Pharmacokinetics of Aztreonam in Healthy Subjects and Patients with Cystic Fibrosis and Evaluation of Dose-Exposure Relationships Using Monte Carlo Simulation[▽]

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Aztreonam (AZM) is a monobactam antibiotic with a high level of activity against gram-negative microorganisms, including *Pseudomonas aeruginosa*. We evaluated AZM pharmacokinetics and pharmacokinetic-pharmacodynamic relationships in patients with cystic fibrosis (CF) and healthy subjects. Pharmacokinetic data in eight CF patients and healthy subjects that were matched for age, gender, weight, and height were obtained and analyzed by using the nonparametric adaptive grid algorithm. Probabilities of target attainment using percentages of time of unbound concentration above the MIC ($fT > MIC$) were obtained by using a Monte Carlo simulation. AZM total body clearance was significantly higher in CF patients (100.1 ± 17.1 versus 76.2 ± 7.4 ml/min in healthy subjects; $P < 0.01$). The pharmacokinetic parameter estimates for terminal half-life (1.54 ± 0.17 h [mean $\pm$ the standard deviation]) and volume of distribution (0.20 ± 0.02 liters/kg in patients with CF patients were not different from those in healthy subjects. Monte Carlo simulations with a target of a $fT > MIC$ of 50 to 60% at a dose of 1,000 mg every 8 h indicated a clinical breakpoint of 4 mg/liter and 1 to 2 mg/liter for healthy subjects and CF patients, respectively. This study using matched controls showed that AZM total body clearance and not the volume of distribution is higher in CF patients as a result of increased renal clearance. Pharmacokinetic parameter estimates in healthy subjects resulted in a clinical susceptibility breakpoint of ≤ 4 mg/liter for a dose of 1,000 mg every 8 h. Patients suspected of having high clearance rates, such as CF patients, should be monitored closely, with dosing regimens adjusted accordingly.

Aztreonam is a monobactam antibiotic with a high level of activity against gram-negative microorganisms, including *Pseudomonas aeruginosa*. The pharmacokinetics (PK) of aztreonam have been extensively studied in healthy subjects (38), as well as in a variety of small cohorts of patients with different underlying disease states (23, 24, 35), including patients with cystic fibrosis (CF) experiencing pulmonary exacerbations due to *P. aeruginosa* (6, 7, 33, 34). Despite the fact that aztreonam has been on the market since the early 1980s, we are not aware of any PK data in the public domain related to adult patients with CF. In a small pediatric CF study aztreonam clearance was found to be higher than in unaffected adults, with no apparent PK differences compared to two children without CF (30, 31). Over the years there has been controversy over whether patients with CF truly exhibit different PK characteristics as a result of their disease or whether the observed differences in part are an artifact and the result of normalizing parameters to total body weight or surface area in a patient group with known altered body composition (36, 37). Most of

the earlier published PK studies in CF patients lack adequate control groups that match for age, gender, height, and weight.

An important focus of antimicrobial pharmacology is the identification of relationships between antibiotic PK and pharmacodynamic (PD) characteristics. In recent years various strategies have been sought to correlate a microorganism's susceptibility (as indicated by the MIC) with the efficacy of an antimicrobial drug. For different drug classes PD indices such as the exposure of unbound free (f) drug in relation to the MIC ($fAUC/MIC$ or $fT > MIC$) have been shown to correlate well with efficacy (11) and now contribute significantly to the establishment of MIC breakpoints that differentiate between high and low probabilities of cure (1).

A statistical technique that recently has found its way into drug development is Monte Carlo simulation (MCS) (1, 5, 14, 26). MCS can be used to determine the probability of target attainment (PTA) of PD indices by taking the inherent variation within different populations into account (2, 13, 14, 25, 28, 29). MCS differs from traditional simulation in that the model parameters are treated as stochastic or random variables rather than as fixed values. Between-patient variability in population PK parameter estimates has only recently been recognized as a factor in predicting the outcome in individual patients and establishing breakpoint and targets for clinical susceptibility (28). To date, many MCS studies use PK parameter estimates obtained in healthy subjects or in sub-

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jects other than the target patient population to evaluate target attainment with different dosing regimens. It has been shown that this may well lead to over- or underestimation of the PTAs (8, 28).

