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Pharmacokinetics and Pharmacodynamics of Aerosolized Antibacterial Agents in Chronically Infected Cystic Fibrosis Patients

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SUMMARY 753
INTRODUCTION 754
Current Challenges in Antibiotic Resistance 754
Antibacterial activities of ciprofloxacin, levofloxacin, tobramycin, amikacin, colistin, and aztreonam 754
Heteroresistance, a Resistance Reservoir without Selective Pressure of Antibacterials 755
Implications for Susceptibility Testing 756
PULMONARY PHARMACOKINETICS 756
Confounding Factors 756
Chemistry and Formulation 756
Serum and Sputum Pharmacokinetics 757
Fluoroquinolones 757
Aminoglycosides 758
Aztreonam 758
Colistin 758
Correlation of Sputum Concentrations to Antibacterial Activities 758
PHARMACODYNAMICS 759
Definition of Endpoints and Use of Appropriate Matrices 759
Preclinical and Clinical PK/PD Studies in CF Patients 759
Fluoroquinolones 759
Aminoglycosides 760
Beta-lactams 760
Colistin 761
Emergence of Resistance in PK/PD Studies and in CF Patients 761
Emergence of resistance in *P. aeruginosa* 761
Emergence of resistance in *S. aureus* and *H. influenzae* 763
Bacteriophage induction and promotion of resistance spread 763
EFFECT OF AEROSOLIZED ANTIBACTERIAL THERAPY ON LUNG FUNCTION AND *P. AERUGINOSA* SPUTUM LOAD 764
Fluoroquinolones 764
Aminoglycosides 766
Aztreonam 767
Colistin 767
Correlation of Sputum Concentrations to Antipseudomonal Efficacy and Improvement of Lung Function 768
OPEN QUESTIONS AND CAVEATS 769
The CF Inflammasome 769
The CF Transmembrane Conductance Regulator Protein 770
Signaling and Virulence 770
The CF Microbiome 770
CONCLUSIONS 771
REFERENCES 771
AUTHOR BIO 782

SUMMARY

Bacteria adapt to growth in lungs of patients with cystic fibrosis (CF) by selection of heterogeneously resistant variants that are not detected by conventional susceptibility testing but are selected for rapidly during antibacterial treatment. Therefore, total bacterial counts and antibiotic susceptibilities are misleading indicators of infection and are not helpful as guides for therapy decisions or efficacy endpoints. High drug concentrations delivered by aerosol may maximize efficacy, as decreased drug susceptibilities of the pathogens are compensated for by high target site concentrations. However, reductions of the bacterial load in sputum and improvements in lung function were within the same ranges following aerosolized and conventional therapies. Furthermore, the use of conventional pharmacokinetic/pharmacodynamic (PK/PD) surrogates correlating pharmacokinetics in serum with clinical cure and presumed or proven eradication of the pathogen as a basis for PK/PD investigations in CF patients is irrelevant, as minimization of systemic exposure is one of the main objectives of aerosolized therapy; in addition, bacterial pathogens cannot be eradicated, and chronic infection cannot be cured. Consequently, conven-

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tional PK/PD surrogates are not applicable to CF patients. It is nonetheless obvious that systemic exposure of patients, with all its sequelae, is minimized and that the burden of oral treatment for CF patients suffering from chronic infections is reduced.

INTRODUCTION

Aerosolized antibacterials have long been used for the treatment of respiratory tract infections in general and of cystic fibrosis (CF) in particular. It is assumed that inhalation of antibacterials deposits high drug concentrations at the focus of infection, thus compensating for the poor penetration of, e.g., β-lactams or aminoglycosides into the respiratory tract upon enteral or parenteral administration so that difficult-to-treat pathogens are in principle exposed to adequate drug concentrations (1–6).

In addition to the poor penetration of antibacterials into the focus of infection, antibacterial therapy of CF patients is aggravated by several factors, such as biofilm formation, which impairs drug penetration and protects the pathogens from immune defense (7–14); the development of hypermutator strains, characterized by high mutation rates, which result in pronounced resistance against antimicrobials and pathoadaptation (15–27); drug adsorption to sputum components (28, 29), which limits antibiotic activities; and slow growth as small-colony morphotypes (30–34), which facilitates recurrent and persistent infections and reduces the antibacterial activities of several antimicrobials (35, 36). The result of the multiplicity of factors impairing the activities of antibacterials is that the pathogen cannot be eliminated, and infection persists in a chronic state. Nevertheless, in many studies, treatment of CF patients with antibacterials was associated with improvement in lung function (37), prolongation of the time to the next acute respiratory exacerbation (38), and a moderate reduction of viable bacterial counts so that improvement of lung function is more pronounced following antibiosis than following chest physiotherapy and administration of bronchodilators (39). Due to the lack of eradication, high doses of antibacterials should be administered to chronically infected CF patients, which have to be given to CF patients for most of their lives (6, 7); furthermore, the pharmacokinetics of agents are modified in many patient populations, including CF patients, necessitating dose adjustments (40).

Aggravating this problem is the continuous increases in resistance to any antibacterial agent used for the treatment of CF patients. Fluoroquinolone, penicillin, carbapenem, and aminoglycoside resistance rates exceeding 30% and even multidrug resistance rates have been reported (40–47). Resistance development was associated with patient age, and resistance mechanisms accumulated sequentially, so that resistance rates gradually increased over time (42). Furthermore, resistance development is amplified by high mutation frequencies in hypermutable isolates from CF patients (16). Acquisition of β-lactamase expression, increased efflux pump activity, and permeability changes due to alterations of lipopolysaccharides or porins occur during the chronic state of infection. No single mutation compromises every drug. Nevertheless, upregulated efflux can simultaneously compromise fluoroquinolones and most β-lactams. A combination of upregulated efflux, loss of porins, and outer membrane impermeability compromises probably every drug class except the polymyxins (47).

In particular, because of suboptimal drug penetration into the respiratory tract and continuously increasing drug resistance, there is an urgent need to optimize the use of existing agents, considering the lack of new antibacterials. High drug concentrations delivered by aerosol may overcome the decreased susceptibilities of pathogens. Furthermore, aminoglycosides and fluoroquinolones exhibit concentration-dependent pharmacodynamics (48–51), so increased drug concentrations delivered by aerosol may translate into augmented antibacterial activity; thus, inhalation of antibacterials may augment efficacy and reduce systemic toxicities.

Marketing authorizations for inhalation therapy have been granted for colistin, tobramycin, and aztreonam; liposomal amikacin, liposomal ciprofloxacin, ciprofloxacin betaine, levofloxacin hemihydrate, and a new tobramycin formulation are in clinical development for aerosol therapy of Gram-negative pathogens. A combination of tobramycin and fosfomycin is being developed for treatment of patients with *Pseudomonas aeruginosa* and/or staphylococcal airway infections.

This review describes the pharmacokinetic/pharmacodynamic (PK/PD) characteristics of aerosolized administration of antibacterials to CF patients, with special emphasis on novel formulations compared to established aerosol formulations and oral or intravenous (i.v.) administration. PK/PD evaluations are based on the indices of the MIC of an antibacterial agent for the pathogen, quantitating its activity, and the pharmacokinetics of the agent as a measure of drug exposure, so that PK/PD evaluations integrate these two indices. Therefore, the susceptibility patterns of the typical pathogens and the kinetics of the relevant agents are described first, followed by an evaluation of PK/PD targets and a discussion of whether aerosolized antibacterial therapy may augment PK/PD target attainment rates and, in particular, clinical efficacy. For discussion of the changing CF airway microbiome, pathophysiology, treatment, and infection control measures, the reader is kindly referred to recent reviews (see references 52–58 and references therein).

Current Challenges in Antibiotic Resistance

Antibacterial activities of ciprofloxacin, levofloxacin, tobramycin, amikacin, colistin, and aztreonam. *Staphylococcus aureus* and *Haemophilus influenzae* are the most common bacteria isolated initially from the sputum of CF patients, and thereafter, *Pseudomonas aeruginosa* is the prevalent species; several opportunistic bacterial species, such as *Burkholderia cepacia*, *Stenotrophomonas maltophilia*, and *Achromobacter xylosoxidans*, are occasionally associated with CF. These species are the predominant ones, although the CF microbiome is much more complex (48–50). Therefore, the antibacterial activities of agents used for aerosolized therapy against these species are summarized in Table 1. The isolates studied originated from CF patients only; pathogens isolated from non-cystic fibrosis patients were more susceptible than were those from CF patients.

In general, the data summarized in Table 1 demonstrate that colistin, ciprofloxacin, and levofloxacin are the most active agents, whereas tobramycin, amikacin, and aztreonam are less active. The MIC_{50} and MIC_{90} values are scattered over a broad range.

High concentrations of these antibacterials of ≥16 mg/liter inhibited 90% of isolates of *S. maltophilia*, *A. xylosoxidans*, *B. cepacia*, and methicillin-resistant *S. aureus* (MRSA) (59–70).

The *P. aeruginosa* fluoroquinolone resistance rate ranged from 14% to 30%, the amikacin resistance rate ranged from 26% to 36%, the gentamicin resistance rate ranged from almost 50% to

TABLE 1 Antibacterial activities of various agents inhibiting 50% and 90% of strains isolated from CF patients[a]

Organism	Concn (mg/liter)											
	CPX		LVX		Tobramycin		Amikacin		Aztreonam		Colistin	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
P. aeruginosa	1–2	8	1–2	8	0.5–8	8–256	8–16	>128	4–8	64–128	0.5–2	0.5–8
S. maltophilia	4	16	2	8	>32	>32	128	>128	>128	>128	2	8
B. cepacia	4	32	4	32	>32	>32	128	>128	32	>128	IR	IR
A. xylosoxidans	4	16	4	16	>32	>32	>128	>128	64	>128	8	>16
MRSA	16	>32	4	>32	128	>128	8	16	>512	>512	IR	IR

[a] Data were derived from references 59–70. CPX, ciprofloxacin; LVX, levofloxacin; IR, intrinsically resistant.

40%, and the tobramycin resistance rate ranged from 40% to 10% of the total isolates studied (41, 43, 47, 63, 71, 72). Almost all isolates of *A. xylosoxidans* were drug resistant, although the MICs of fluoroquinolones were lower than those of the other drug classes for *S. maltophilia*, *A. xylosoxidans*, and *B. cepacia*; resistance rates amounted to 91% to 98% (61, 63). All agents displayed reduced activities against *S. maltophilia*, with aztreonam and gentamicin resistance rates of 96% and 80%, respectively (72).

It is important to note that susceptibility tests were performed with planktonic bacteria but not with bacteria immobilized in biofilms. Furthermore, criteria classifying the studied isolates as being susceptible or resistant are based on breakpoints derived from PK/PD surrogates adapted for enteral or parenteral therapy, which do not apply for treatment with inhaled antibacterials (73). High local concentrations of antibacterials following inhaled therapy were not considered. Therefore, it has been suggested that susceptibility testing be adapted: intermediate and resistant strains should be retested by using high-range Etest strips and recategorized interpretive criteria (69, 74–76) so that intermediately susceptible and even resistant pathogens may be treatable with aerosolized antibacterials. It should also be considered that in all of the resistance surveillance studies mentioned above, the isolates were not stratified according to the status of infection, i.e., colonization or early or chronic infection. Early isolates of *P. aeruginosa* have not yet acquired the phenotypic and genotypic responses to a chronic infection, so almost all *P. aeruginosa* isolates associated with early infection were drug susceptible (76–78). Thus, conventional susceptibility testing of CF isolates is poorly predictive of the clinical response (31, 79, 80).

Heteroresistance, a Resistance Reservoir without Selective Pressure of Antibacterials

Within a genotypically homogeneous population, there are several mixed populations of low-and high-level drug-resistant and -susceptible or hypersusceptible phenotypes; although the frequency of heteroresistance is variable, its rate is as low as $\leq 10^{-5}$ to 10^{-6}. Therefore, traditional *in vitro* susceptibility testing characterizes the entire population as being susceptible, although a subset expresses phenotypic resistance. Antibiosis diminishes the susceptible subpopulation while the resistant population increases in total numbers, so heteroresistance is considered a precursor stage of resistance. It is also relevant for susceptibility testing that the MIC values reported in Table 1 were generated by using standard methods recommended by the CLSI (81); i.e., the inoculum is prepared by selecting "at least three to five well-isolated colonies of the same morphological type," yielding a final inoculum of 10^5 CFU/ml, so the resistant subpopulation very likely passes unnoticed. The standard CLSI method results in an analysis of very few colonies of the same morphotype per patient and per time point only, whereas a laborious population analysis has to be performed to detect heteroresistance. Detection of heteroresistance, however, is important, as *S. aureus* and *P. aeruginosa* adapt rapidly to growth in CF lungs as disease progresses. During the early phases of colonization, the *P. aeruginosa* population is phenotypically homogeneous and susceptible to antibacterials. Heterogeneity increases as disease progresses so that there are diverse pheno- and genotypes that are heterogeneously resistant in parallel within one patient. Furthermore, it has been demonstrated that there is considerable within-patient diversity per time point (82–87). Consequently, the geno- and phenotypes of strains isolated from CF patients vary both within an individual patient per time point and with time as the disease progresses. Unfortunately, whether the strains analyzed in the course of susceptibility studies have been isolated from colonized or chronically infected patients is almost never described. This differentiation, however, is of clinical relevance, as the numbers of different phenotypes in chronically infected CF patients are 50% higher than those in colonized CF patients; the resistance rates in the chronically infected groups were twice as high as those in the comparator group (88).

The phenotypic and genotypic heterogeneity of pathogens isolated from CF lungs results in considerable heteroresistance of the pathogens. This type of heteroresistance is caused by processes to adapt to growth in a hostile environment and not by selection due to antimicrobial therapy. Heteroresistance, as such, is a species-specific pathoadaptation resulting in a mixed population of drug-resistant and drug-susceptible subpopulations of a single bacterial strain. In general, adaptation of *H. influenzae*, *S. aureus*, *P. aeruginosa*, and *B. cepacia* to growth in cystic fibrosis lungs generates heterogeneous populations, resulting in heteroresistance, a hypermutator phenotype, and small colony variants (SCVs) (16–20, 33–36).

