Once-daily tobramycin in cystic fibrosis: better for clinical outcome than thrice-daily tobramycin but more resistance development?

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Received 10 November 2005; returned 11 May 2006; revised 5 July 2006; accepted 15 July 2006

Objectives: Once-daily administration of aminoglycosides in cystic fibrosis (CF) patients is considered equally efficacious and potentially less nephrotoxic than dosing three times a day. However, the choice of the most suitable PK/PD index (Cmax/MIC versus AUC24/MIC) to ensure optimum clinical outcome in this patient population is not clear.

Patients and methods: In a single-centre, open, randomized, controlled, non-blinded study 33 adult CF patients (20 females, 19–37 years) were treated with intravenous tobramycin (10 mg/kg/day) for 14 days given either as single dose once a day (Q24; 17 patients) or divided into three equal doses every 8 h (Q8; 16 patients). Tobramycin serum concentrations and MICs for Pseudomonas aeruginosa were determined on days 1 and 14. The clinical outcome parameter, correlated to PK/PD indices, was the percentage predicted forced expiratory volume in 1 s (FEV1% pred.).

Results: FEV1% pred. improved significantly for both treatments. There was a log-linear relationship between Cmax/MIC and FEV1% pred. and AUC/MIC and FEV1% pred. for both treatments. For equal values of AUC24/MIC, however, Q24 treatment provided better improvement in lung function than Q8 dosing, whereas Cmax/MIC did not show any dosing interval dependence. A statistically significant increase was observed for MIC (day 1) versus MIC (day 14) for Q24 treatment, however, no such difference was observed for Q8 treatment.

Conclusions: The most important PK/PD parameter for clinical outcome in CF patients was Cmax/MIC. Outcome prediction of AUC24/MIC was dependent on the regimen. The increase of P. aeruginosa resistance after once-daily administration is linked to a long dosing interval. More and larger studies are needed to optimize the dosing regimen for maximum clinical outcome with minimum resistance development.

Keywords: PK/PD, lung function, Pseudomonas aeruginosa

Introduction

Patients with chronic lung diseases such as cystic fibrosis (CF) are frequently colonized with multidrug-resistant bacteria such as Pseudomonas aeruginosa.1 The consequences are a chronic pulmonary infection and recurrent acute exacerbations caused by the bacterium. Accordingly, CF patients receive repeated and prolonged cycles of so-called ‘Pseudomonas-effective’ antibiotics.2-3 Aminoglycosides are worldwide frequently used antibacterial drugs in the treatment of P. aeruginosa infections.4 They generate a higher rate and extent of bacterial killing with increasing concentrations, which is referred to as concentration-dependent activity.5,6 They also exhibit a significant post-antibiotic effect, which is characterized by an effect that persists for some time after the concentrations have fallen below the MIC of the bacteria.7 For aminoglycosides the ideal dosing regimen would maximize concentration of the antibiotic, namely the peak of plasma concentration (Cmax) and the exposure (AUC), because the higher the concentration, the more effective and the faster is the degree of bacterial killing.5,6,8,9 Once-daily administration of the total daily dose has been proposed to best realize this concept.10 Clinical studies in patients without CF have demonstrated equal