In the present study we sought to explore the likelihood of treatment success with specific dosing regimens of aztreonam using data from well-defined adult CF patient and healthy subject populations. Patient PK parameter estimates were compared to data obtained in a cohort of healthy subjects who were matched to the CF patients for age, gender, height, and weight. The data were used to develop integrated PK-PD stochastic models and were used in a MCS study to determine the PTA and evaluate current aztreonam breakpoints.

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MATERIALS AND METHODS

Study population. Patients with CF were recruited from the Adult Cystic Fibrosis Center at Haga Hospital, The Hague, The Netherlands, and healthy subjects were recruited from Leiden University Medical Center. Subjects were matched for age, gender, weight, and height. Patients and healthy subjects were enrolled in a single-dose PK study as part of a clinical trial evaluating the PK data, safety, and efficacy of aztreonam administered by ambulatory continuous infusion in patients with CF. The study was approved by the hospital's institutional review board and was conducted in accordance with the principles of the Declaration of Helsinki. Details of the study were fully explained to patients and healthy subjects who gave their written informed consent. CF patients were eligible only if the bacteria isolated from the last sputum culture were susceptible to aztreonam (MIC of ≤ 8 mg/liter). CF was diagnosed in all patients in early childhood based on pathological sweat tests, pancreatic insufficiency, and genotyping.

Dosing and sample collection. Volunteers and patients received a single 2,000-mg dose as a short 20-min infusion using a programmable pump (Terufusion, model STC 521; Terumo Corp., Tokyo, Japan) with samples collected over an 8-h period. The infusion line was primed with drug solution to ensure full dose delivery. Blood samples were taken prior to and after the start of a 20-min infusion of 2,000 mg of aztreonam at 0.33, 0.5, 0.67, 0.83, 1, 1.5, 2, 3, 4, 5, 6, 7, and 8 h. Samples were collected on ice and centrifuged within 1 h after collection, with serum stored at -70°C until analysis.

Urine samples were collected every 2 h for up to 8 h after the aztreonam dose. Patients and volunteers were encouraged to drink during the PK study day. After each void the volume was measured, after which a portion of the urine was stored in polypropylene bottles at -70°C until analysis.

Aztreonam concentrations in serum and urine were determined with a validated high-pressure liquid chromatography method (18, 42). The lower limit of quantification was 0.75 mg/liter. Calibration curves in serum were found to be linear over the working range of 2.5 to 250 mg/liter. Precision within and between runs showed a coefficient of variation (CV) of 0.5 to 1% ($n = 10$). The intra- and interday CVs were 1 to 4.7%, respectively ($n = 10$). Recovery from serum was $99.1 \pm 2.0\%$. The assay error pattern (SD) over the working range was calculated as $\text{SD} = 0.110473 + 0.009535C + 0.0000452C^2$, where SD is the standard deviation of the assay, C represents the measured aztreonam concentration, and C^2 is the square of C .

Aztreonam serum protein binding was determined by ultrafiltration by means of the Centrifree micropartition system (MPS-1 4010; Amicon Corp., Lexington, MA). The filtration was performed at room temperature at $1,000 \times g$ in a 35° fixed angle rotor centrifuge. Aztreonam serum protein binding was expressed as $1 - \text{the free aztreonam/total aztreonam ratio} \times 100\%$.