Carbapenem or polymyxin-colistin heteroresistance has been described for *Acinetobacter baumannii* and *P. aeruginosa*, occurring with frequencies ranging from 2×10^{-4} to 7×10^{-8}; heteroresistance to both drug classes was rare (89–92), as was heteroresistance to colistin in multidrug-resistant *A. baumannii* (93, 94). Although polymyxin heteroresistance was uncommon, several isolates presented heterogeneous subpopulations with increased and thus borderline susceptible MICs, which might cause treatment failures (92, 95). Heteroresistance to carboxy- and ureidopenicillins, cephalosporins, carbapenems, aminoglycosides, colistin, fluoroquinolones, as well as the piperacillin-tazobactam and

ticarcillin-clavulanate combinations in *P. aeruginosa* has been reported (96–101).

Comparative genome sequencing of *S. aureus* isolates obtained sequentially from the airways of CF patients revealed variations in phage content, growth rate, hemolytic activity, and other pathogenicity factors as well as genetic polymorphisms in genes that influence antibiotic resistance. The majority of polymorphisms were isolate specific, suggesting the existence of a heterogeneous *S. aureus* population that evolved from a single infecting strain. The increased frequency of genomic alterations is due to phage mobilization (102–106). Genomic plasticity of *S. aureus* facilitates adaptation to various and changing conditions in CF lungs (103). Carriage of MRSA and infection are becoming increasingly common among CF patients; the prevalence of MRSA in CF patients in the United States is approximately 25%. The molecular epidemiology and Panton-Valentine leukocidin gene carriage are complex (107–109). MRSA possess an intrinsic ability to generate highly methicillin-resistant subclones at a frequency of 10^{-4} to 10^{-8}, so any MRSA population represents a heterogeneously β-lactam-resistant population (110, 111), even in the absence of β-lactam exposure. Glycopeptide-intermediate methicillin-susceptible *S. aureus* (MSSA) and glycopeptide-intermediate MRSA strains, including heteroresistant ones, have been isolated from CF patients. The first glycopeptide-intermediate *S. aureus* strain with a vancomycin MIC of 4 to 8 mg/liter was isolated from a CF patient in 1999. Since then, several additional cases in CF patients were reported; vancomycin heteroresistance has been observed in approximately 3% to 5% of CF patients harboring *S. aureus* (112–117).

Implications for Susceptibility Testing

The intraindividual diversity of bacterial populations has significant implications for susceptibility testing, selection of resistance, and pharmacokinetic/pharmacodynamic considerations; the latter two aspects are discussed below. Basically, heteroresistance implies that the bacterial population in CF airways is significantly undersampled, resistance rates are underestimated, and there are subpopulations with different MICs. Therefore, routine susceptibility testing is not applicable, as the results are not representative of a heterogeneous population of *P. aeruginosa* (118). Consequently, data generated by routine methods are poorly predictive; ciprofloxacin susceptibility and resistance of a mixed-morphotype strain of *P. aeruginosa* from a CF patient were correctly predicted in 87.0% and 41.7% of cases, respectively (119), and another analysis revealed that the conventional approach of susceptibility testing indicated susceptibility in 45% of the isolates studied, although resistant subpopulations were identified by population analysis in all of these samples classified as being susceptible (120). Furthermore, conventional testing had no prognostic value for the clinical response to parenteral antibiotic treatment in CF patients (121, 122). Consequently, routine susceptibility testing of CF pathogens is not representative, as the test provides a considerable underestimation of resistance (31, 118) and has a limited prognostic value for clinical efficacy. Detection of heteroresistance would probably improve the situation. Detection of heteroresistance by, e.g., population analysis profiling is difficult and labor-intensive, so this method cannot be used under routine conditions. An alternative to conventional test methods could provide direct plating of sputum samples onto agar plates; direct plating increased the detection rate of resistant subpopulations to almost 100%, compared to ~40 to 60% with conventional testing (118, 119, 123).

Therefore, a clear understanding of within-patient population dynamics during the progression of disease as well as during every treatment cycle and the development of tools for analysis of intraspecies diversity are of utmost importance. Furthermore, interpretive criteria for susceptibility testing should be adapted to relevant concentrations of unbound agents administered by inhalation, and the longitudinal change in susceptibility of CF bacterial isolates has to be considered.

PULMONARY PHARMACOKINETICS

Confounding Factors

As reviewed recently, drug levels in the respiratory tract cannot be extrapolated from the nominal dose placed in the delivery system (124, 125). The reasons for this are manifold. First, the agent may adhere to the delivery system so that the emitted dose is lower than the nominal dose. Second, the fate of a particle, once inhaled, is influenced by a variety of factors, such as the anatomy of the respiratory tract and the aerodynamic characteristics of an aqueous aerosol, which may be altered by the relative humidity of the airway. Third, inspiratory and expiratory breathing patterns of the CF patient population may vary within one patient from day to day and between patients. Furthermore, reliable measurements of the dose deposited are difficult to perform, so different techniques used for quantitation of pulmonary deposition may generate contradictory data (124, 125). Residual amounts of inhaled drug may be deposited in the oral cavity and may be recovered during sputum sampling. Drug concentrations in serum, respiratory secretions, as well as urine provide useful information about the systemic bioavailability of inhaled agents. However, analyses of serum concentrations and urinary recovery may be affected by the swallowed drug absorbed from the gastrointestinal tract, so drug concentrations in serum or urine may in part arise from swallowed drug deposited extrapulmonarily, and another part will arise from pulmonary deposition. Methods to reduce these pitfalls, such as the use of charcoal to absorb residual drug in sputum, have been described and used in recent studies, as described below. Furthermore, rapid clearance via pulmonary macrophages and mucociliary clearance make effective pulmonary deposition difficult.

Chemistry and Formulation

One of the determinants of intrapulmonary distribution and absorption as well as antibacterial activity is the solubility of an agent; only the soluble and free fraction diffuses through membranes and reaches its target. Most fluoroquinolones are barely water soluble at a physiological pH; their solubilities increase at an acidic or alkaline pH, as fluoroquinolones are zwitterions. Ciprofloxacin hydrochloride, used in the liposomal formulation of ciprofloxacin, is moderately water soluble at pH 7 (54 to 86 mg/liter) (126–128), whereas ciprofloxacin betaine, used in the dry powder formulation, is "slightly soluble," i.e., <50 mg/liter (129). The water solubility of levofloxacin at pH 6.9 is a thousandfold higher, amounting to 50 g/liter, (130). Aminoglycosides, colistin, aztreonam, vancomycin, and fosfomycin are highly water soluble (131).

On the other hand, water solubility may affect therapeutic efficacy negatively, as it reduces the residence time of the agent in the respiratory tract significantly. For example, following intratra-

TABLE 2 Pharmacokinetics of various antibacterials in adult CF patients following oral, intravenous, or aerosolized administration

Agent, dose, and route	C_{max} (mg/liter) in serum	C_{max} (mg/liter) in sputum	C_{max} (mg/liter) in lung or ELF	$t_{1/2}$ (h) in serum/sputum	Urinary recovery (%)	Reference(s)
CPX, 500 mg, p.o.	2.5–3.8	0.7–1.9	3.9–29	3.7–5.1	35.7	144–146
CPX, 750 mg, p.o.	3.4–4.5		3.6–4.5			144, 145
CPX, 1,000 mg, p.o.	4.6–5.6	1.8		3.7–5.1	40.8	144–147
CPX, 200 mg, i.v.	4.9		16.9			148
CPX, 32.5 mg, ae	0.056	33.0		9.5/9.04		149
LVX, 200 mg, p.o.	2.1		3.9			151
LVX, 500 mg, p.o.	4.1–5.3 (4 h)		9.9–15.2*			152–155
LVX, 750 mg, p.o.	12.0 (4 h)		22.1*			152–155
LVX, 500 mg, i.v.	6.6		18.3			156
LVX, 180 mg, ae	0.95–1.3	2,563-2,932		6.4–6.8/3.5–4.3		157
TOB, 1.7–3.5, mg/kg q.d. i.v.	3.6–11.3 2		1.1-0.7		85	158,160
TOB, 10 mg/kg, q.d. i.v.	22–29			1.7–2.2		221, 223, 224, 369
TOB, 600 mg, ae[b]	1.3			13	17.5	164
TOB, 300 mg, ae[b]	<4	489–695	3.6–5.5	8.9–11.2	5.5	165–168
TOB, 112 mg, ae[c]	1.02	1,048		3.1/2.2		169
TOB, 300 mg, ae[b]	1.04	737		3.0/1.7		169
AMI, 30 mg/kg, i.v.	83–121 (AUC = 235 mg · h/liter)	6.3–10.9 (AUC = 83.7 mg · h/liter)		0.6–2.6	83	369, 225, 299, 370, 371
AMI, 500 mg, ae	AUC = 8.3 mg · h/liter	AUC = 3,830 mg · h/liter		2.9		170
AMI, 560 mg, ae	1.29	2,286			25–50	171
AZM, 2 g	80–228	5.2		1.8	72	172–175
AZM, 75 mg, ae	0.42–0.49	324–677		2.1		176, 177
COL, 2.4 mg/kg	2.5–10 (range)			4.2		179
COL, 66 mg	0.17–0.18	~40		4.1–4.5	4.3	180

[a] The broad range of ciprofloxacin lung tissue concentrations following oral (p.o.) or i.v. administration is due to the fact that some investigators administered the drugs once and others administered them repeatedly; the higher values represent steady-state concentrations. The range of levofloxacin concentrations following aerosolized (ae) administration is due to the fact that the dose of 180 mg was administered in two different formulations (50 mg/ml and 100 mg/ml) (lung, lung tissue homogenate; ELF, epithelial lining fluid; AUC, area under the concentration-versus-time curve; $t_{1/2}$, half-life, either in serum or in sputum; CPX, ciprofloxacin; LVX, levofloxacin; TOB, tobramycin; AMI, amikacin; AZM, aztreonam; COL, colistin). Asterisks indicate values for ELF.

[b] Tobramycin solution for inhalation.

[c] Tobramycin dry powder for inhalation.

cheal administration to experimental animals, moderately soluble ciprofloxacin hydrochloride was rapidly absorbed from the lungs into the systemic circulation, with a half-life of only 1 h. Consequently, ciprofloxacin lung tissue concentrations declined too rapidly to achieve effective treatment. In order to overcome the rapid clearance of ciprofloxacin hydrochloride from the lungs, an experimental liposomal formulation was developed. The elimination half-life of liposomally encapsulated ciprofloxacin in experimental animals was 3 h, which resulted in therapeutic efficacy against *Francisella tularensis* and *P. aeruginosa* infections (132–135).

Two liposomal formulations of inhaled ciprofloxacin (Pulmaquin and Lipoquin) that differ in the proportions of a rapidly available aqueous solution of ciprofloxacin hydrochloride and slow-release liposomal ciprofloxacin are in development. Dual-release ciprofloxacin for inhalation (DRCFI) (Pulmaquin or ARD-3150) uses the slow-release liposomal formulation (also called ciprofloxacin for inhalation [CFI] [Lipoquin or ARD-3100]) mixed with a small amount of ciprofloxacin hydrochloride dissolved in an aqueous medium. ARD-3100 was developed for the management of infections in CF patients, and ARD-3150 was developed for the management of infections in non-cystic fibrosis bronchiectasis patients. ARD-1100 is a liposomal formulation for defense purposes such as treatment of inhaled tularemia, pneumonic plague, and Q fever (Aradigm Corp., Hayward, CA). Likewise, a liposomal formulation of amikacin (Arikace) is in clinical development.

An alternative approach is to administer poorly soluble ciprofloxacin betaine as a dry powder. Delivery of a nominal dose of 35 mg in theory deposits a high total drug concentration of 466 mg/liter in the epithelial lining fluid (ELF). The water-soluble fraction is released continuously over prolonged periods of time (136). Tobramycin (Tobi Podhaler) (137, 138), colistin (colistimethate) (Colobreathe) (139), vancomycin hydrochloride (AeroVanc) (140), and the combination of fosfomycin and tobramycin-leucine (141) are also administered as dry powders. These agents, in contrast to ciprofloxacin, are freely water soluble.

Aztreonam lysine (Cayston) and levofloxacin hemihydrate (MP-376; Aeroquin), which are also freely water soluble, are delivered as aqueous solutions (142, 143).

Serum and Sputum Pharmacokinetics

Fluoroquinolones. Fluoroquinolones in general are characterized by marked tissue penetration activities, surpassing the corresponding serum concentrations 2- to almost 10-fold upon oral or intravenous administration.

Concentrations of ciprofloxacin in lung tissue homogenates or ELF are as high as 15 to 29 mg/liter (Table 2) (144–148).

Following aerosolized administration of 50 mg dry powder ciprofloxacin, corresponding to 32.5 mg ciprofloxacin betaine, maximal sputum concentrations amounted to 33.0 mg/liter, declining with a half-life of 9.04 h (149). As described previously (149), sputum concentrations following a dose of 100 mg dry powder were not significantly higher. The corresponding serum concentrations were negligibly low, with maximal concentrations of 0.08 and 0.3 mg/liter following the 50-mg and 100-mg doses, respectively. Low ciprofloxacin serum concentrations and a long half-life in sputum indicate minimal absorption and, thus, minimal systemic exposition following aerosolized delivery of ciprofloxacin (149). Ciprofloxacin sputum and ELF

concentrations following oral or intravenous administration of ciprofloxacin hydrochloride and aerosolized ciprofloxacin betaine, respectively, are comparable and are near the limit of solubility of ciprofloxacin.

Data on the pharmacokinetics of the liposomal ciprofloxacin formulation have not yet been reported, except for a plasma half-life of 10.5 h (150).

Levofloxacin lung tissue or ELF concentrations upon oral or intravenous administrations are similar to those of ciprofloxacin (Table 2) (151–156). However, its sputum concentrations measured following aerosolized administration surpass lung tissue or ELF concentrations achieved following oral or intravenous administration by 100- to 1,000-fold, reaching sputum concentrations of almost 3,000 mg/liter (157).

Although serum concentrations following aerosolized administration of 180 mg levofloxacin are low, the levels correspond to a serum concentration following oral administration of 100 mg levofloxacin. This phenomenon is likely due to the high solubility of levofloxacin and, thus, relatively good absorption from the lung.