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clinical efficacy and equal or better aminoglycoside tolerability after once-daily dosing compared with the traditional regimens, i.e. administration in two to three divided doses per day by intermittent infusion. In CF patients the largest clinical study previously published confirms these results. Another possibility to evaluate clinical efficacy and to optimize dosing regimens of antibiotics are the so-called ‘MIC-based PK/PD indices’. Based on the two bacterial killing patterns (‘concentration-dependent’ and ‘time-dependent’ killing) three different PK/PD indices have been developed to evaluate efficacy and to optimize dosing regimens of antibiotics, the time above the MIC (t > MIC), the peak of plasma concentration to MIC ratio (C_{max}/MIC) and the area under the concentration during a 24 h interval versus time curve to MIC ratio (AUC_{24}/MIC). For instance, AUC_{24}/MIC has been used successfully to evaluate the efficacy of fluoroquinolones and the efficacy of β-lactam antibiotics is primarily related to t > MIC. On the other hand, for aminoglycosides the best predictive PK/PD parameter is much disputed. Early in vitro studies showed that C_{max}/MIC is the most important PK/PD index explaining efficacy and in clinical trials it has been shown that the target is a C_{max}/MIC ratio of at least 10–12 in order to maximize clinical response. However, in a retrospective study with unselected hospitalized patients higher AUC/MIC ratios correlated to a better clinical outcome. Only one study of the pharmacodynamics of tobramycin in a small population of 13 patients with CF exists. There, the results showed a clear relationship between all three known PK/PD indices and clinical efficacy, with the highest predictive value shown for AUC/MIC. However, only one dosing regimen was investigated and the interdependence of the PK/PD indices may prevent a differentiation. Therefore, the most suitable PK/PD index to ensure optimum clinical outcome in CF patients is still not clear. Another problem concerns the development or increase of P. aeruginosa resistance in chronically infected CF patients after repeated treatments with aminoglycosides. In previously published studies the emergence of resistant P. aeruginosa isolates before and after treatment was not evaluated. Apart from the comparison of the clinical efficacy, the main purpose of our study was to evaluate prospectively a possible correlation between the PK/PD parameters and clinical outcome of CF patients after once- versus thrice-daily tobramycin administration. In addition we investigated the microbiological changes in tobramycin susceptibility in both treatment arms.

Patients and methods

Patients

A total of 33 adult CF patients (20 females, 19–37 years), in our institution regularly treated with intravenous tobramycin in combination with a β-lactam antibiotic for chronic pulmonary infection with P. aeruginosa, were included in the trial. Patients were excluded if they had pre-existing renal insufficiency or hearing impairment (>20 dB hearing level at any two frequencies between 2 and 8 kHz on the standard audiogram). Patients were not enrolled if they had a history of allergies to aminoglycosides or β-lactam antibiotics. Female patients were offered a pregnancy test before enrolment and excluded if test results were positive. Inhaled antibiotics such as tobramycin or colistin were maintained during the study period. The study was approved by the local Ethics Committee. All patients were given a detailed description of the study, and their written consent was obtained. The study was performed in accordance with the Declaration of Helsinki and the Good Clinical Practice Guideline of the European Commission.

Study design and treatment assignment

The study was a single-centre, open-label, randomized, controlled, non-blinded study. Patients were randomly assigned to receive 10 mg/kg tobramycin in 100 mL 0.9% physiological saline administered either once a day (Q24; 17 patients) or divided in three doses every 8 h (Q8; 16 patients) by 30 min infusion for 14 days. Because peak and trough concentrations of tobramycin were not outside of target range (trough level: ≤1 mg/L (Q24) and ≤2 mg/L (Q8); peak level: 20–40 mg/L (Q24) and 5–20 mg/L (Q8)) a dose adjustment was not necessary. In 17 (51.5%; Q24: 9 and Q8: 8) cases tobramycin was given in combination with meropenem at a dose of 50 mg/kg three times a day and in 16 (48.5%; Q24: 8 and Q8: 8) cases in combination with ceftazidime (200 mg/kg three times a day).

Study procedures and clinical outcome parameters

Baseline examination included a complete physical examination, a body plethysmography, an audiometric investigation, and an extensive laboratory profile for evaluation of renal and liver functions as well as measurement of the inflammation parameters in blood. Creatinine clearance was calculated according to the Cockcroft and Gault’s formula. All these tests were repeated at the end of therapy. The primary outcome parameters for clinical efficacy were the proportional improvement in forced expiratory volume in 1 s (FEV1 % pred.) and maximal inspired vital capacity (VC_{max} % pred.), expressed as a percentage of the predicted normal values for age, sex, and height, during 14 days of treatment. Secondary outcome parameters were the change in C-reactive protein, blood leucocyte count and IgG.