PK analysis. Individual PK data were analyzed with WinNonlin (WinNonlin Professional, version 4.0.1; Pharsight Corp; Mountain View, CA) by using a two-compartment model with zero-order input and first-order elimination from the central compartment. The concentration-time profiles were analyzed by nonlinear regression analysis using a weighted least-squares simplex algorithm, with data weighted to the reciprocal of the observed values. Model discrimination was done by using Akaike's information criterion (43). The apparent volume of the central compartment (V_1), the first-order rate constant from the central compartment (k_{10}), and the intercompartmental transfer rate constants (k_{12} and

k_{21}) were identified by the program. Hybrid parameters were calculated by standard PK equations. Renal clearance was estimated by noncompartmental analysis using the urinary data model in WinNonlin.

Population PK analysis. Data sets were analyzed by using the nonparametric adaptive grid with adaptive gamma (NPAG) algorithm described by Leary and coworkers (9, 22). A two-compartment open model with zero-order intermittent drug input and first-order elimination was fitted to the data. Concentrations were weighted by the reciprocal of their variance (Fisher information index) by incorporating the assay error pattern into the appropriate section of the NPAG program. This polynomial is multiplied by a scalar value of gamma that is iteratively determined with each cycle. Maximal a posteriori probability (MAP)-Bayesian estimates were obtained by using the "population of one" utility within NPAG. Model fit was examined by regression analysis and by visual inspection of individual residual plots after the MAP-Bayesian step. The weighted mean error was used as a measure of bias, and the bias-adjusted weighted mean squared error was used as a measure of precision (21).

Model assessment. The final model was assessed by an inspection of standard diagnostic plots of observed versus model-predicted concentrations and separate plots of weighted residuals. A predictive check was performed by simulating a new data set from the original data set by a Monte Carlo population model generator/analyzer (GENMM.exe, USC*PACK; Laboratory of Applied Pharmacokinetics, University of Southern California). Nonparametric population PK models were simulated for aztreonam, giving concentration-time profiles for 999 patients that were randomly generated by MCS. These patient profiles were subsequently analyzed with NPAG. Mean and median parameter vectors and dispersion factors were evaluated by comparison with the respective values estimated in the final model in terms of percentage differences and the 90% confidence intervals (CI).

MCS. MCS of PTA was performed by using MicLab (version 2.33; Medimatics, Maastricht, The Netherlands), a program designed specifically for evaluating target attainment using antimicrobial indices ($T > \text{MIC}$, $C_{\text{max}}/\text{MIC}$ ratio, and AUC/MIC ratio). The program allows inclusion of the covariance matrix (or correlation matrix) of the parameter estimates used in the simulations. The output consists of a probability distribution, a cumulative probability distribution, and specific CIs over user-defined MIC and $T > \text{MIC}$ ranges. The clinically recommended dosing regimens of aztreonam in patients with CF (1,000 to 2,000 mg given every 8 h [q8h]), respectively, were used in the simulation in patients and healthy subjects using a 10,000-subject population and a log-normal distribution of the parameters. Protein binding was included in the model; $42.1 \pm 2.7\%$ for CF patients and $51.5 \pm 3.1\%$ for the healthy subjects, respectively. Regimens were simulated both with and without the correlation matrix of the parameters. The dosing regimens were assumed to include an infusion time of 20 min and a total of 10 consecutive doses. The percentage of $fT > \text{MIC}$ was determined for MICs ranging from 0.03 to 64 mg/liter. A PK-PD target of 50 to 60% $fT > \text{MIC}$ was used since it is considered to give near-maximal bactericidal activity (10, 26).

Statistical analysis. PK data were tested for normal distribution by using a normality test (SPSS 15.0 for Windows; SPSS, Inc., Chicago, IL). The Fisher exact test was used in case of non-normal distribution. For normally distributed data, the two-tailed Student t test for unpaired data was used to compare results between patients and controls. Bias was evaluated with the Wilcoxon signed-rank test. A probability value (P) of <0.05 was considered significant. All results are expressed as mean $\pm$ the SD unless specified otherwise.