Aminoglycosides. Although aminoglycosides are distributed almost freely in the extracellular space of most tissues because of their minimal protein binding and high water solubility, they cross biological membranes poorly, so drug concentrations in bronchial secretions are low, thus restricting their efficacy in the treatment of respiratory tract infections (158–162). Low concentrations of aminoglycosides in the respiratory tract upon intravenous dosing are due to their polycationic charge and their lipid insolubility (163). Data summarized in Table 2 demonstrate that sputum or lung tissue concentrations were significantly increased upon ultrasonic (164), pneumatic (165), and Pari LC Plus Jet (166, 167) nebulization using the previous formulations and devices compared to intravenous administration. Recently, a novel tobramycin dry powder formulation was launched. A study comparing the pharmacokinetics of this dry powder formulation with the pharmacokinetics of the previous tobramycin solutions for inhalation revealed that mean sputum concentrations as high as 1,048 mg/kg of body weight were measured upon inhalation of 112 mg, compared to 737 mg/kg following aerosolized administration of 300 mg of the aqueous formulation. The systemic bioavailability of tobramycin dry powder is 12% (168, 169). Measurable and slowly declining tobramycin serum concentrations were recorded, independent of the nebulizer type used. This prolonged half-life may likely be due to the delayed absorption of tobramycin from the lung into the bloodstream. Comparisons of the pharmacokinetic characteristics of tobramycin delivered by either by previous or actual inhalation devices demonstrate that both the formulation administered as well as the inhalation device have significant impacts on pharmacokinetics and the drug amounts delivered and deposited.

No study results have been posted at ClinicalTrials.gov (registration no. NCT00794586) for the evaluation of the pharmacokinetics of the fosfomycin-tobramycin combination as of 3 June 2014.

Amikacin concentrations in sputum were about 45-fold higher upon aerosolized administration of 500 mg of a liposomal formulation than after parenteral administration of 30 mg/kg of body weight; mean sputum concentrations increased ~3- to 5-fold from day 1 to day 14 (170, 171). In supplemental material published previously (158), it was reported that mean serum concentrations as low as 1.29 μg/liter and mean sputum concentrations of 2,286 μg/g were recorded following aerosolized administration of 560 mg (171).

Aztreonam. Aztreonam displayed the typical β-lactam pharmacokinetics, with a short half-life and slow penetration into bronchial secretions, corresponding to a penetration ratio of 20% upon a single intravenous administration (172–177). High sputum concentrations were achieved upon aerosolized administration; sputum concentrations increased dose proportionally from 383 to 879 to 985 mg/kg following aerosol delivery of 75, 150, and 225 mg. Systemic exposure was low but occurred rapidly (Table 2) (171–177).

Colistin. Analysis of colistin pharmacokinetics, in particular tissue kinetics, is problematic and may generate conflicting data because of the different methods used and also due to the fact that polymyxins adsorb to various matrices. The pharmacokinetics of either 25 mg colistimethate dry powder or 158 mg of a nebulized solution were evaluated in a crossover study (178). Maximal serum concentrations and area under the concentration-time curve (AUC) values of 66.3 mg/liter and 200.7 mg · h/liter, respectively, were recorded following aerosolized administration of dry powder, and the corresponding values for nebulized administration were 144.3 mg/liter and 459.5 mgh/liter; sputum concentrations were not reported in that study. Data reported in Table 2 (178–180) should be interpreted with caution, and urinary recovery observed after aerosolized therapy indicating absorption from the lung is disputed (181–183).

Correlation of Sputum Concentrations to Antibacterial Activities

Achievable drug levels at the focus of infection are often correlated to MICs of the relevant pathogens; a drug is considered to be effective and the pathogen is considered to be susceptible if the drug concentrations exceed the MIC. In general, aerosolized administration of the agents described above, except for ciprofloxacin betaine administered with a dry powder inhaler (ciprofloxacin DPI), generates sputum concentrations exceeding those following oral or intravenous administration, as higher doses can be administered topically than enterally or parenterally. Comparison of the sputum levels with the MIC_{90} values for the relevant pathogens summarized in Table 1 reveals that the sputum levels of levofloxacin and colistin surpass the MIC_{90} values for all the relevant species by severalfold. The aminoglycoside and aztreonam sputum concentrations correspond to or exceed 2-fold MIC values of >128 mg/liter.

However, such correlations should be interpreted with caution, as sputum concentrations are not a predictive marker for lung deposition (1, 2), nor is the relevant site for antibiotic penetration clearly defined. Concentrations of antibacterials in sputum and bronchi are presumably most relevant for bronchial infections, while drug levels in lung parenchyma, epithelial lining fluid, and alveolar macrophages or neutrophils are probably more important for pneumonic infections (184). Although local concentrations of an antibacterial agent are relevant for therapeutic efficacy, it is also important to consider whether the activity of the agent may be impaired by certain local conditions such as acidic pH, which prevails in some pneumonic areas of the lung. Growth at an acidic pH increased the MICs of aminoglycosides, commercially available fluoroquinolones, colistin, and macrolides but not aztreonam (185–191). Furthermore, polycations such as poly-

myxin-colistin and aminoglycosides bind to DNA, so they are inactivated in the presence of, e.g., pus and cell debris (192, 193). Fluoroquinolones also bind to DNA and cell debris (194–197). In addition, infected areas with poor ventilation may be protected from aerosolized agents. These factors, in addition to those mentioned briefly above in the introduction and in addition to the drug concentrations at the focus of infection, have an impact on the clinical efficacy of antibacterial agents.

As discussed in "Implications for Susceptibility Testing," above, reported MICs of antibacterials for pathogens isolated from CF patients are not representative of the heterogeneously susceptible bacterial populations isolated from CF patients. As discussed below, the exposition of heterogeneous populations to fluctuating drug concentrations simulating lung tissue levels results in the elimination of susceptible but selection of resistant subpopulations. Not only population dynamics but also physical structure, such as biofilm formation and deposition of DNA and cell debris, have an impact on the activities of antibacterial agents (198).

PHARMACODYNAMICS

Definition of Endpoints and Use of Appropriate Matrices

During the past 20 years, investigators have been able to identify clinical outcome predictors based on the correlation of the pharmacokinetics and pharmacodynamics (PK/PD) of an agent; pharmacokinetic parameters are derived from serum kinetics, and pharmacodynamic parameters are derived from MIC values. Surrogates for clinical cure and eradication of pathogens have been defined, and quantitative values that predict the probabilities of success or failure of antibiotic regimens have been assigned (199–204). In addition, PK/PD parameters offer a way to expedite the antibiotic development process and to provide a rationale for the dose selected.

In general, antimicrobial agents can be categorized on the basis of the PK/PD surrogates that correlate best with clinical or bacteriological efficacy. The three most frequently used PK/PD surrogates for antibacterials are the duration of time that a drug concentration remains above the MIC ($T>$MIC), the ratio of the maximal drug concentration to the MIC (C_{max}/MIC ratio), and the ratio of the area under the concentration-time curve at 24 h to the MIC (AUC/MIC ratio). Based on the compilation of PK/PD targets derived from *in vitro* models such as exposure of indicator strains to fluctuating drug concentrations simulating serum or target site concentrations, animal infection models, computer-based modeling, and clinical data, the magnitudes of exposure necessary for clinical and bacteriological efficacy were defined. The endpoints analyzed in clinical studies were clinical cure and presumed or proven eradication of the pathogen. The PK/PD surrogates most predictive of the efficacy of aminoglycosides and fluoroquinolones are the C_{max}/MIC and AUC/MIC ratios, and the most predictive PK/PD measure for β-lactams is $T>$MIC (199–204). In principle, the antibacterial activity and clinical efficacy of aminoglycosides and fluoroquinolones are concentration dependent, so increasing concentrations are paralleled by increasing activities. Consequently, high maximal serum concentrations and high AUCs correlate best with their activity profiles. C_{max}/MIC and AUC/MIC ratios of ≥10 and ≥125 h, respectively, were found to be predictive of the efficacy of aminoglycosides and fluoroquinolones in the treatment of Gram-negative infections. A C_{max}/MIC ratio of ≥10 was found to minimize the emergence of fluoroquinolone resistance. It has also been suggested that aminoglycoside sputum concentrations should exceed the MICs 25 times to achieve a bactericidal effect (205). The efficacy of β-lactams is determined predominantly by $T>$MIC, which should correspond to 30% to 50% of the dosing interval (200–204). These PK/PD measures should be attained with a probability of ≥90%. Based on these concepts and well-defined PK/PD measures, it is possible to preclinically identify treatment regimens that will optimize the probability of clinical and bacteriological outcomes as new antimicrobial agents or new formulations of old agents are developed. Therefore, these principles have also been applied for the development of aerosolized antibacterials.

The use of the PK/PD surrogates correlating pharmacokinetics in serum with clinical cure and presumed or proven eradication of the pathogen as a basis for PK/PD investigations in CF patients is problematic for two reasons. First, clinical cure and/or eradication of *P. aeruginosa*, with the exception of early *P. aeruginosa* infection (77), cannot be achieved in CF patients. Second, serum concentrations are irrelevant in CF patients treated by inhalation, as the minimization of systemic exposure but optimization of target-site-specific exposure is one of the primary objectives of aerosolized therapy. Therefore, it may be obvious that sputum instead of serum kinetics should be used as a basis for PK/PD considerations. So far, however, a systematic analysis of target-site-specific data generated *in vitro*, *in vivo*, and in clinical studies and a correlation of PK/PD measures with clinical efficacy have not been done.

Therefore, the endpoints clinical cure and/or eradication of the pathogen that are to be evaluated clinically and correlated to drug exposure differ between non-CF patients and CF patients, so the endpoint to be met should be redefined. Furthermore, the matrix, i.e., serum or sputum, to be used for pharmacokinetic evaluations and the PK/PD target to be achieved in CF patients treated with aerosolized antibacterials should be reconsidered. Actually, pharmacokinetic analysis of aerosolized antibacterials is based on drug measurements in sputum. However, drug concentrations in sputum provide only an approximation, as sputum is not a homogeneous matrix. Sputum viscosity and contents of cell debris and pus, etc., are variable, and the origin of expectorated sputum is unknown.

Preclinical and Clinical PK/PD Studies in CF Patients

Fluoroquinolones. Based on the pioneering studies of Forrest et al., PK/PD surrogates for intravenous ciprofloxacin treatment of respiratory tract infections in seriously ill patients have been defined (206). Serum samples for pharmacokinetic analysis and specimens from the site of infection for microbiological assessments were withdrawn from every patient daily so that kinetic parameters, the presence or absence of the pathogen, and the MIC could be determined. By correlating exposure to total drug levels (AUC [mg · h/liter]) and susceptibility of the pathogen (MIC [mg/liter]) to clinical and bacteriological responses, it was demonstrated that an AUC/MIC ratio of ≥125 h (corresponding to about 75 h for the free fraction) was associated with a high probability of clinical response and in particular eradication of the pathogen from the lung. Those authors stated clearly in the discussion of these data that it is difficult to achieve an AUC/MIC ratio of ≥125 h for bacteria with ciprofloxacin MICs of 0.5 mg/liter and above, such as *Staphylococcus* spp. and *Pseudomonas* species (206).

Subsequent studies with ciprofloxacin confirmed that an AUC/

MIC ratio of ≥125 h was associated with clinical and bacteriological efficacy against highly susceptible *P. aeruginosa* isolates only (207–211). The probability of meeting the PK/PD target, i.e., an AUC/MIC ratio of ≥125 h for isolates with MICs of >0.3 mg/liter, was suboptimal, resulting in a target attainment rate of 53 to 59% for routinely isolated *P. aeruginosa* strains in 2002 (212). Therefore, an AUC/MIC target of >350 h has been set for *P. aeruginosa* (206, 207). However, although the PK/PD target of fluoroquinolones for *P. aeruginosa* has been increased from ≥125 h to >350 h, an AUC/MIC ratio of ≥125 h is still used by almost every author as a predictor of clinical or bacteriological efficacy in patients infected with *P. aeruginosa*, including CF patients (213, 214), or in preclinical models (215). The use of an increased PK/PD target of >350 h implies that only highly susceptible isolates are treatable: the serum AUCs of ciprofloxacin following an oral administration of 500 mg or 750 mg to CF patients amount to 11 mg · h/liter and 19 mg · h/liter, respectively (144, 146), and the serum AUC of levofloxacin following an oral dose of 500 mg administered to CF patients is 71.3 mg · h/liter (155). Consequently, the PK/PD target of >350 h would be attained for isolates with a ciprofloxacin MIC of <0.05 mg/liter to be treated with 750 mg ciprofloxacin and isolates with a levofloxacin MIC of <0.2 mg/liter to be treated with 500 mg levofloxacin. Susceptibility data compiled by the EUCAST reveal that the susceptible wild-type population of *P. aeruginosa* (n = 19,046) is characterized by ciprofloxacin MICs ranging from 0.125 to 4 mg/liter and a median MIC of 1 mg/liter, thus indicating that an AUC/MIC ratio of >350 h can be attained for only a small minority of susceptible wild-type strains (203). Nevertheless, orally administered ciprofloxacin and levofloxacin were and still are clinically effective in the treatment of *P. aeruginosa* infections in non-CF and CF patients. As the minimization of systemic exposure and the optimization of target site exposure are the basic principles for aerosolized administration, pharmacokinetic parameters derived from serum concentrations cannot be used, but it seems to be obvious that parameters derived from sputum concentrations should be used for PK/PD investigations. If sputum AUCs of 72.5 mg · h/liter and approximately 2,000 mg · h/liter following aerosolized administration of 32.5 mg ciprofloxacin DPI and 180 mg levofloxacin hemihydrate, respectively, are used as the basis for these calculations, the PK/PD target would be attained for strains with ciprofloxacin MICs of 0.2 mg/liter and levofloxacin MICs of 5.0 mg/liter. These ratios are much higher than those calculated for oral therapy, but still, borderline susceptible and resistant isolates will not be covered by aerosolized administration of fluoroquinolones. As summarized in Table 1, the MIC_{90} value of ciprofloxacin and levofloxacin for aggregated CF isolates is 8 mg/liter; early colonizers should be more susceptible, while isolates from chronically infected patients should have higher MIC values.

An *in vitro* study (215) and an *in vivo* mouse lung infection model (216) revealed that levofloxacin exhibited a concentration- or dose-dependent reduction of viable *P. aeruginosa* counts. Aerosolized levofloxacin doses of 15, 30, and 60 mg/kg of body weight in a chronic lung infection model reduced viable counts by 0.07, 0.62, and 1.25 CFU/lung, whereas the antibacterial effects of intraperitoneally applied identical doses of levofloxacin were significantly lower.