Pharmacokinetic analysis

For Q8 regimen blood samples were collected before and 0.5, 1, 2, 6 and 8 h after end of the first infusion and 8 h after the third infusion. For Q24 regimen blood samples were taken before and 0.5, 1, 2, 8, 12 and 24 h after end of the first infusion. The last plasma sample for both treatment groups was taken on day 14 before the last administration. All tobramycin concentrations in plasma were measured with an automated fluorescence polarization immunoassay (TDx, Abbott Laboratories, Park, IL, USA). Population kinetic analysis was performed for all 33 patients using NONMEM (Version V, Globomax). Based on an initial examination of the tobramycin time–concentration curves, potential pharmacokinetic models considered were 1- and 2-compartment models. For the 2-compartment model, parameterization with macro constants B, C, β and γ (Advan 3 Trans 5 subroutine) was used. Interindividual variability in model parameters (e.g. β) was modelled using an exponential error model as follows:

\[ \beta_j = TV\beta \ast \text{Exp}(\eta\beta) \]

where \( \beta_j \) is the hypothetical true macro rate constant for the jth individual as predicted by the regression model, TV\( \beta \) is the typical population value for the macro constant and \( \eta\beta \) represents the deviation of the jth individual’s macro constant and that predicted by the regression model. \( \eta\beta \) is assumed to be an independent, identically distributed, normal random variable with a zero mean and variance \( \sigma^2 \). First order (FO) method was used throughout the analysis. For residual error or within subject variability model (WSV), constant coefficient of variation (CCV) or a combination of additive and CCV
error model was used. Generalized additive modelling (GAM) as implemented in XPOSE was used to screen for covariates that significantly related to the pharmacokinetic parameters. Information criteria (AIC) were used for model selection. At each step, the model is changed by addition or deletion of the covariate that resulted in largest decrease in AIC. The search stops when the AIC has reached a minimum value. The covariates screened from GAM were then subjected to univariate analysis. Likelihood ratio test was performed and covariates that resulted in a drop of 6.63 $U (P < 0.01, df = 1, \chi^2$ test) in objective function were included to build the full model. Stepwise backward deletion of the covariates from the full model was performed and deletions ($P < 0.001, df = 1$, increase in objective function = 10.83) determined the final model. FO method was used for the analysis. This conservative approach ensured that only the most meaningful covariates entered the model. Diagnostic scatter plots were used to evaluate the goodness of fit throughout the model-building procedure. The area under the concentration–time curve (AUC$_{ss-24}$) over 24 h in steady-state was calculated for each subject from the PK parameters obtained by population analysis.

\[
\text{AUC}_{ss-24} = \frac{B}{\beta} + \frac{C}{\gamma} \cdot \ln(x)
\]

**Microbiology**

Microbiological cultures and antibiotic susceptibility testing were performed before start of the treatment and at the end of the study. From all patients sputum samples were obtained and inoculated into Columbia Blood and MacConkey agar plates (Difco, Detroit, MI, USA) and screened after 24 and 48 h of incubation for the presence of *P. aeruginosa* strains and other pathogens. MICs from recovered pathogens were determined using standard CLSI microtiter MIC methods. In case of different *P. aeruginosa* strains in the sputum sample the MIC of the most resistant strain was used for the evaluation.

**PK/PD investigations**

The individual $C_{\text{max}}$ and AUC$_{24}$ values were correlated with microbiological susceptibility data (MIC) of the least susceptible *P. aeruginosa* strain on day 1 as well as the following clinical outcome parameters: (i) FEV$_1$ % pred. on day 14 and (ii) change in inflammatory parameters (C-reactive protein, blood leucocyte count and IgG). For PK/PD correlation, FEV$_1$ [%] versus $C_{\text{max}}$/MIC and FEV$_1$ [%] versus AUC$_{24}$/MIC were fitted using a log linear model with the following equation:

\[
Y = Y_0 + a \cdot \ln(x)
\]

For some patients, an improvement in FEV1 was observed even for low $C_{\text{max}}$/MIC and AUC$_{24}$/MIC ratio, which cannot be attributed to drug treatment. Therefore, data for patients with $C_{\text{max}}$/MIC < 2 and FEV1 $\geq$ 40 and AUC$_{24}$/MIC < 20 and FEV1 $\geq$ 40 were excluded for model fitting.