RESULTS

Eight patients with CF (age range, 26 to 35 years) and eight healthy volunteers (age range, 22 to 35 years) were enrolled. Of the CF patients, six were homozygous for the CF gene mutation ($\Delta F508$), a 3-bp deletion in the CF transmembrane conductance regulator (CFTR) gene, resulting in the loss of amino acid 508 of the CFTR protein, and two patients had a one allele with the $\Delta F508$ mutation and either a different type of CF mutation or an unknown mutation (J. Zielenski et al., Cystic Fibrosis Mutation Data Base [http://www.genet.sickkids.on.ca/cftr/app]). The demographic data for the two groups are summarized in Table 1. Healthy subjects were well matched for age, gender, weight, and height, with no differences in demographic data except for the calculated creatinine clearance

TABLE 1. Demographic characteristics of CF patients and control subjects^a

Characteristic	Mean $\pm$ SD		<i>P</i>
	Subjects with CF (<i>n</i> = 8)	Controls (<i>n</i> = 8)	
Gender	5 F, 3 M	6 F, 2 M	NA
Age (yr)	29.8 $\pm$ 3.2	26.6 $\pm$ 4.0	0.13
Wt (kg)	54.9 $\pm$ 6.6	58.8 $\pm$ 6.9	0.29
Ht (cm)	167.9 $\pm$ 6.1	167.9 $\pm$ 5.6	1.00
LBM (kg)	43.9 $\pm$ 5.7	45.6 $\pm$ 4.9	0.56
BSA (m ²)	1.62 $\pm$ 0.12	1.66 $\pm$ 0.10	0.38
CL _{CR} (ml/min/1.73 m ²)	159.6 $\pm$ 28.2	106.3 $\pm$ 16.8	<0.001

^a Values are means except as noted. F, female; M, male; NA, not applicable; LBM, Hallynck et al. (17); BSA, body surface area (15), the body mass index, calculated as weight/(height in meters)²; CL_{CR}, creatinine clearance calculated according to the methods of Jelliffe and Jelliffe (20) and Touw et al. (40).

(Table 1). Mean serum concentration-time profiles of aztreonam in CF patients and healthy subjects are shown in Fig. 1. The concentration-time curves show a comparable bi-exponential profile in both patients and healthy subjects with data best described by an open two-compartmental model. Peak concentrations at the end of the infusion were 228 $\pm$ 49 mg/liter in CF patients and 242 $\pm$ 38 mg/liter in healthy subjects and were not significantly different (*P* = 0.18). The postinfusion concentrations were lower in the CF patients than in the healthy subjects. Table 2 summarizes the mean PK parameter estimates in CF patients and matched healthy subjects. No significant differences were observed in the distribution half-life (*t*_{1/2α}), elimination half-life (*t*_{1/2β}), and the apparent volumes of distribution *V*₁, *V*_{ss}, and *V*_β. Total body clearance (CL) and renal clearance (CL_R) estimates were significantly increased in CF patients (*P* < 0.01). Clearance normalized for body weight in CF patients was 41% higher (*P* < 0.01). Mean AUCs in CF patients were 23% lower than those observed in the healthy subjects (*P* < 0.01).

The mean fraction of the dose recovered from the urine in CF patients was 72.0% (range, 55.0 to 78.9%) and was not different from that in healthy subjects (Table 2). Urine collections after a single dose were complete in all patients but

TABLE 2. Aztreonam PK parameter estimates in patients with CF and in matched healthy subjects