Aminoglycosides. Evaluations of the pharmacodynamic characteristics of aminoglycosides are scarce. C_{max}/MIC and AUC/MIC ratios of 8 to 10 and 80 h to 100 h, respectively, are reasonable to predict the likelihood of efficacy in the treatment of nosocomial infections, including respiratory tract infections (217–220). The most predictive PK/PD efficacy parameter in CF patients was the C_{max}/MIC ratio (221).

In a mouse lung infection model comparing aerosolized tobramycin and levofloxacin, it was shown that bacterial loads of *P. aeruginosa* in the lungs of chronically infected animals were reduced following twice-daily treatment for 3 days with 60 mg/kg of levofloxacin or tobramycin by 3.33 $\log_{10}$ titers and 2.94 CFU/lung, respectively, compared to the preexposure level (216).

Intravenous tobramycin treatment of CF patients with chronic *P. aeruginosa* infections revealed that the C_{max}/MIC ratio correlated best with clinical efficacy, with a log-linear relationship between the C_{max}/MIC ratio and percent forced expiratory volume in 1 s (FEV_1) being predicted. However, *P. aeruginosa* was recovered in all patients treated, and a PK/PD analysis of bacteriological outcome has not been reported (154). These findings are in agreement with findings of other clinical tobramycin studies (164, 209, 210). Aerosolized administration of liposomal amikacin to CF patients did not result in a PK/PD-driven change in lung function tests, but a concentration-dependent reduction of bacterial load was correlated to the AUC/MIC ratio (170, 171, 222).

Assuming that an AUC/MIC ratio of 100 is predictive of the likelihood of clinical success, and based on AUC values of 110.5 and 235 mg · h/liter for tobramycin (223) and amikacin (224), respectively, isolates with a tobramycin MIC of ≤1 mg/liter and an amikacin MIC of ≤2 mg/liter can be treated effectively with the standard doses administered intravenously. The MICs of tobramycin and amikacin for the *P. aeruginosa* wild-type population range from 0.016 to 512 mg/liter and 0.25 to 256 mg/liter, respectively, with median MICs of 0.5 mg/liter and 4 mg/liter, respectively (http://www.escmid.org/), thus indicating that the activities of the two aminoglycosides are suboptimal, particularly against strains isolated from CF patients; the MIC_{90} values for these isolates are as high as 8 to >256 mg/liter (Table 1). The AUC of amikacin in sputum following aerosolized administration increased almost 45-fold compared to that following i.v. dosing (225), so isolates with MICs of up to 32 mg/liter could be adequately exposed. If PK/PD assessments are based on the suggestion that aminoglycoside sputum concentrations should exceed the MICs 25-fold, aerosolized tobramycin and amikacin would cover pathogens with MICs of up to 16 mg/liter.

Beta-lactams. In a chronic *P. aeruginosa* lung infection model, aztreonam (400 mg/kg) reduced the lung burden by 1.25 CFU/lung compared to the preexposure level (216). The probabilities of clinical success were calculated for non-CF and CF patients dosed intravenously with 1 g aztreonam every 8 h using a PK/PD target of 50 to 60% T>MIC (172). Isolates with MICs of up to 1 mg/liter and 2 mg/liter, respectively, were reliably treated with 1 g aztreonam four times a day (q.i.d.).

Three additional PK/PD studies are worth mentioning, although neither piperacillin nor ceftazidime has been developed for aerosolized therapy. Time above the MIC is the relevant parameter for these β-lactams. The PK/PD of piperacillin has been studied in hospitalized non-CF patients and in CF patients (226, 227). A PK/PD target of 50% T>MIC was set for both patient populations, who were dosed with two different regimens. The MIC that was covered for 50% of the dosing interval by either regimen was evaluated; a PK/PD analysis of drug effects has not been performed. In the non-CF population, the target was met by

administration of 3 g piperacillin every 6 h for hypothetical isolates with a numerical MIC of ≤8 mg/liter, while administration every 4 h resulted in a more robust target attainment and covered hypothetical isolates with numerical MICs of ≥16 mg/liter (226), which corresponds to the resistance breakpoint set by the EUCAST. A piperacillin dose of 3 g every 4 h achieved robust target attainment in CF patients for hypothetical isolates with MICs of ≤12 mg/liter (227). These calculations suggest that piperacillin can be used for the empirical treatment of CF patients. However, the probabilities of target attainment were different for a population of clinical isolates of *P. aeruginosa* sampled from both non-CF and CF patients. The target of 50% *T*>MIC was attained for *P. aeruginosa* isolates from patients admitted to one hospital in North America, while surveillance isolates collected throughout North or South America and two German institutions were not reliably covered. Target attainment rates differed by 50% between North and South American isolates and by 30 to 35% between isolates from the two German institutions (227).

An analysis of PK/PD characteristics of ceftazidime in non-CF and CF patients resulted in an analogous outcome (228). These data suggest that PK/PD analysis and calculation of target attainment rates should be performed on a regional/local level by using "real-life" PK and PD data from the relevant patient group. Target attainment rates should be recalculated regularly, as the MICs shift over time in parallel with a continuous increase in resistance rates.

Colistin. PK/PD parameters of colistin were derived from an *in vitro* model simulating 5 different intermittent administrations and a continuous infusion regimen of the drug.

The AUC/MIC ratio fitted best with antibacterial activity. The AUC/MIC ratios required for 2-log reductions of viable counts of *P. aeruginosa* ranged from approximately 36 h to 7 h, although the MICs of colistin for the three indicator strains tested were almost identical (0.5 to 1 mg/liter). The reduction of viable counts was independent of the dosing schedule, with once-daily (q.d.) twice-daily (b.i.d.), and three-times-daily (t.i.d.) administrations being equally active. However, the t.i.d. regimen minimized the emergence of resistance most effectively (229, 230). These findings were confirmed in a murine thigh and lung infection model (231). The MICs of colistin for the susceptible wild-type population range from 0.032 to 8 mg/liter, with a median MIC of 0.25 mg/liter (http://www.escmid.org/). The serum AUCs following aerosolized dry powder or liquid solution administration amount to 200.7 mg · h/liter and 459.5 mg · h/liter, respectively, so AUC/MIC ratios of 20 h (approximation from the reported ranges of colistin PK/PD surrogates) provide exposures of isolates with MICs of up to 10 mg/liter following dry powder inhalation and approximately 20 mg/liter following administration of a nebulized solution. Thus, colistin may likely expose the entire wild-type population to adequate drug concentrations.

It is worth mentioning that calculations of PK/PD targets, i.e., AUC/MIC ratios of fluoroquinolones, aminoglycosides, and colistin on the one hand and *T*>MIC of β-lactams on the other hand, have been performed by using two different methods: the former by deterministic and the latter by probabilistic methods. The deterministic method correlates mean pharmacokinetic parameters to one numerical value, usually the MIC_{90} value, so that variabilities in drug exposures are not taken into account. The probabilistic method considers the variabilities of drug exposures of the relevant patient populations and the variabilities of the susceptibility pattern of the causative pathogen. Data generated by using the deterministic method tend to be too favorable, as only discrete numerical values (i.e., mean C_{max} or MIC_{90}) are used. Data generated by probabilistic methods tend to be more disadvantageous, as the entire patient and pathogen populations, with all their variabilities, such as high and low drug exposures or high and low MICs, are considered (203). Therefore, it cannot be concluded on the basis of these calculations that one drug class may provide a better target attainment rate than the other.

Emergence of Resistance in PK/PD Studies and in CF Patients

In vitro mutational resistance to antibacterials in pathogens isolated from CF patients will not be reviewed. The frequency with which resistance to antibacterials emerges *in vitro* is routinely determined by the exposure of a bacterial population to a given concentration of the test agent. This procedure results in the quantification of the number of mutant bacteria in a population and describes the frequency with which resistant bacterial phenotypes arise *in vitro*, expressed as mutation rates or mutation frequencies. Such data are considered to provide important information about the risk of resistance emerging in a treated patient. However, pathoadaptive processes favor the emergence of bacteria displaying a hypermutator phenotype (15–27), exhibiting significantly increased rates of mutation, or a small-colony morphotype (30–34), facilitating recurrent and persistent infections. Both adaptive responses confer antibiotic resistance, which, however, is not analyzed by routine procedures. Therefore, *in vitro* studies assessing mutation rates or mutation frequencies are not reviewed.

Emergence of resistance in *P. aeruginosa*. In general, resistance to antibacterials developed rapidly in *P. aeruginosa*. This fact is mirrored by analyses of strains isolated either from patients with long-term administration of fluoroquinolones, aminoglycosides, and β-lactams, including carbapenems and aztreonam, or from colonized CF patients. A marked increase of resistance was demonstrated in the long-term-treated patients but not in the colonized patients (232). Growth of a highly adaptive bacterium like *P. aeruginosa* in CF lungs is characterized by a variable nutrient and oxygen supply resulting in decreased oxygen consumption and increased nitrogen utilization. These metabolic shifts confer resistance to tobramycin and ciprofloxacin even without preceding exposure to these antibacterials (233). In addition to growth in a hostile environment, fluctuating inflammatory and immunological responses trigger the development of various growth morphotypes, such as a biofilm mode of growth and small colony variants (SCVs), and the establishment of a hypermutation phenotype (234). Higher mutation rates in biofilm-associated *P. aeruginosa* have also been found (235). Dormant persisting bacteria are tolerant to antibacterial agents because of the reduced rates of DNA replication, translation, cell wall synthesis, and metabolism. Persisters isolated from CF patients were found to contribute to relapsing chronic infections and may represent a pool of adaptively evolving organisms from which resistant mutants can emerge. The molecular mechanisms relating to antibiotic tolerance and resistance were reviewed recently (235–237). In general, mutations play an important role in the adaptation of *P. aeruginosa* to the specific environmental conditions in the lungs of CF patients, and hypermutators can be isolated frequently. It was demonstrated that 37% of CF patients chronically infected with *P. aerugi-*

TABLE 3 Population analysis of four *P. aeruginosa* strains isolated in 1983 from adult CF patients treated twice daily with 500 mg ciprofloxacin orally[a]

Strain	Day	CLSI MIC (mg/liter)	Bacterial load determined by population analysis (log_{10} CFU/ml)				
			1× CLSI MIC	2× CLSI MIC	4× CLSI MIC	16× CLSI MIC	64× CLSI MIC
1	1	0.25	7.6	6.3	6.8	5.5	ND
	3	1.0	ND	ND	7.3	7.3	ND
	5	2.0	ND	ND	4.1	8.3	8.8
9	1	0.03	6.6	6.5	ND	ND	ND
	3	1.0	ND	6.0	6.7	5.5	ND
	5	1.0	ND	ND	6.2	7.7	ND
19	1	0.12	8.3	8.6	8.6	6.5	ND
	3	0.5	ND	7.5	7.5	6.3	5.9
	5	1.0	ND	7.3	7.3	8.1	8.3
36	1	0.03	8.0	7.2	6.9	5.3	ND
	3	0.25	ND	8.3	7.3	6.0	5.9
	5	0.5	ND	5.5	6.9	9.1	9.3

[a] These isolates were exposed *in vitro* to fluctuating ciprofloxacin concentrations simulating an oral twice-daily dose of 500 mg. The CLSI MIC was generated by selecting at least three to five well-isolated colonies of the same morphological type for preparation of the inoculum. Population analysis was performed by selecting only one colony for the inoculum preparation (viable counts represent the number of colonies grown on agar plates containing a ciprofloxacin concentration one or two times, etc., the concentration equivalent to the CLSI MIC) (ND, not detected; i.e., viable counts were below the limit of detectability of 2 log_{10} CFU/ml). See reference 97 for the method used to analyze the data.

nosa harbor hypermutators (16, 20–22). All these features consistent with adaptive responses to growth in CF lungs create an environment facilitating pathoadaptive mutations as well as increased rates of resistance acquisition and perhaps also transmissibility (237, 238). Although exposure to either ciprofloxacin or tobramycin was adequate, hypermutator strains not only persisted but also multiplied (50,000-fold), in contrast to the nonhypermutator strains (239), and acquired antibiotic resistance more frequently, followed by deterioration of lung function (240). Exposure of *P. aeruginosa* to subinhibitory concentrations of β-lactams, aminoglycosides, and fluoroquinolones led to 3- to 14-fold increases in mutation frequencies (241). The practical impact of the presence of hypermutators in CF patients is that within a large population of pathogens in the CF lung, resistant subpopulations are present prior to the commencement of treatment and will be rapidly selected as soon as the patient is treated.

In addition, exposure of biofilm-grown *P. aeruginosa* to the optimized PK/PD parameter of an AUC/MIC ratio of 384 h for ciprofloxacin failed to reduce total viable counts and rapidly selected for resistance development (242). This study suggests that, in agreement with the data discussed in "Preclinical and Clinical PK/PD Studies in CF Patients," above, conventional and even optimized PK/PD measures are not predictive of antibacterial activity in CF patients and fail to suppress resistance development. As summarized in "Preclinical and Clinical PK/PD Studies in CF Patients," above, almost all isolates, except for the highly susceptible subpopulations that may prevail during the early phases of colonization, are exposed to suboptimal concentrations of the antibacterials studied so far, except probably colistin.