**Statistical analysis**

For all variables, statistical tests were performed using GraphPad PRISM (version 4.00). At baseline, the two treatment groups were compared using Mann–Whitney two-tailed test. Statistical analysis of the mean changes in clinical outcome from baseline (day 1 before start of therapy) to day 14 between Q8 and Q24 regimens was performed with Mann–Whitney two-tailed test. Statistical analysis for difference in MICs from day 1 to day 14 for each regimen was performed using Wilcoxon signed-rank test. In all tests, $P < 0.05$ was regarded as significant.
between 0.5 and 32 mg/L. At day 14 the total number of patients with tobramycin MICs ≥16 mg/L as resistance breakpoint increased from 1 to 5 for the Q24 regimen and from 2 to 3 for the Q8 treatment. The number of patients with general increase in MIC for Q8 and Q24 were 6 and 8, respectively. MIC values significantly increased during treatment for Q24 by an average of 6.8 mg/L (Wilcoxon signed-rank test, \( P = 0.034 \)), whereas they remained unchanged for Q8 (\( \Delta \text{MIC} = 0.6 \text{mg/L} \)) (Figure 1a and b). Before and after the end of treatment all Pseudomonas isolates were susceptible to meropenem or ceftazidime, i.e. no resistance development for the used \( \beta \)-lactam antibiotics was observed. Isolation of fungi or potential bacterial pathogens such as Staphylococcus aureus and Stenotrophomonas maltophilia in sputum samples of patients did not increase after 14 days of therapy.

**Population pharmacokinetics**

A two-compartment disposition model best described the population observations. This was evident from a drop in the objective function value by 86 U and improvement in diagnostic plots. The initial short distribution phase for aminoglycosides (alpha) could not be characterized for the present tobramycin observations. Therefore, the two-compartmental model actually describes the beta and gamma phases of a three-compartment model of aminoglycosides.\(^{34} \) The estimate for the half-life of the late gamma phase (return from the deep compartment) was found to be 23 h, because only 10% of gamma phase were seized by blood collecting procedure. Therefore, the representative parameter in the model (macro rate constant) was fixed so as to achieve a \( \gamma \) half-life of 100 h.\(^{35,36} \) The covariate analysis indicated only body weight to have a significant effect in explaining between-subject variability for the \( \gamma \) half-life. However, it bears no significance for interpretation as the estimate for gamma half-life was fixed. The residual error was best described using an additive and CCV in the final model. Concentration–time profiles of the individual observations and population-predicted profiles for Q8 and Q24 treatment regimens are shown in Figures 2 and 3. The diagnostic plots and final estimated parameters for tobramycin are shown in Figure 4 and Table 3, respectively. Combination therapy with ceftazidime or meropenem did not affect the pharmacokinetics of tobramycin.

**Table 2.** Median (range) change in lung function (in percent) and inflammatory parameters after 14 days treatment with tobramycin once-daily (Q24) versus thrice-daily (Q8) administration

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Q24 (( n = 17 ))</th>
<th>Q8 (( n = 16 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV1% pred. (% )</td>
<td>11.3 (–13.7–50.0)</td>
<td>7.4 (–14.7–37.2)</td>
</tr>
<tr>
<td>IVC% pred. (% )</td>
<td>3.1 (–6.3–54.0)</td>
<td>7.9 (–2.6–28.6)</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>–42.9 (–87.1–237.5)</td>
<td>–8.5 (–1.0–16.0)</td>
</tr>
<tr>
<td>Leucocytes (( \times 10^9/\mu \text{L} ))</td>
<td>–2.5 (–0.9–5.6)</td>
<td>–2.6 (–7.9–1.3)</td>
</tr>
<tr>
<td>IgG (g/L)</td>
<td>–1.4 (–6.9–1.0)</td>
<td>–1.8 (–27.0–1.0)</td>
</tr>
</tbody>
</table>

FEV1% pred., proportional improvement in predicted forced expiratory volume in 1 s; IVC % pred., proportional improvement in predicted inspiratory vital capacity.