Parameter	Mean $\pm$ SD		<i>P</i>
	CF patients (<i>n</i> = 8)	Healthy volunteers (<i>n</i> = 8) ^b	
<i>t</i> _{1/2α} (h)	0.23 $\pm$ 0.07	0.32 $\pm$ 0.11	NS
<i>t</i> _{1/2β} (h)	1.54 $\pm$ 0.17	1.81 $\pm$ 0.33	NS
<i>V</i> ₁ (liters/kg)	0.109 $\pm$ 0.012	0.112 $\pm$ 0.025	NS
<i>V</i> _{ss} (liters/kg)	0.199 $\pm$ 0.023	0.175 $\pm$ 0.037	NS
<i>V</i> _β (liters/kg)	0.241 $\pm$ 0.030	0.206 $\pm$ 0.050	NS
AUC (mg · h/liter)	336.7 $\pm$ 54.7	414.1 $\pm$ 40.8	0.0095
CL (ml/min)	100.1 $\pm$ 17.1	76.2 $\pm$ 7.4	0.0044
CL (ml/min/kg)	1.84 $\pm$ 0.33	1.31 $\pm$ 0.13	0.0013
CL _R (ml/min)	72.8 $\pm$ 4.1	55.2 $\pm$ 5.3*	0.0036
CL _{NR} (ml/min)	27.4 $\pm$ 9.9	27.9 $\pm$ 21.4*	0.11
Fe	0.72 $\pm$ 0.08	0.71 $\pm$ 0.05*	NS
Protein binding (%)	42.1 $\pm$ 2.7	51.5 $\pm$ 3.1	<0.0001

^a NS, not significant; *t*_{1/2α}, distribution half-life; *t*_{1/2β}, terminal elimination half-life; *V*₁, volume of distribution of central compartment; *V*_{ss}, volume of distribution at steady state; *V*_β, volume of distribution during the elimination phase; AUC, area under the curve; CL, total body clearance; CL_R, renal clearance; CL_{NR}, nonrenal clearance; Fe, fraction of the dose recovered in the urine.

^b *, Based on urinary data from seven subjects.

incomplete in one healthy subject. Nonrenal clearance calculated as the difference between the total body clearance and the renal clearance in CF patients was 0.54 $\pm$ 0.22 ml/min/kg. Aztreonam protein binding in patients with CF was significantly lower than in healthy subjects: 42.1 $\pm$ 2.7% versus 51.5 $\pm$ 3.1%, respectively (*P* < 0.001). Albumin concentrations in CF patients were lower than in controls: 38.1 $\pm$ 3.0 and 42.0 $\pm$ 5.0 g/liter, respectively (*P* < 0.05). Aztreonam infusion was well tolerated, with no phlebitis or other side effects noted in patients and healthy subjects. Table 3 summarizes population PK parameter estimates generated with NPAG for CF patients and healthy subjects. Overall, data for the two groups were well described by the models (Fig. 2). Predictive performance as evaluated by regression analysis using mean parameter estimates yielded the following results: observed = 0.243 + 0.995 $\times$ predicted (*R*² = 0.99; *P* < 0.001) and observed = -1.744 + 1.029 $\times$ predicted (*R*² = 0.99; *P* < 0.001) for CF

FIG. 1. Mean serum concentration-time curves of aztreonam in eight adult patients with CF and eight matched healthy subjects during and after a 20-min infusion of 2,000 mg of aztreonam. Mean datum points ($\pm$ the SD) are graphically connected for each group.

TABLE 3. Population PK parameter estimates for azteonam in healthy volunteers and in patients with CF

Population ^a	Individual estimates and measures of dispersion		Correlation between estimates			
	Mean (SD)	%CV	<i>V</i> _c	k12	k21	CL
Healthy subjects						
<i>V</i> _c (liters)	7.075 (1.229)	17.4	1			
k12 (h ⁻¹)	0.569 (0.246)	43.3	-0.5869	1		
k21 (h ⁻¹)	1.241 (0.393)	31.6	-0.3473	0.7264	1	
CL (liters/h)	4.590 (0.443)	9.7	0.5625	-0.0749	0.3916	1
Patients with CF						
<i>V</i> _c (liters)	5.993 (0.734)	12.3	1			
K12 (h ⁻¹)	1.339 (0.608)	45.4	-0.5238	1		
K21 (h ⁻¹)	1.586 (0.310)	19.5	-0.2922	0.8585	1	
CL (liters/h)	6.052 (1.062)	17.6	0.4738	0.3234	0.4298	1

^a %CV, percent coefficient of variation; *V*_c, volume of central compartment; k12 and k21, rate constants between central and peripheral compartments; CL, total body clearance.