Furthermore, pathogen heterogeneity contributes significantly to resistance development. *In vitro* exposure of *P. aeruginosa* to fluctuating enoxacin serum concentrations following oral doses of 500 mg/day administered either once or twice daily for 2 days resulted in rapid emergence of resistance due to the selection of moderately resistant subpopulations (243). A population analysis of four *P. aeruginosa* strains isolated from adult CF patients who were treated in 1983 for the first time in their lives with 500 mg ciprofloxacin orally twice daily (145) revealed that the preexposure isolates were heterogeneously susceptible to ciprofloxacin, with MICs for the subpopulations ranging from ≤1- to up to 16-fold the MIC of ciprofloxacin, as determined for the entire population by the CLSI method (97). These strains were exposed *in vitro* for 5 days to fluctuating ciprofloxacin concentrations simulating an oral 500-mg b.i.d. dose. Viable counts of subpopulations with MICs of one to four times the overall MIC were either reduced or totally eliminated during exposure to fluctuating drug concentrations over 5 days, while the viable counts of highly resistant subpopulations with MICs up to 64 times the initial MIC increased continuously (97). Data summarized in Table 3 illustrate the shifts in population densities of the various subpopulations. Although on day 1, the highly susceptible subpopulations with MICs ranging from 0.03 to 0.25 mg/liter were predominant, less susceptible subpopulations with MICs 2, 4, and 16 times as high were isolated in smaller numbers. On consecutive days of exposure to fluctuating ciprofloxacin concentrations, the susceptible subpopulations were eradicated, and the moderately resistant and resistant subpopulations prevailed (Table 3). This finding is remarkable insofar as these CF patients were treated with ciprofloxacin for the first time in their lives and years prior to the launch of ciprofloxacin and levofloxacin, but still, resistance already existed in the pretherapy isolates. Similar results were generated for tobramycin; however, the propensity for resistance selection was ~2 orders of magnitude lower than for ciprofloxacin (my unpublished data). These data were confirmed recently: three clinical *P. aeruginosa* isolates were exposed to fluctuating ciprofloxacin concentrations simulating AUC/MIC ratios ranging from 55 h to 180 h. Resistant subpopulations already existed prior to exposure and were enriched during exposure (234). A genomic analysis of one *P. aeruginosa* strain revealed that 35 different genes were involved in ciprofloxacin resistance; 10 of these were regulated by ciprofloxacin (235). Three studies (145, 243, 244) demonstrated that heterogeneous bacterial populations provide a reservoir for resis-

tance selection, which occurs rapidly during drug exposure. The selection of resistant subpopulations within the first dosing interval provides the basis for a continuously decreasing drug effect of the second and following doses and a rapid amplification of highly resistant populations.

Previous colistin therapy significantly increased the number of bacteria exhibiting colistin heteroresistance in *A. baumannii* (95). Although polymyxin heteroresistance was uncommon, several isolates presented heterogeneous subpopulations with increased and, thus, borderline susceptible MICs, which might cause treatment failures (92); only four successive *in vitro* passages in the presence of colistin increased the proportion of resistant subpopulations from 0.000023% to 100% (93).

Emergence of resistance in *S. aureus* and *H. influenzae*. Early infections in CF airways are most frequently caused by *H. influenzae* and *S. aureus*. The annual prevalences of methicillin-susceptible and methicillin-resistant *S. aureus* (MSSA and MRSA, respectively) isolated from CF patients in 2008 in North America, Europe, and Australia ranged from approximately 15% to 56% for MSSA and 3% to 24% for MRSA. Health care- and community-associated MRSA strains were isolated from 70% and 17% of pediatric CF patients (109, 245, 246).

The mechanism of methicillin resistance is due to the acquisition of an altered penicillin binding protein 2A (247, 248); the corresponding *mecA* gene is located on the so-called staphylococcal cassette chromosome *mec* (SCC*mec*) element. Expression of methicillin resistance is heterogeneous: in a small subpopulation expressing a low level of methicillin resistance, other highly resistant subpopulations segregate (249). The SCC*mec* element contains genes for aminoglycoside, tetracycline, and macrolide-lincosamide-streptogramin B (MLSB) resistance (249–251). Furthermore, the *mecA* gene is located in close vicinity to DNA gyrase (252–254). Therefore, mutations in genes coding for antibiotic resistance select for methicillin resistance, and, vice versa, methicillin resistance almost always coincides with multidrug resistance. Consequently, fluoroquinolones used for oral, intravenous, or aerosolized treatment of CF patients coselect for MRSA. Ciprofloxacin is a better MRSA selector than levofloxacin. Aminoglycosides and tobramycin in particular are unlikely to be effective because the *aadD* gene, encoding 4′,4″-adenylyltransferase, conferring tobramycin resistance is present within SCC*mec* (255–258). Aztreonam selects for aminoglycoside resistance in an MRSA background (259). Adaptation of *S. aureus* to growth in lungs of CF patients results in the appearance of SCVs, which facilitate recurrent and persistent infections and coexistence in the presence of *P. aeruginosa* (30–36). Coinfection with *P. aeruginosa* enhances SCV formation (260–262). In general, SCVs are characterized by permeability changes in the cell wall, reducing the uptake of fluoroquinolones such as ciprofloxacin and levofloxacin but not moxifloxacin, so that the MICs of ciprofloxacin for SCVs are higher than for the normal phenotype; furthermore, the lower electrochemical gradient across the cell membrane of SCVs reduces the transport of aminoglycosides significantly so that aminoglycosides are not active against SCVs (36, 263–265).

The thymidine-dependent SCV phenotype of strains isolated from CF patients represents a specific pathoadaptive response to growth in airways of CF patients; this phenotype is significantly more hypermutable than that of thymidine-independent SCVs (18). Both hypermutability and any SCV phenotype contribute to resistance to macrolides, aminoglycosides, rifampin, fosfomycin, and ciprofloxacin (18, 23, 266, 267). Furthermore, growth of *S. aureus* in CF lungs is associated with hypermutability (22, 266), *in vitro* exposure of hypermutators to gentamicin resulted in an increased emergence of SCVs (268), and antibacterials other than gentamicin have not been studied. *S. aureus* hypermutators were found to be more virulent (27). Thus, SCV colonial morphotype, heteroresistance, and hypermutability serve as catalysts for persistent *S. aureus* infections in CF patients.

Likewise, long-term colonization of CF lungs with *H. influenzae* favors the emergence of hypermutators (269, 270). More than 20% of hypermutable *H. influenzae* isolates from CF patients were β-lactamase producers or fluoroquinolone resistant (269–271). A multiresistance phenotype was associated with long-term colonization (269).

Fluoroquinolone treatment of CF patients fosters quinolone resistance development in *H. influenzae* isolates (272, 273); long-term azithromycin maintenance in CF patients was associated with increased macrolide resistance in *H. influenzae*, *S. aureus*, and streptococci isolated from CF sputa (274–277). The rate of macrolide resistance increased from 10% f patients with staphylococcal colonization prior to the start of maintenance therapy to 83%, 97%, and 100% in the consecutive years after initiation of azithromycin administrations (277); macrolide resistance in *H. influenzae* increased within 4 years of azithromycin maintenance therapy, from 3.7% to 37.5% (275). Approximately 50% of macrolide-resistant *S. aureus* strains expressed macrolide-lincosamide-streptogramin resistance (277).

Bacteriophage induction and promotion of resistance spread. Bacteriophages play important roles in adaptive processes of *S. aureus* growing in the lungs of CF patients. Extensive phage dynamics lead to the emergence of heterogeneous *S. aureus* subpopulations with various virulence and resistance phenotypes (104–106). Phage mobilization plays an important role in CF patients not only during pathoadaptive processes but also in particular during exposure to antibacterials. Ciprofloxacin and trimethoprim were found to induce phage mobilization in *S. aureus* isolates from CF patients (103), thus increasing virulence and selecting for antibiotic resistance. The emergence and spread of a specific MRSA strain isolated from a CF patient in 2002 in Marseille, France, are worrying. This atypical strain, named "*S. aureus* strain CF-Marseille," was susceptible to gentamicin but resistant to tobramycin, kanamycin, erythromycin, lincomycin, and ofloxacin.

Furthermore, this strain had a hetero-glycopeptide-intermediate phenotype of resistance. This strain is closely related to vancomycin-intermediate strain Mu50 and spreads rapidly in CF patients (278). It is worrying that this strain carries an antibiotic-inducible (e.g., imipenem, tobramycin, and ciprofloxacin) bacteriophage, which may cause a high frequency of transfer and the unintended spread of virulence and resistance determinants.

As reviewed recently, several antibacterial agents may induce phages in *S. aureus* and *P. aeruginosa* strains isolated from CF patients (279). In particular, tobramycin, ciprofloxacin, cotrimoxazole, and imipenem induced phages in *S. aureus* strain CF-Marseille. Furthermore, ciprofloxacin strongly induced phage production in *P. aeruginosa* strains isolated from CF patients, and high numbers of free phages from *P. aeruginosa* were detected in direct preparations of sputum samples from CF patients. These phages are characterized by a specific metabolic profile reflecting pathoadaptation. Among the sequences that were specific to CF

viromes were genes related to virulence. As summarized by Rolain et al. (279), many proteins associated with antimicrobial resistance genes were identified in the virome. These specific CF virome sequences contain many sequences presumably encoding a variety of different antibiotic resistance mechanisms, such as efflux pumps, fluoroquinolone resistance, and β-lactamase production, in much higher numbers than in non-CF viromes. Phages contribute to bacterial genome alterations facilitating pathoadaptation. As phages are mobilized by antibacterial therapy, antibiotic-mediated phage induction results in the selection of highly virulent, multidrug-resistant, and well-adapted strains.

Thus, on the one hand, a vicious circle is driven by pathoadaptive responses leading to antibiotic recalcitrance, even in the absence of exposure to antibacterials, and on the other hand, antibacterials transiently reduce the bacterial load minimally and improve lung function but promote the selection of small-colony morphotypes, biofilm formation, and hypermutable and more virulent populations, all of which are significantly less susceptible to antibacterials and are prone to develop resistance. It is an inevitable consequence of adaptive responses to growth in airways of CF patients that resistance to antimicrobials of any class will develop even in the absence of antibacterial therapy and in particular under selective antibiotic pressure, irrespective of whether drug exposure of the pathogens is adequate or not.

EFFECT OF AEROSOLIZED ANTIBACTERIAL THERAPY ON LUNG FUNCTION AND *P. AERUGINOSA* SPUTUM LOAD

Traditional endpoints of clinical studies are clinical cure and eradication of the pathogen, which, however, are irrelevant and cannot be attained in CF patients. Eradication of *P. aeruginosa* from the lungs is achievable by early aggressive antibacterial therapy of young colonized CF patients (77). Young patients treated early with antipseudomonals remained free of chronic colonization for up to 2 years. During disease progression, bacteria adapt to the hostile environment so that bacterial populations protecting themselves from attack by host defense mechanisms and antibacterials emerge rapidly. Once chronic infection is established, it is almost impossible to eradicate *P. aeruginosa*. Improvement of lung function and reduction of bacterial load are used instead. Because of the questionable value of MIC testing, not being representative of the heterogeneity of the bacterial population and not being clinically relevant; longitudinal genotypic and phenotypic changes of the pathogens leading to within-patient and, in particular, interpatient diversity of colonial morphology as well as diversity of antigenicity and drug susceptibility; and undefined CF-specific PK/PD targets to be met, it is impossible to predict the probability of clinical or bacteriological success of enteral, parenteral, or aerosolized antibacterial treatment. Therefore, it is an open question whether high target site concentrations due to aerosolized administration of antibacterials may translate into better clinical or microbiological efficacy or an attenuated propensity for resistance development compared to oral or intravenous administration. Only trials investigating the efficacies of novel formulations of antibacterials for aerosolized therapy are summarized below and compared with previous clinical studies with orally or parenterally administered agents; the effect of aerosolized antipseudomonal treatment on *P. aeruginosa* sputum load, drug susceptibility and/or resistance development as a pharmacodynamic parameter, as well as lung function expressed as the change in the forced expiratory volume in 1 s (FEV_1) compared to baseline are summarized as efficacy endpoints. Changes in FEV_1 and reductions of the *P. aeruginosa* sputum load have been analyzed as the primary and secondary endpoints, respectively, in almost all clinical trials. Most studies have been designed as placebo-controlled trials, and only a few studies have been designed as comparative trials. Different CF patient populations have been studied in these trials, as relatively young patients colonized with homoresistant populations of susceptible *P. aeruginosa* strains or patients suffering from mild lung infection but also patients >30 years of age with advanced lung disease infected by a heteroresistant population of *P. aeruginosa* have been included. Both patient age and bacterial population dynamics affect clinical and bacteriological efficacies.

Furthermore, a few authors reported CF-specific comedications, some of which may affect the antimicrobial action of antibacterials, but this information is not provided in the majority of publications. Another aspect worth mentioning is the nonuniform evaluation of *P. aeruginosa* sputum load reductions. In most studies, a change at day 28 from baseline is reported. Others report a change of the sputum load compared to placebo; however, viable counts in sputa of placebo recipients increased in almost all of the reported studies, so a reduction of viable counts due to aerosolized treatment may be statistically significant compared to placebo but not compared to baseline. Multiple factors have effects on disease progression, impairment of lung function, and the virulence and susceptibility pattern of the causative pathogen, so clinical and bacteriological outcomes are different in different studies, thus making historical comparisons difficult and inaccurate. Therefore, data compiled in Table 4 should be compared with caution.

