ± 0.15 ($n = 9$) was not significantly different ($P = 0.14$) from the previously obtained ratio of 0.33 ± 0.15 ($n = 9$). The saturation of the efflux rate at high amoxicillin levels in the MEF following large local intra-ME doses decreased the CL_{out} , leading to a greater ratio of CL_{in} to CL_{out} .

A less-than-unity distribution ratio between MEF and plasma was observed in the current as well as the previous study. This may be evidence for the existence of an efflux transporter on the apical side (MEF side) of the ME mucosal

epithelium, pumping amoxicillin towards the basolateral side (blood side) of the epithelial membrane.

In this present study, the systemic dose was the same for all chinchillas. In contrast there were three different ranges of local intra-ME doses administered—low (13 to 19 $\mu\text{g/kg}$), medium (66 to 96 $\mu\text{g/kg}$), and high (164 to 235 $\mu\text{g/kg}$). These stratified local doses were employed to examine the possible existence of saturable efflux transport kinetics. The amoxicillin concentration-time profiles in dosed and nondosed ears in each individual chinchilla clearly indicated nonlinear efflux kinetics. In the dosed ear, a log-linear declining concentration-time profile in the MEF was more evident at low doses, as opposed to the extended elimination half-life and downward concave shape observed at the medium to high doses. In comparison, the concentration-time profiles in the nondosed ear were largely parallel regardless of the actual levels of amoxicillin. This observation prompted the adoption of a nonlinear model to characterize influx and efflux clearances, in addition to the linear model involving noncompartmental analysis. The fitted K_m (mean, 29.3 $\mu\text{g/ml}$) fell well within the range of MEF levels of amoxicillin in the dosed ear. However, since peak amoxicillin levels in the nondosed ear ranged from only 2 to 27 $\mu\text{g/ml}$, no saturation would be expected in those concentration-time profiles.

The ME is a relatively remote, small, peripheral tissue space that is not highly perfused by blood flow. During systemic amoxicillin therapy at usual doses, levels in the ME mucosa would not be high enough to saturate efflux. The analysis that incorporated Michaelis-Menten parameters also produced distribution clearances and distribution ratio parameters that agreed better with the previous study. Given the fact that nonlinear kinetics are often attributed to enzyme-mediated or active transport mechanisms, the results of the current study provide new evidence to support the existence of an active transporter in the ME mucosal epithelium membrane.

What was discovered previously and reproduced in the current study was that no marked distinction was seen between the infected and NC animals in terms of the MEF/plasma distri-

FIG. 7. Type 3 *S. pneumoniae* growth and kill curves in MEF; summary of five chinchillas.

bution. Tissue that is infected and inflamed would be expected to have a more porous vascular endothelial membrane. The lack of difference in the distribution ratios suggested that the infection did not play a big role in changing the permeability of this physical barrier or that infection enhances permeability to the same degree in the two directions. How infection affects the passive and active transport processes remains a complex scenario that requires further explorations.

PD. It is known that PD evaluation in an in vivo setting will encounter more variables than in vitro studies (19, 20). The variability comes from multiple factors including host immune condition, experimental procedures, and interanimal variability. The variation may cause complexity in the interpretation of the bacterial growth or killing kinetics based on cultures of tissue fluid samples from the animal model.

From the five chinchillas studied, in the 10 ears (five dosed and five nondosed) infected by type 3 *S. pneumoniae*, the general trends of the growth-kill curves were in agreement with the previously reported data (17, 22, 24). The continuing growth of pneumococcus in the nondosed control ear was limited; indeed, a relatively stable plateau was observed. It is speculated that *S. pneumoniae* had entered a stationary phase 3 to 7 days after living, encapsulated bacteria were inoculated into the nutrient-limited ME environment. In a study conducted by Sato et al. (22), the growth of *S. pneumoniae* reached a plateau in the chinchilla infected ME when the bacterial count rose to around 7 to 8 log units. Likewise in the current study, the steady-state bacterial turnover in the nondosed ME clearly represented a control of the killing process in the ear dosed with amoxicillin.

The bacterial count declined markedly following the dosing with amoxicillin. Within 5 h after dosing, all bacterial culture readings were reduced by at least 5 log units at all dose levels. The extremely rapid kill rate did not appear to be dose related. It has been well acknowledged that amoxicillin, or beta-lactams in general, belongs to the antibiotics classified by their concentration-independent bacterial killing nature, i.e., the kill rate is not determined by the dose or concentration of the antibiotics, instead being driven more by the time or duration of treatment at which drug levels remain above the MIC. The difference in kill rate among these chinchillas may be attributed to the individual host immune response, which carries a relatively large interindividual variability.

For the sake of experimental simplicity, amoxicillin was studied as monotherapy in the current study, although it is known that amoxicillin is commonly prescribed in combination with clavulanic acid.

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