FIG. 2. Population model (A) and MAP-Bayesian individual predicted versus observed concentrations (B) based on the final population PK model developed showing data for healthy subjects (●) and CF patients (○). The lines of best fit were not statistically significant different from the line of identity.

patients and healthy subjects, respectively. Bias and precision estimates were -0.08 and 1.06 mg/liter and -0.06 and 1.11 mg/liter, respectively. There were no significant differences between models generated with the patient data and the simulated models obtained with the GENMM.exe model generator. The final mean parameter vectors, variances, and correlation matrices were subsequently used to simulate concentration-time profile distributions in patients receiving different aztreonam dosing regimens. In order not to underestimate the CV due to the small patient sample size, a larger CV was used for clearance ($CV = 30\%$) in the MCS of aztreonam dosing regimens in CF patients. The percentage chosen is comparable to the clearance variability observed in studies with larger numbers of CF patients that were sampled with similar precision (28, 41) and is in line with the results of our Monte Carlo model validation (using GENMM.exe).

Table 4 shows the PTA in the conventional manner using mean parameters taking into account parameter variability (expressed as SD values) and parameter interdependence (correlation matrix). The data are presented as 30, 40, 50, and 60% of $fT > MIC$ with 100% PTA for each $fT > MIC$ summarized at the bottom of the table. At a 1,000-mg aztreonam dose and a level of 50 to 60% $fT > MIC$, a PTA of 100% was reached at 2 and 1 mg/liter for healthy subjects and CF patients, respec-

tively. At an aztreonam dose of 2,000 mg q8h, the corresponding values were 4 and 2 mg/liter, respectively.

Figure 3 gives the full probability distribution expressed as percentages of $fT > MIC$ over the MIC range. Due to increased aztreonam clearance in patients with CF the mean percentages of $fT > MIC$ in patients tend to be lower than those in healthy subjects. Increased variability in clearance in this patient population resulted in widening of the CI. When applying the target of 50 to 60% $fT > MIC$, a PTA of $\geq 99\%$ in patients with CF is reached at relatively low MICs (a 95% CI of ca. 1 to 2 mg/liter versus a mean of ca. 2 to 4 mg/liter at the 1,000-mg

TABLE 4. PTA after MCS for two aztreonam dosing regimens in healthy subjects and in patients with CF summarizing four target percentages of time (30, 40, 50, and 60%) that the unbound fraction of aztreonam remained above the MIC (% $fT > MIC$)

Dosage, population, and parameter	% $fT > MIC$ at:			
	30% of time	40% of time	50% of time	60% of time
Aztreonam at 1,000 mg q8h				
Healthy subjects				
MIC (mg/liter)				
0.5	100	100	100	100
1	100	100	100	100
2	100	100	100	99
4	100	100	98	86
8	100	91	52	17
16	37	3	0	0
32	0	0	0	0
100% PTA	8	4	2	1
Patients with CF				
MIC (mg/liter)				
0.5	100	100	100	100
1	100	100	100	99
2	100	100	98	89
4	100	97	82	56
8	94	62	24	7
16	10	0	0	0
32	0	0	0	0
100% PTA	4	2	1	0.5
Aztreonam at 2,000 mg q8h				
Healthy subjects				
MIC (mg/liter)				
0.5	100	100	100	100
1	100	100	100	100
2	100	100	100	100
4	100	100	100	100
8	100	100	98	86
16	100	91	52	17
32	38	3	0	0
100% PTA	16	8	4	4
Patients with CF				
MIC (mg/liter)				
0.5	100	100	100	100
1	100	100	100	100
2	100	100	100	99
4	100	100	98	91
8	100	98	83	56
16	95	63	28	10
32	18	3	0	0
100% PTA	8	4	2	1

FIG. 3. Means (—●—) with 95% (---) and 99% (—) CIs for the percentage of time the unbound concentration of aztreonam remained above the MIC (% fT > MIC), based on the mean PK parameter vectors, variances, and correlation matrix, as a function of the MICs for healthy subjects receiving 1,000 and 2,000 mg q8h (A) and patients with CF receiving 1,000 and 2,000 mg q8h (B).

dose and a 95% CI of ca. 2 to 4 mg/liter versus a mean of ca. 4 to 8 mg/liter at the 2,000 mg-dose level). Overall, the break-points were approximately one twofold dilution lower in patients with CF; this is in accordance with the data presented in Table 4.