Fluoroquinolones

Two ciprofloxacin formulations are or were under phase II/III development in CF patients. Ciprofloxacin betaine administered with a dry powder inhaler (ciprofloxacin DPI) was evaluated in a randomized, double-blind, placebo-controlled, multicenter study for efficacy and safety at doses of 32.5 and 48.75 mg/kg b.i.d. for 28 days. The primary endpoint was the change in FEV_1 at days 28 to 30 from baseline (280). Administration of both ciprofloxacin DPI and a placebo powder formulation matching the corresponding dose of ciprofloxacin DPI resulted in a reduction of FEV_1 values at days 28 to 30. Changes of the *P. aeruginosa* sputum density at days 28 to 30 from baseline were 0.73 and 0.78 $\log_{10}$ CFU/g in the ciprofloxacin DPI groups, compared to 0.07 and 0.63 $\log_{10}$ CFU/g in the two corresponding placebo groups. Ciprofloxacin-resistant mucoid *P. aeruginosa* strains were isolated at days 56 to 60 in 21 to 25% of CF patients treated with either dose of ciprofloxacin DPI, while ciprofloxacin-resistant strains were isolated from 21% and 10% of the placebo recipients (280). The dose-independent reduction of viable counts and deterioration of lung function may likely be due to the finding that ciprofloxacin sputum concentrations did not differ between the two dose groups (see "Fluoroquinolones," above) (Table 2). Ciprofloxacin hydrochloride liposomes allow for sustained release so that the liposomal ciprofloxacin formulation can be administered once a day at a dose of 6 ml of a 50-mg/ml formulation. Efficacy was evaluated in 22 CF patients. The primary endpoint was the decrease in sputum density of *P. aeruginosa* after 14 days (281). Inhaled liposomal ciprofloxacin hydrochloride improved lung function significantly and reduced viable counts of *P. aeruginosa* in sputum by 1.43 $\log_{10}$ CFU/g; resistance development was not assessed or reported

TABLE 4 Antibacterial efficacies of various aerosolized regimens used to treat *P. aeruginosa* lung infections in patients with cystic fibrosis compared to oral or intravenous treatment[a]

Drug and route	Age of patients (yr) (range or mean)	Dose, regimen	Treatment duration (wk)	*P. aeruginosa* change from baseline at day 28 (log_{10} CFU/g)[g]	FEV_1 change from baseline at day 28 (%)	Reference(s)
CPX DPI	12–>18	32.5 mg, b.i.d.	4	−0.98[c]	−1.0	280
CPX DPI	12–>18	48.75 mg, b.i.d.	4	−1.0[c]	−0.62	280
CPX lip		300 mg, b.i.d.	2	−1.43[c]	+6.86	281
CPX p.o.	>18	500 mg, b.i.d.	4	0[b]	+6.23	191
LVX	28	120 mg, q.d.	4	−0.31[b]	+1.93	284, 285
LVX	27.5	240 mg, q.d.	4	−0.31[b]	+2.56	284, 285
LVX	29.2	240 mg, b.i.d.	4	−0.73[b]	+6.25	284, 285
LVX p.o.	23.8	500 mg, q.d.	2	−5 (2), −2 (1), −1 (3)[d]	NR	155
TOB TIP	6–21	112 mg, b.i.d.	12	−2.4[b]	+4.9	288, 372
TOB TIP	6–21	112 mg, b.i.d.	12	−1.91[b]	NR	289, 292
TOB TIP	<13	112 mg, b.i.d.	12	−1.11[b]	+10.4	289, 292
TOB TIP	≥13–<20	112 mg, b.i.d.	12		+6.8	289, 292
TOB TIP	≥20	112 mg, b.i.d.	12		+0.3	289, 292
TOB TSI	<13	300 mg, b.i.d.	12	−1.54[b]	+9.4	289, 292
TOB TSI	≥13–<20	300 mg, b.i.d.	12		+3.9	289, 292
TOB TSI	≥20	300 mg, b.i.d.	12		+0.9	289, 292
TOB TSI	6–12	300 mg, b.i.d.	4	−0.86[b]	+11.51	294
TOB TSI	13–17	300 mg, b.i.d.	4		+14.43	294
TOB TSI	>18	300 mg, b.i.d.	4		+1.77	294
TOB TSI	6–>18	300 mg, b.i.d.	4	−1.66[b]	+7.07	295
TOB i.v.	19–37	10 mg/kg, q.d.	2	NR	+11.3	221
TOB i.v.	19–37	3.3 mg/kg, t.i.d.	2	NR	+7.4	221
TOB i.v.	7.4–17.2	5 mg/kg, t.i.d.	2	NR	+14.9	296
TOB i.v.	5.6–19.3	15 mg/kg, q.d.	2	NR	+15	296
AMI	14–38	500 mg, q.d.	2	−0.32[b]	+5.05	170
AMI	17.4	560 mg, q.d.	4	−0.74[b]	+7.5–+7.9	171, 296, 373
AMI		590 mg, q.d.	4	−1.25[b]	+4.7	295
AMI i.v.	1.8–22	35 mg/kg, q.d.	2	77% erad.[e]	+15	299
AZLI	27.2	75 mg, b.i.d.	2	−0.39[b]	<1	303
AZLI	23.9	225 mg, b.i.d.	2	−0.37[b]	1	303
AZLI	7–74	75 mg, t.i.d.	4	−1.4[b]	+7.9	78, 304
AZLI	10–50	75 mg, b.i.d.	4	−0.45[b]	+5.8	305, 306
AZLI	7–50	75 mg, t.i.d.	4	−0.36[b]	+5.8	305, 306
AZLI	27.3	75 mg, b.i.d.	4	−0.2[b]	+4.9	307, 308
AZLI	29.0	75 mg, t.i.d.	4	−0.8[b]	+8.0	307, 308
AZLI	19.2	75 mg, t.i.d.	4	−1.35[b]	+0.29	309, 310
AZLI	25.8	75 mg, t.i.d.	4	NR	+8.35	311
AZT i.v.	6–>18	50 mg, q.i.d.	2	−0.41[b]	+8.5	312
COL DPI	6–12	80 mg, b.i.d.	4	−0.6[b]	−8.11	294
COL DPI	≥13–17	80 mg, b.i.d.	4		+6.01	294
COL DPI	≥18	80 mg, b.i.d.	4		+0.79	294
COL DPI	>6	1.7 MU, b.i.d.	24	NR	+0.64	318
COL DPI	14.2	1.0 MU, b.i.d.	12	NR	−11	319
COL i.v.	21.7	2.0 MU, t.i.d.	2	NR	+9.2	323
COL i.v.	26	2.0 MU, t.i.d.[f]	2	NR	+6.9	324

[a] Efficacy endpoints are expressed as the change from the baseline, i.e., reduction, in *P. aeruginosa* CFU (log_{10} CFU/g) in sputum or change (percent) in FEV_1 values (+, increase; −, decrease) (CPX DPI, ciprofloxacin dry powder for inhalation; CPX lip, ciprofloxacin liposomal formulation for inhalation; LVX, levofloxacin; TOB TIP, tobramycin inhalation dry powder; TOB TSI, tobramycin solution for inhalation; AMI, amikacin; AZLI, aztreonam lysine for inhalation; AZT, aztreonam; COL DPI, colistin dry powder for inhalation; COL, colistin).

[b] Change from baseline at day 28.

[c] Recorded at days 14 to 16.

[d] Two patients had a 5-log_{10} reduction of titers, etc.

[e] *P. aeruginosa* was not detectable at day 14 in 77% of patients.

[f] Combination therapy with another antipseudomonal agent.

[g] erad., eradicated; NR, not reported.

(281). For comparison, oral treatment of CF patients with 500 mg b.i.d. resulted in an improvement of lung function but no reduction of the *P. aeruginosa* sputum load; ciprofloxacin resistance developed in 75% of the isolates tested (282, 283).

Levofloxacin inhalation solution (MP-376) was studied in 151 patients with CF who were randomized to receive one of three doses of aerosolized levofloxacin (120 mg once daily, 240 mg once daily, and 240 mg twice daily) or placebo for 28 days (284, 285).

The primary efficacy endpoint was the change in sputum *P. aeruginosa* density. Mean sputum densities decreased from baseline values in the three treatment groups by 0.31, 0.31, and 0.73 $\log_{10}$ CFU/g sputum, respectively. As viable counts of *P. aeruginosa* increased in the placebo group by 0.23 $\log_{10}$ CFU/g sputum, a statistically significant treatment difference of 0.96 $\log_{10}$ CFU/g sputum compared to placebo was recorded on day 28 in the 240-mg b.i.d. group; a statistical analysis for changes in *P. aeruginosa* densities in the two other treatment groups has not been reported. A dose-dependent increase in FEV_1 was observed between the 240-mg MP-376 twice-daily group (6.25% improvement) and placebo (2.36% deterioration). The difference between the placebo group and the twice-daily regimen was statistically significant. The improvement in FEV_1 for the group administered 240 mg MP-376 twice a day was still statistically significant on day 42, but values returned to baseline by day 56. Furthermore, a significantly reduced need for other anti-*P. aeruginosa* antibiotics was observed in all MP-376 treatment groups compared to the placebo group. Importantly, there were no significant changes in levofloxacin MIC_{50} or MIC_{90} values for *P. aeruginosa* during the 28-day treatment and the follow-up period of the study. It is important to note that >67% of the patients treated in any of the three levofloxacin groups or the placebo group were comedicated with azithromycin, which has been demonstrated to improve clinical outcomes in *P. aeruginosa*-infected CF patients despite its lack of antibacterial activity against this pathogen (286). Two different phase III trials are being performed with MP-376 with the aim to evaluate either the safety, tolerability, and efficacy or the efficacy of MP-376 compared to tobramycin for inhalation (ClinicalTrials.gov no. NCT01270347), with the primary endpoints of evaluating the change of FEV_1 from baseline or monitoring changes in *P. aeruginosa* sputum density from baseline to the end of treatment. No study results have yet been posted.

Aminoglycosides

A tobramycin solution specifically formulated for inhalation (TSI) (Tobi) is frequently used for b.i.d. treatment of chronic *P. aeruginosa* infections in CF patients at a dose of 300 mg/ml; its efficacy was summarized by Cheer et al. (287). A tobramycin inhalation dry powder formulation (TIP) administered via a novel inhaler has been developed. The efficacy of TIP was compared to that of placebo in two studies (288, 289) and to that of Tobi in another study (290). TIP was compared to placebo through 3 cycles of 28 days on and 28 days off drug or placebo; cycle 1 was designed as a double-blind placebo-controlled extension, and cycles 2 and 3 were designed as open-label crossover extensions. TIP and the matching placebos were administered in four capsules, each containing 28 mg dry powder to be inhaled twice daily. The ages of the patients ranged from 6 to 21 years so that relatively treatment-naive patients were enrolled. In the third study, 112 mg TIP twice daily was compared to 300 mg/5 ml Tobi twice daily in an open-label randomized trial. Unlike both placebo-controlled studies, the comparative study included patients who were extensively pretreated with inhaled antipseudomonals. In particular, 82.1% of patients receiving TIP and 82.3% of patients receiving Tobi had prior TSI exposure. The study results generated in these three clinical trials (288–290) were critically reviewed by Lam et al. (291).

Unfortunately, the data described above (288–291) are reported only as relative measures, which are not appropriate for comparison with data from other studies. Therefore, changes in *P. aeruginosa* sputum densities and FEV_1 are supplemented with the absolute changes in FEV_1 from baseline at the end of treatment retrieved from publicly accessible assessment reports by the EMA (292) and the Australian Government Department of Health (293) and the ClinicalTrials.gov identifier (Table 4). TIP in the placebo-controlled study improved the FEV_1 by 12.8% at day 28 of cycle 1 and by 11.4% at day 28 of the third cycle, compared to almost 0% and almost +13%, respectively, in the placebo group (289). TIP decreased the sputum density of the nonmucoid phenotype of *P. aeruginosa* at day 28 of cycle 1 by 1.91 $\log_{10}$ CFU/g, versus 0.15 $\log_{10}$ CFU/g in the placebo group; the mucoid phenotype was reduced by TIP by 2.61 $\log_{10}$ CFU/g, versus 0.43 $\log_{10}$ CFU/g in the placebo group (289); and the *P. aeruginosa* sputum density was reduced by 2.4 $\log_{10}$ CFU/g at day 29 of cycle 1 (285) and by 1.2 $\log_{10}$ CFU/g at day 28 of cycle 3 (288) in the other placebo-controlled study (288). Overall, comparable increases in FEV_1 with each tobramycin treatment were observed in the controlled study (290), with mean relative improvements on day 28 of the third cycle of 5.8% in the TIP treatment arm and 4.7% in the TSI arm (293). The magnitude of the treatment difference was greater in children than in adults. Lung function of patients 6 to 13 years of age improved by 10.4% and 9.4% compared to 6.8% versus 3.9% and 0.3% versus 0.9% in patients aged 13 to 20 and >20 years in the TIP arm versus the TSI arm, respectively (292) (Table 4). The mean reductions in sputum *P. aeruginosa* density at day 28 of cycle 3 were comparable between the TIP and DPI groups and across all age groups (290, 292). Neither the change of FEV_1 from baseline at day 28 of cycle 1 nor the change in *P. aeruginosa* sputum densities was statistically significantly different between the TIP and TSI groups, respectively. In general, the magnitude of the reduction of *P. aeruginosa* sputum density during the on-treatment phase decreased with each successive cycle in all studies (288–290, 293). Previously, tobramycin solution for inhalation was compared with colistin in a randomized trial; patients were subgrouped according to age (294). Overall, FEV_1 improved by 6.7% in the TSI group, compared to 0.37% in the colistin group; younger patients benefitted most from TSI treatment (11.5% to 14.4%), whereas for patients >18 years of age, FEV_1 improved by 1.77%. *P. aeruginosa* isolates with MICs of ≥4 mg/liter at baseline and at day 28 were isolated from 38% and 49% of tobramycin-treated patients, respectively (294). In another phase III study, TSI was compared with a liposomal formulation of amikacin (295). TSI reduced the *P. aeruginosa* sputum load by 1.66 $\log_{10}$ CFU/g and improved FEV_1 values by 7.07%, compared to values of 1.25 $\log_{10}$ CFU/g and 4.7%, respectively, in the amikacin arm (295). For comparison, tobramycin administered intravenously either once daily (10 mg/kg q.d.) or three times daily (3.3 mg/kg t.i.d.) improved lung function comparably well (Table 4); however, a statistically significant increase in MICs was observed for the once-daily group compared to the three-times-daily group (221). Comparable results were generated by others (296–298).

Aerosolized administration of liposomal amikacin reduced the *P. aeruginosa* sputum load by 0.32 to 1.25 $\log_{10}$ CFU/g, and pulmonary function increased from baseline by 4.7% to 7.9% (Table 4) (170, 171, 295, 296). Aerosolized administration of liposomal amikacin to CF patients did not result in a PK/PD-driven change in tests of lung function but resulted in a concentration-dependent reduction of the bacterial load, which was correlated to the AUC/MIC ratio (170, 171). Compared to TSI, liposomal amikacin

reduced bacterial sputum load less effectively and had a less marked improvement in lung function, but patients maintained on amikacin reported significantly greater improvements in their respiratory symptoms at the end of each treatment cycle and throughout the study period (295). Intravenously administered amikacin reduced viable counts of *P. aeruginosa* in the sputa of 14 out of 18 patients to below the limit of detectability; clearance persisted in 11 patients for 1 month and in 9 patients for 11 months after cessation of therapy (299).

Aztreonam

As reviewed recently, the efficacy of aztreonam lysine has been studied in several placebo-controlled phase II and phase III trials (300–302); the data are summarized in relative terms. The study authors reported changes in *P. aeruginosa* sputum load and FEV_1 in comparison to values for the corresponding placebo group but not as a change from baseline, so the data summarized in Table 4 have been deduced from ClinicalTrials.gov registration data. The efficacies of two doses of aztreonam were compared in a placebo-controlled, randomized, phase II study (303). Patients were dosed with either 75 mg or 225 mg aztreonam twice daily for 14 days. Viable counts of *P. aeruginosa* were reduced by both regimens dose independently by approximately 0.4 $\log_{10}$ CFU/g. Likewise, FEV_1 improved almost dose independently by 6% and 2% in the 75-mg and 225-mg dose groups, respectively, on day 14, i.e., cessation of therapy, and by <1% at day 28. It is important to note that 46%, 32%, and 23% of patients in the 75-mg, 225-mg, and placebo dose groups, respectively, received bronchodilators; patients using bronchodilators had greater reductions of *P. aeruginosa* sputum loads and better improvement of lung function. The susceptibility pattern of the isolates did not change from day 0 to day 14 or 28 (303).

The studies AIR-CF1 to AIR-CF4 were designed as placebo-controlled phase III trials. AIR-CF1 was a double-blind, multicenter, multinational, placebo-controlled trial. Patients were randomized to receive either 75 mg aztreonam or placebo t.i.d. for 28 days (78, 304). The *P. aeruginosa* sputum density decreased in treated patients by 1.4 $\log_{10}$ CFU/g and remained at baseline for placebo recipients. The MIC_{90} values of aztreonam and comparators remained unchanged throughout the study period. Pulmonary function improved in the verum group by 10.3% compared to placebo (78) or by 7.9% compared to baseline (304); FEV_1 values declined in the placebo group by 2.4% (304).

AIR-CF2 was a randomized, double-blind, placebo-controlled trial designed to assess the efficacy of aztreonam administered twice or three times daily compared with placebo after a run-in phase during which 300 mg of inhaled tobramycin was administered to all the patients twice daily for 28 days (305, 306). Due to tobramycin administration during the run-in phase immediately preceding aztreonam administration, the *P. aeruginosa* sputum load was decreased by 0.28 $\log_{10}$ CFU/g, and FEV_1 was improved by 0.9%. Decreases in *P. aeruginosa* sputum densities for the twice- and three-times-daily aztreonam regimens were comparable and significantly better than those for the placebo group. Improvements in FEV_1 measurements were not different between the two treatment groups, so the treatment courses were pooled. In the pooled treatment group, the mean improvement of FEV_1 was 6.3% compared to placebo or 3.9% compared to baseline; the mean reductions of *P. aeruginosa* sputum load were 0.43 $\log_{10}$ CFU/g compared to baseline and 0.66 $\log_{10}$ CFU/g compared to placebo. Aztreonam susceptibility remained unchanged up to day 56 (305, 306).

To answer the question of whether repeated courses of inhaled aztreonam may have an impact on safety and efficacy, an open-label follow-up trial that enrolled patients from the previous two phase III placebo-controlled trials was performed (AIR-CF3) (307, 308). CF patients were treated with 75 mg aztreonam three times daily using a 28-day-on and 28-day-off schedule unless they were originally randomized to receive twice-daily treatment (n = 85) in AIR-CF2. The use of additional treatments, including azithromycin (n = 147 [53.6%]), was allowed throughout this study. Sustained improvements in FEV_1 were observed over the 18-month course of therapy. Changes in FEV_1 from baseline of 4.9% and 8.0% in the b.i.d. and t.i.d treatments groups, respectively, were recorded after completion of the first course on day 28; FEV_1 values decreased to 1.2% and 4.2%, respectively, at the end of the ninth course. Viable counts of *P. aeruginosa* in sputa of the b.i.d. and t.i.d. groups decreased from baseline to day 28 by 0.2 and 0.8 $\log_{10}$ CFU/g and at the end of all treatment cycles by 0.5 and 0.6 $\log_{10}$ CFU/g, respectively. Transient increases in MIC_{90}s of aztreonam by two titration steps from a baseline MIC of 128 mg/liter were observed. The increases prevailed in the b.i.d. group but overall remained unchanged in both groups at the end of therapy (307, 308). These data indicate that, in contrast to the AIR-CF2 study, a dose-response relationship may exist.

Recently, a randomized, double-blind, placebo-controlled trial of inhaled aztreonam (75 mg t.i.d.) in CF patients with mild lung disease was completed (309, 310). The *P. aeruginosa* sputum load was reduced by 1.4 $\log_{10}$ CFU/g. The MIC_{90} of aztreonam remained unchanged in the placebo group but increased by 2 $\log_{10}$ titers in the aztreonam group. FEV_1 increased by 2.5% compared to placebo (309) but by only 0.29% compared to baseline (310).

An open-label, randomized, head-to-head study comparing aztreonam with tobramycin inhalation over 6 months with three treatment cycles of 28 days on and 28 days off was completed recently (311). The study design consisted of 2 treatment arms of 28-day, intermittent, repeating treatment regimens: aztreonam inhalation solution or tobramycin inhalation solution (TSI). The total study period was 26 weeks. The *P. aeruginosa* sputum load was not assessed. At day 28 of the first cycle, FEV_1 improved in the aztreonam group by 8.35% compared to 0.55% in the tobramycin group compared to baseline. The mean changes from the baseline across three treatment courses were +2.05% in the aztreonam arm and −0.66% in the tobramycin arm. In CF patients who received tobramycin for ≥84 days in the 12 months prior to randomization, the mean changes at day 28 were +10.04% and +0.54% in the aztreonam and tobramycin arms, respectively (311). These data indicate that aztreonam may be superior to tobramycin inhalation solution; a final analysis of these study data has not yet been reported. Intravenous aztreonam improved FEV_1 by 8.5%, compared to 12.9% in the azlocillin-plus-tobramycin comparator group. Aztreonam reduced the *P. aeruginosa* sputum load by 40.7%, versus 37% in the azlocillin-plus-tobramycin comparator group; MICs remained "fairly constant," although the incidence of resistance to all study drugs increased with therapy (312).

Colistin

The efficacy of colistin following intravenous or aerosolized administration of colistin sulfomethate was reviewed previously

(313–317); since then, several controlled studies have been published, which are summarized below. Hodson et al. (294) compared the efficacy of colistin (80 mg dissolved in 3 ml, b.i.d.) with that of TSI; patients were subgrouped according to age and treated for 4 weeks. Overall, lung function did not improve significantly; the mean FEV_1 improved by 0.37%, compared to 6.7% in the TSI group. The lung function of younger patients deteriorated under colistin treatment by −8.11%, whereas this patient group benefitted most from TSI treatment (11.5% to 14.4%). Patients 13 to 17 years of age showed an improvement of FEV_1 by 6.01%, compared to 14.43% in the TSI group. Both treatments produced almost no improvement in patients aged >18 years (+0.79% versus +1.77%). Overall, colistin treatment reduced the *P. aeruginosa* sputum load by 0.6 $\log_{10}$ CFU/ml (294). In another study, the efficacy of colistin (1,662,500 IU b.i.d.) was compared with that of TSI (300 mg in 5 ml b.i.d.) in three 28-day cycles in patients aged >6 years (308). Mean changes in FEV_1 at week 24 compared to baseline were +0.964% in the colistin group, compared to +0.986% in the TSI group. Changes in the sputum load were not reported (318). Aerosolized colistin treatment of CF patients chronically infected with *P. aeruginosa* caused a deterioration of FEV_1 values by −11%, versus −17% in the placebo group (319).

The impacts of antibiosis on sputum load and microbial susceptibility patterns were evaluated (320) in the course of the comparative trial of inhaled colistin versus TSI (294). In the colistin group, the percentage of isolates with a colistin MIC of ≥4 mg/liter remained at 34%, whereas the percentage of isolates with a tobramycin MIC of ≥4 mg/liter decreased from 27 to 16%. In the TSI group, the percentage of patients carrying isolates with a tobramycin MIC of ≥4 mg/liter increased from 38 to 49%, and the percentage of isolates with a colistin MIC of ≥4 mg/liter remained at 55%. Furthermore, the clinical and bacterial response to TSI and colistin was independent of the MIC at baseline. Neither antimicrobial therapy was associated with infection by *B. cepacia* or other inherently resistant pathogens (320). The author of this study concludes that in agreement with previous studies on the use of aerosolized combination therapy of tobramycin and azlocillin as well as the triple combination of tobramycin plus azlocillin and ticarcillin-clavulanic acid (Timentin), conventional measures of antimicrobial resistance may underestimate the clinical efficacy of tobramycin and colistin when delivered at the high concentrations achieved with the TSI formulation (320–322). These findings are in agreement with the poor predictability of clinical success by conventional susceptibility testing (31, 79, 80, 118–123).

For comparison, intravenous administration of 2 megaunits (MU) of colistin t.i.d. for 12 days resulted in an improvement of FEV_1 values by 9.2%, and therapy of patients with colistin plus another antipseudomonal agent improved lung function by 18.5% (323); in another study performed by this group, an improvement of lung function of 6.9% due to combination therapy was reported (324).

Correlation of Sputum Concentrations to Antipseudomonal Efficacy and Improvement of Lung Function

Comparison of target site concentrations, summarized in Table 3, and antipseudomonal activity and improvement of lung function, summarized in Table 4, following aerosolized administration compared to enteral or parenteral administration presents a puzzling paradox:

- antipseudomonal activities of all the agents studied are independent of their sputum levels,
- antibacterial activity and clinical efficacy are independent of the susceptibility of the isolate and resistance development under therapy, respectively, and
- improvements of FEV_1 values are comparable, except for ciprofloxacin DPI, in patients treated with aerosolized antibacterials and those treated orally or intravenously and thus are independent of the route of administration.

Although historical comparisons of data generated in clinical trials are burdened with several imponderabilities, as mentioned above, intravenously administered aminoglycosides tend to be more efficacious than aerosolized aminoglycosides. Likewise, oral administration of fluoroquinolones seems to be as efficacious as aerosolized therapy. These trends are remarkable insofar as both aminoglycosides and fluoroquinolones exert concentration-dependent *in vitro* activity and efficacy in preclinical infection models (215, 216), so it is tempting to assume that aerosolized therapy should result in markedly ameliorated efficacy. Indeed, within-study analyses reveal that dose-dependent antipseudomonal activities of either levofloxacin (284, 285) or amikacin (170, 171) as well as improvements of lung function were demonstrated although within a narrow range only. Nevertheless, high sputum concentrations of aerosolized aminoglycosides or fluoroquinolones exceeding those following intravenous administration, 26- to 45-fold and 100-fold, respectively, do not translate into more marked antipseudomonal activity or a better improvement of FEV_1 values in CF patients. Reductions of sputum load and improvements of lung function are within the same order of magnitude following either aerosolized and oral or intravenous administration, respectively.

Sputum concentrations of intravenously administered aztreonam never exceed the lowest reported MIC_{50} value of 4 mg/liter, so aztreonam never achieves its PK/PD target following intravenous administration; as demonstrated previously, only isolates with MICs of up to 1 mg/liter and 2 mg/liter, respectively, could be reliably treated with 1 g q.i.d. aztreonam (172). However, it is clinically and antibacterially effective in the treatment of *P. aeruginosa* lung infections of CF patients, although the MICs of aztreonam for the majority of isolates exceed 2 mg/liter. The approximately 65- to 135-fold-higher sputum concentrations following aerosolized administrations result in significantly prolonged exposure of the pathogens to aztreonam. Aerosolized aztreonam meets the PK/PD target but is as efficacious as intravenously administered aztreonam not achieving its PK/PD target.

Colistin is the most active agent against *P. aeruginosa* and is characterized by the most favorable PK/PD attainment rate compared to the fluoroquinolones, aminoglycosides, or aztreonam. Therefore, it should offer the most reliable treatment option. However, it was therapeutically inferior to tobramycin (294).

Although all the studies mentioned above provide evidence for the benefit of aerosolized antibacterials in the treatment of CF patients, none of the studies prove it. Almost all of these studies have been designed as placebo-controlled studies, but only two studies comparing the efficacies of inhaled and systemic antibacterial treatments have been performed. Placebo-controlled studies are adequate, as this design controls for an improved standard of care; in some cases, increases in FEV_1 values may be due to special

care of treated patients, which becomes evident only if a placebo control group is included. Patients in the verum as well as placebo groups will benefit if an improved standard of care is provided. However, this was not observed in any of the studies mentioned in Table 4. However, a placebo-controlled design does not address the question of whether a PK/PD-based rationale for aerosolized therapy, i.e., the link between high drug exposure and clinical or microbiological effect, may translate into the clinical arena. At present, this question cannot be answered because of missing proof.

Previously, two pilot studies comparing inhaled and systemic administration of antibacterials were performed. In the first study, patients 0.5 to 16 years of age with clinically stable disease and early colonization with *P. aeruginosa* were treated intravenously with either ceftazidime and tobramycin for 2 weeks or inhaled tobramycin for 4 weeks; three subjects randomized to the systemic group could not be treated intravenously and were therefore dosed orally with ciprofloxacin plus inhaled tobramycin for 2 weeks (325). The primary outcome was a change in the percentage of neutrophils in lavage fluid. Unfortunately, only a few patients agreed to be enrolled in this trial, so this study was underpowered. Nevertheless, baseline characteristics of patients, such as age, immune status, and disease severity, were well matched in both groups. Fifteen subjects (inhaled = 6; systemic = 9) completed the study. Patients having received systemic treatment showed a modest median change in the percentage of neutrophils (−7%), which was not statistically significant compared to the inhaled group (+5.4%; $P = 0.07$). However, the systemic group had significantly greater reductions in total numbers of cells (−50% versus −3%; $P < 0.01$) and neutrophils (−74% versus −10%; $P = 0.02$) per ml lavage fluid. Reductions of *P. aeruginosa* sputum load after treatment were not significantly different in both groups. Sputum levels of the antibacterials were not analyzed. Patients benefitted most from combined ciprofloxacin-plus-tobramycin treatment. Overall, in clinically stable children with CF, systemic antibacterials caused a greater short-term reduction in lower airway inflammation than inhaled agents (325). However, numbers of patients were too small, so large multicenter studies comparing aerosolized administration to oral or systemic administration in patients of different age groups should be performed. In the second study, aerosolized administration of either gentamicin dry powder or gentamicin solution for inhalation was compared with intravenous gentamicin in a single-dose, triple-crossover design (326). Patients aged >18 years either were dosed with 160 mg gentamicin via a dry powder inhaler or a small-volume nebulizer or received an intravenous dose of 5 mg/kg of body weight. All patients harbored gentamicin-susceptible *P. aeruginosa*. Viable counts were quantitated at baseline and 2 h after administration. *P. aeruginosa* sputum loads were reduced by 0.49 $\log_{10}$ CFU/ml sputum by gentamicin dry powder inhalation, 0.38 $\log_{10}$ CFU/ml sputum by gentamicin solution for inhalation, and 0.46 $\log_{10}$ CFU/ml sputum following intravenous administration. These antipseudomonal effects have to be put into perspective, as dry powder inhalation resulted in a 7-fold-lower sputum concentration than the aerosolized gentamicin solution; sputum concentrations following intravenous administration were 19 and 139 times lower than those after aerosolized administration of the dry powder or the solution for inhalation. Nevertheless, the antipseudomonal short-term efficacies were comparable irrespective of whether high or low sputum concentrations were obtained (326).