DISCUSSION

There has been ongoing discussion as to whether patients with CF display altered drug disposition compared to their healthy peers. Increased total body clearance and larger volumes of distribution have been reported for many drugs, including the β -lactam antibiotics (12, 37). In the present study the clearance in our control group was comparable to aztreonam clearance observed in healthy adults (1.31 versus 1.27 ml/min/kg) (24, 39). Larger volumes of distribution have been primarily attributed to an increased amount of lean body mass (LBM) per kilogram of body weight and when corrected for LBM, or with allometric scaling when appropriate, most of these differences will disappear (3, 32). It is important to note that for ethical and practical reasons, especially the older PK studies lack adequate non-CF comparator groups. In studies that did include controls, subjects seldom were matched for age, gender, body weight, and height. Since growth and devel-

opment (or the lack thereof) are two linked colinear processes in children, not correcting for body size will introduce important bias and may explain in large part claimed PK differences in patients with CF. Our study is unique in that aztreonam PK data were studied in patients in comparison to a well-matched control group.

We found no difference in the aztreonam volume of distribution between patients with CF and matched healthy subjects (Table 2). Volume estimates in our patients were in between those reported in children with CF (0.25 ± 0.05 liter/kg) and those observed in healthy adults (0.16 ± 0.2 liter/kg) (23, 24). Increased total body clearance of β -lactams has been attributed to increased renal clearance, particularly tubular secretion. To date, no pathological abnormalities have been identified that could fully explain enhanced renal clearance (32). Our data show that the total body clearance is ca. 30% higher in patients with CF and that this increase is the result of a significantly higher renal clearance (Table 2). Aztreonam is predominantly eliminated by glomerular filtration and in part by tubular secretion (38). We found 72% of the dose excreted as unchanged aztreonam in the urine, a finding in accordance with earlier data reported in healthy volunteers. The lower protein binding in our CF patients can partly explain the ob-

served increased renal aztreonam clearance. When “correcting” renal clearance for protein binding, ca. 67% of the difference could be accounted for. We found the free fraction to be ca. 20% higher in our patients. It has been reported that in healthy volunteers the renal clearance of unbound aztreonam exceeded the glomerular filtration rate and that probenecid diminished tubular secretion, indicating that active tubular secretion does occur (23, 24). In the present study the renal clearance was about half the creatinine clearance, a surrogate marker for the glomerular filtration rate (Table 2). Taking into account plasma protein binding, this is suggestive of a small fraction being eliminated via active tubular secretion. However, since the glomerular filtration rate was not simultaneously measured, no further analysis was possible. Since aztreonam is completely ionized at urinary pH values (pKa of -0.5 , 2.7 , or 3.7), the rate of reabsorption was assumed to be negligible. Aztreonam also exhibits hepatic metabolism and biliary secretion and the amount eliminated by nonrenal mechanisms is ca. 20% (38). The nonrenal clearance in the present study was not significantly different between patients and controls and accounted for ca. 28% of the total clearance. Based on this we postulate that the major part of the increase in renal clearance is attributable to higher free concentrations.

The second aim of the present study was to evaluate the impact of differences in PK, such as increased aztreonam clearance on dosing requirements. In addition, we sought to evaluate the impact of between patient variability on PK-PD indices and breakpoints. For this analysis, we developed population models and used MCS to generate robust estimates of probability of attaining predefined PD targets, taking into account important between-patient PK variability. In contrast to analyses based solely on mean parameter estimates to evaluate whether a particular dosing regimen would achieve the desired target, the present study highlights the importance of incorporating random PK variability.