This phenomenon is remarkable insofar as, in principle, aminoglycosides exhibit a concentration-dependent effect, which, however, was not observed under these conditions. The authors of the latter study discuss whether aerosolized antibacterials may be redistributed or could diffuse along a gradient from the central airways to peripheral areas of the lung, which are poorly accessible. However, this hypothesis is not supported by clinical findings (326). As the origin of expectorated sputum was not defined, it remains unknown if the antibacterial in the sputum originated from the central or peripheral airways. Therefore, it is questionable whether a correlation of sputum concentrations to improvements in lung function or reductions in bacterial sputum load represents an adequate parameter to describe PK/PD relationships of aerosolized antibacterials.

OPEN QUESTIONS AND CAVEATS

The cornerstones of PK/PD analysis are the MICs of the pathogen and the C_{max} and/or AUC values of the antibacterial agent. As discussed above, MICs are neither representative nor clinically relevant. Serum-derived pharmacokinetic constants are inappropriate as surrogates for clinical efficacy of aerosolized antibacterials; sputum levels correlate poorly with efficacy. Furthermore, pathoadaptive responses of bacteria as well as the selective pressure exerted by antibacterials result in a rapid emergence of highly resistant bacterial populations protecting themselves from attack by host defense mechanisms and antibacterials so that bacteria persist in the CF lung in high numbers. Nevertheless, antibacterial therapy is clinically effective in CF patients. This raises the question of whether the antibacterials studied probably exert additional actions apart from direct interactions with their targets, i.e., penicillin binding proteins, ribosome, and type II topoisomerases, resulting in reductions of the bacterial sputum load.

The following topics, which may contribute to the clinical efficacy of antibacterials, have not yet been analyzed systematically and have not yet been subjected to pharmacodynamic analysis, so an in depth discussion of these topics would be beyond the scope of this review; only those studies that are directly linked to clinical significance are mentioned below.

The CF Inflammasome

In the CF lung, airway epithelial cells have been shown to produce proinflammatory cytokines in response to stimulation by, e.g., *P. aeruginosa*, which activate the CF inflammasome. Inflammasome responses depend on NF-κB signaling, which ultimately leads to the upregulation of specific inflammasome components as well as the expression of proinflammatory cytokines. Almost any class of antibacterial exerts immunomodulatory effects. Macrolides and fluoroquinolones exert their immunomodulatory effects via modulations of transcription factors, such as NF-κB, activator protein 1, NF–interleukin-6, and nuclear factor of activated T cells, as well as cyclic AMP, leading to the downregulation of proinflammatory and upregulation of colony-stimulating factors (327–332). Beta-lactams conjugate to inflammation-promoting cytokines, thus reducing their inflammatory activity (333–335). Aminoglycosides may trigger signaling cascades by disturbing membrane fluidity or by binding specifically with protein kinase C isoforms and phospholipase C (327). Macrolides were shown to exert beneficial clinical effects on CF patients with *P. aeruginosa* lung infection (286, 331, 332), despite their lack of *in vitro* activity against *P. aeruginosa*. Fluoroquinolones are known to modulate

immunoresponses, in particular in preclinical infection models of and in patients with respiratory tract infections (328–330); however, a contribution of immunomodulatory activities of fluoroquinolones to clinical or bacteriological success has not yet been proven. Likewise, the impact of immunomodulatory activities of β-lactams and aminoglycosides on successful treatment of respiratory tract infections has not been studied.

The CF Transmembrane Conductance Regulator Protein

Among the agents targeting the cystic fibrosis transmembrane conductance regulator (CFTR) gene are aminoglycosides; different pharmacological approaches to correct this gene defect were reviewed recently (336, 337). Premature termination codons or nonsense codons, i.e., class I mutations, account for 5% to 10% of all CFTR mutations. Class I CFTR nonsense mutations can be overcome by using agents that affect the interaction of mRNA with the ribosome, thus causing readthrough of premature stop codons during translation of the CFTR gene so that a full-length, intact CFTR protein is synthesized. Since the first demonstration of gentamicin-mediated *in vitro* readthrough (338) and several preclinical studies (336, 337), an initial open-label pilot study followed by a double-blind, placebo-controlled study of 24 patients showed significant improvements of the typical electrophysiological abnormalities caused by CFTR dysfunction. Patients were dosed for 2 weeks intranasally with two drops of a gentamicin solution (3 mg/ml) per nostril three times daily, resulting in a total daily dose of 0.9 mg (339–341). The restoration of CFTR function was also demonstrated following intravenous injection of gentamicin. In one study, patients were dosed initially with 2.5 mg/kg t.i.d. so that peak levels of between 8 and 10 mg/liter and trough levels of <2 mg/liter were achieved; patients were treated for 7 days (342). In another study, gentamicin was given in a dose of 10 mg/kg q.d. for 2 days, and on the third day, dosing was increased, aiming at a peak concentration ranging from 20 to 40 mg/liter and trough levels of <2 mg/liter; patients were treated for 15 days (343). Improvement of CFTR function was recorded for four out of five (342) and for six out of nine (343) patients. These data demonstrate that suppression of premature termination codons with aminoglycosides may be a rational approach to restore CFTR function and may contribute to the clinical efficacy of aminoglycosides.

Signaling and Virulence

It has long been known that antibacterials modulate bacterial cells at low concentrations in a multifaceted manner. On the one hand, antibacterial agents exhibit the phenomenon of hormesis independent of their mode of action and affect transcription at low concentrations (344). On the other hand, bacteria produce low-molecular-weight metabolites, including antibacterial agents that play roles as sensors of environmental stress and as signaling molecules. Most microbial metabolites modulate gene transcription at low concentrations in response to environmental stress, such as changes in bacterial population density; in response to inflammation; or for protection from antibacterial treatment. The primary effect of the signaling molecules is the preservation of growth and population diversity in the environment; i.e., the metabolites act as quorum sensors. Thus, a large number of quorum sensors act as cell signaling molecules that regulate gene expression within as well as between microbial populations and in the host (345–347). The extent of biofilm formation and synthesis of pathogenicity factors correlated with species-specific levels of quorum sensors (348–350). *P. aeruginosa* quorum sensors were isolated from sputa of CF patients (351, 352). It was demonstrated that azithromycin, ceftazidime, and ciprofloxacin at low concentrations inhibited quorum-sensing-regulated synthesis of pathogenicity factors in *P. aeruginosa* (353, 354). Others could not reproduce the effects on quorum-sensing-controlled synthesis of pathogenicity factors; subinhibitory concentrations of tobramycin, norfloxacin, tetracycline, and azithromycin induced biofilm production and upregulated the synthesis of several pathogenicity factors (355, 356). Higher concentrations of azithromycin close to the MIC reduced the production of several virulence factors of *P. aeruginosa* (356). These discrepant findings indicate that the effects of antibacterials on quorum-sensing-controlled virulence are due to pleiotropic effects of the agents on bacterial sensing and communication systems. An important clinical aspect, however, is that administration of these agents improves respiratory function in CF patients, even if either *P. aeruginosa* is intrinsically resistant to macrolides or highly resistant subpopulations are selected rapidly by aminoglycosides or fluoroquinolones.

An interesting and clinically relevant placebo-controlled trial investigating the anti-quorum-sensing properties of azithromycin in intubated patients colonized by *P. aeruginosa* was performed recently (357). Those authors hypothesized that quorum-sensing-negative and, hence, less virulent and less fit mutants benefit during an infection-free interval from quorum-sensing-controlled production of pathogenicity factors. During growth in the hostile environment of the lung, quorum-sensing-negative mutants of *P. aeruginosa* benefit from the exoproducts provided by wild-type isolates so that the total sputum density of *P. aeruginosa* increases, thus providing relative protection from colonization by other microorganisms. Furthermore, those authors hypothesized that inhibition of quorum-sensing-mediated gene expression removes this growth advantage of less virulent and less fit quorum-sensing-negative mutants so that more virulent quorum-sensing-positive wild-type bacteria would predominate. Azithromycin administration (300 mg/day) significantly reduced quorum-sensing-controlled gene expression measured directly in tracheal aspirates. Concomitantly, the advantage of quorum-sensing-negative mutants was lost, and virulent wild-type isolates predominated during azithromycin treatment.

These data generated in a clinical trial were confirmed *in vitro*. The authors of this study conclude that these *in vivo* and *in vitro* results demonstrate that antivirulence interventions based on a quorum sensing blockade diminish natural selection toward reduced virulence and therefore may increase the evolution of highly virulent bacteria. Furthermore, this study confirms that azithromycin interferes with quorum sensing under clinically relevant conditions (357). This study, taken together with the discrepant findings described above, also indicates that quorum sensing inhibition as a novel target for antibacterial therapy is a very complex strategy.

The CF Microbiome

The microbial ecology of CF is not only changing but also becoming increasingly complex, as demonstrated by a combination of culture-dependent and culture-independent approaches (52–58). Furthermore, CF-associated microbial communities are not only complex but also diverse in anatomically distinct regions of the CF lung (358). Very few bacterial pathogens, such as *S. aureus* and *H.*

influenzae initially and *P. aeruginosa* thereafter, are the prevalent species, and several others, such as *B. cepacia*, *S. maltophilia*, and *A. xylosoxidans*, are considered opportunistic pathogens. However, other organisms missed by conventional microbiology also contribute to disease (359–365). Of a total of 170 bacterial genera identified so far in the CF microbiome, 12 accounted for >90% of the total bacterial load (366). These species may contribute significantly to the vigorous immune response triggered by the presence of bacteria in CF patients. Moreover, signaling cascades within the entire CF microbiome and not only between well-established CF pathogens may stimulate both positive- and negative-feedback mechanisms, which determine the frequency and severity of CF infective exacerbations (359–365). For example, it was demonstrated in experimental animals that the presence of avirulent, oropharyngeal flora significantly increased the pathogenicity of *P. aeruginosa* (345). Another example is the need for antibiotic therapy of the *Streptococcus milleri* group as a new pathogen in the CF lung in order to prevent a loss of lung function and reduce the frequency of exacerbations (367). The actual culture-based techniques to identify causative pathogens of CF lung disease are inadequate to identify microbial community richness. As CF lung infections are not only polymicrobial but also confined in space, treatment regimens may be ineffective against several bacterial species and in some areas of the lung. Conversely, actual antibacterial regimens may affect the complex microbiome in an unknown way, either with interactions with viable counts of several bacterial species or with interspecies interactions, which could hypothetically result in a clinical benefit despite a negligible effect on the predominant culturable species. Studies on the antibacterial effects of aerosolized agents should not only quantitate shifts in viable counts compared to baseline but also integrate CF microbiome composition and function with therapy. The challenge in the future is to study the complex interactions between microbial consortia and the patient as well as the impact of antibacterial therapy on this polyfactorial interplay. A recent review has addressed this topic by discussing first whether novel diagnostic strategies can be used to better define the CF microbiome; second whether the new strategies can be applied to characterize, without subculture, the longitudinal impact of antimicrobial therapy in order to assess treatment efficacy and to optimize antibiotic regimens; and third whether biomarkers that are predictive of health status, disease progression, or response to therapy can be indentified (368).

The open question, therefore, is if we ask the right questions.

There is strong evidence that these four topics have significant relevance for disease progression as well as treatment. Thus, the CF inflammasome, CFTR, CF microbiome, and quorum sensing represent targets for antibacterials, in addition to the bacterial species considered so far to represent the pathogen. Studies that link microbiome composition and function, CFTR function, quorum sensing, virulence expression, as well as immunology with efficacy endpoints and the host response should be designed. Such a system-based approach would generate several main outcome measures. In contrast, the main outcome measures of traditionally designed studies are clinical response and microbiological eradication of the pathogen. The classical microbe-outcome association implies monocausality based on Koch's postulate that one microbe infects an otherwise sterile tissue or organ so that its presence at the site of infection indicates pathogenicity. However, an association of complex microbiomes with several chronic diseases, in particular CF, has been reported, thus necessitating a multifactorial outcome analysis. As all the outcome measures generated in a system-based analysis of CF treatment are quantifiable, they can be linked to each other, and their correlation to pharmacokinetics and clinical efficacy can be analyzed.

CONCLUSIONS

New formulations of old antibacterials have been demonstrated to be efficacious, but proof that high target site concentrations following aerosolized administration compared to oral or intravenous administration translate into greater clinical and/or bacteriological efficacy is missing. The respiratory tract of CF patients represents a complex local microbiome in which many different bacterial species either coexist or compete with each other. Longitudinal, multistaged changes in the CF microbiome are associated with a deterioration of lung function; within-patient pathoadaptive responses select for resistant genotypes and phenotypes even in the absence of antibacterials. Therefore, total viable counts of CF pathogens and their susceptibility patterns are probably misleading markers of infection or exacerbation and not helpful as guides for therapy decisions. Furthermore, the use of conventional PK/PD surrogates correlating pharmacokinetics in serum with clinical cure and presumed or proven eradication of the pathogen as a basis for PK/PD investigations in CF patients is irrelevant, as the minimization of systemic exposure but optimization of target-site-specific exposure is one of the main objectives of aerosolized therapy. Thus, disease-specific PK/PD surrogates should be derived from adequately designed studies. Susceptibility data that are representative of the entire bacterial population should be determined, and the relevant matrix for PK analysis as well as the PK/PD target to be met should be defined. Other patient populations with chronic lung infections may benefit from such efforts, as aerosolized therapies are currently being examined, e.g., for the treatment of chronic obstructive pulmonary disease (COPD).

Although it is not precisely known at present if CF patients benefit from aerosolized therapy more than from oral or intravenous therapy, it is nonetheless obvious that systemic exposure of patients and, thus, the risk of systemic adverse reactions as well as resistance acquisition by the resident flora are minimized. Aerosolized therapy may reduce the burden of treatment for CF patients.

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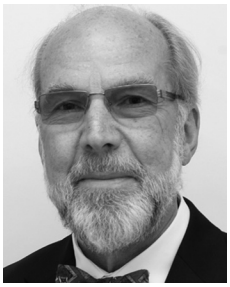

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