Patients with CF exhibit on average higher aztreonam clearance (Fig. 1), resulting in lower $\%fT > MIC$ values over the MIC range. In addition, variability in clearance estimates as expressed by CVs was larger in patients with CF than in healthy subjects, corresponding to our earlier observations for ceftazidime (28). These differences give rise to broadening of the target attainment distribution and were especially pronounced at the lower end of the distributions (99% CI, Fig. 3). In terms of dosing requirements, this means that in order to obtain equal exposure to aztreonam, patients with CF may require higher and/or more frequent dosing. Clinically, it is of particular importance to be able to identify patients that exhibit much higher than normal drug clearance and for whom dose adjustment would be warranted. The data presented in this study provide the basis for a PK-PD model-based approach, for instance, by using glomerular filtration estimates and other clinical indicators for changes in the volume of distribution and elimination to predict drug exposure or, ultimately, by obtaining one or two concentration measurements for Bayesian estimation of the patient's individual PK-PD profile given the chosen dosing regimen (19). For tobramycin, a clear relationship between plasma drug concentrations, susceptibility of the *P. aeruginosa* strain, and effect for the treatment of infectious exacerbations in patients with CF has been shown. Using a quantitative analysis and an Emax model, the effect of therapy could be well described and was dependent on

the AUC/MIC ratio (27). Along with such PK-PD approaches, particularly the mucoid form of growth of *P. aeruginosa* and the diffusion barrier by the CF sputum itself will require intensive dosing. However, the relationship between antibiotic concentrations in pulmonary secretions and clinical effect has not been well studied or documented. Sputum concentrations of β -lactam antibiotics in CF remain disappointingly low despite high intermittent dosages (36). Despite the general idea that with intermittent dosing the high peak concentrations reached in plasma would facilitate penetration of the antibiotic in sputum, the diffusing antibiotic typically shows a relatively flat concentration-time profile that does not follow the concentration profile in plasma (4). Diffusion may be maximized, leading to sustained supra MIC concentrations in the lung by using different administration techniques, e.g., the continuous infusion of β -lactam antibiotics (41).

The final objective of the present study was to evaluate current breakpoints for aztreonam as recommended by the Clinical and Laboratory Standards Institute, EUCAST, and other organizations. Some of these breakpoints were set more than 10 years ago, before tools such as MCS and PK-PD information were available. The results of the MCS analysis in the healthy population indicate that a 95 to 100% PTA is reached at an MIC of 4 mg/liter for a target of 50 to 60% $fT > MIC$ for a dose of 1,000 mg q8h and 8 mg/liter for a dose of 2,000 mg q8h. This indicates that the clinical susceptibility breakpoint for aztreonam would be either 4 or 8 mg/liter, depending on the dose that is used clinically. Aztreonam is most commonly used for infections due to *P. aeruginosa*, where the recommended dosing regimen is 2,000 mg q8h. A clinical breakpoint of 8 mg/liter thus appears to be justified and compares to the current Clinical and Laboratory Standards Institute breakpoint provided a high dose is used. The EUCAST recently harmonized breakpoints for aztreonam and other cephalosporins and came up with non-species-related breakpoints of 4 mg/liter for susceptible and 8 mg/liter for resistant, based on the clinical use of both 1,000 and 2,000 mg q8h used in a number of countries (16).

This is the first study using matched controls to show that aztreonam total body clearance, and not the volume of distribution, is significantly higher in CF patients as a result of increased renal clearance of patients with CF. The PK parameter estimates for aztreonam based on data from a small group of healthy subjects resulted in a clinical susceptibility breakpoint comparable to the breakpoint obtained for CF patients and, based on the present study, would be ≤ 4 mg/liter. Patients suspected of having unusually high rates of clearance should thus be monitored closely.

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