No statistical significant differences between the two study groups (two-sided Mann–Whitney test, \( P > 0.05 \)).

**PK/PD investigations**

To quantify the relationship between PK/PD indices of tobramycin and clinical efficacy, a log-linear model was fit to the data.
There was a good log-linear relationship between AUC$_{24}$/MIC and $C_{\text{max}}$/MIC versus FEV$_1$% pred. on day 14 for both treatment regimens (Figure 5). It was observed that for equal values of AUC$_{24}$/MIC, Q24 treatment provided better improvement in lung function than Q8 dosing, whereas $C_{\text{max}}$/MIC did not show any dosing interval dependence. No correlation was found between PK/PD parameters and change in inflammatory parameters (C-reactive protein, blood leucocyte count and IgG).

**Discussion**

The quality of life and life expectancy of CF patients have improved considerably as a result of the control of bronchopulmonary bacterial colonization and acute infectious exacerbations. P. aeruginosa is the major prognostic factor in chronic pulmonary infection of CF patients, because the negative effect of the bacterium on pulmonary function is well known.38–40 P. aeruginosa can only be eradicated in the early stage of infection.

**Table 3.** Estimates of population parameters for tobramycin in CF patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Population estimate (% SE)</th>
<th>BSV (% SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta$ (h$^{-1}$)</td>
<td>0.428 (13.20)</td>
<td>18.50% (35.20)</td>
</tr>
<tr>
<td>$t_{1/2}\beta$ (h)</td>
<td>1.70</td>
<td>–</td>
</tr>
<tr>
<td>$\gamma$ (h$^{-1}$)</td>
<td>0.00693 (fixed)</td>
<td>34.64% (66.60)</td>
</tr>
<tr>
<td>$t_{1/2}\gamma$ (h)</td>
<td>100 (fixed)</td>
<td>–</td>
</tr>
<tr>
<td>$\omega t$ (kg$^{-1}$)</td>
<td>3.44 (40.70)</td>
<td>–</td>
</tr>
<tr>
<td>B/C</td>
<td>44.00 (34.80)</td>
<td>NE</td>
</tr>
<tr>
<td>V$_1$ (L)</td>
<td>15.70 (8.90)</td>
<td>25.20% (28.20)</td>
</tr>
<tr>
<td>Residual variability</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportional error</td>
<td>16.70% (68.20)</td>
<td></td>
</tr>
<tr>
<td>Additive error</td>
<td>0.22 µg/mL (44.80)</td>
<td></td>
</tr>
</tbody>
</table>

BSV, between subject variability; V$_1$, volume of distribution in the central compartment; NE, not estimated.

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**Figure 3.** Population-predicted and individual-observed concentration–time profile for tobramycin following Q8 regimen.

**Figure 4.** Diagnostic plots for population pharmacokinetic analysis. (a) Population-predicted concentration versus observed concentration. (b) Individual-predicted concentrations versus observed concentration. (c) Weighted residuals versus population-predicted concentration. (d) Weighted residuals versus time post-dose.
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Figure 5. Relationships between $C_{\text{max}}$/MIC (Q8: $r^2 = 0.17$, Q24: $r^2 = 0.31$) or AUC$_{24}$/MIC (Q8: $r^2 = 0.63$, Q24: $r^2 = 0.62$) and FEV$_1$ % pred. (Day 14) for Q8 and Q24 dosing regimens.

colonization, while reduction of bacterial density is desirable during chronic colonization. To reduce bacterial density and prevent acute exacerbations, both connected with a degradation of lung function, many CF centres worldwide treat patients chronically infected with the pathogen with intermittent courses of intravenous antipseudomonal antibiotics, i.e. tobramycin at fixed time, e.g. 4 times per year for 2–3 weeks, with good clinical results.

Several unique characteristics of aminoglycosides make once-daily dosing an attractive and possibly superior alternative to multiple-daily dosing. These features include concentration-dependent bactericidal activity, post-antibiotic effect, decreased risk of adaptive resistance, and diminished accumulation in renal tubules and the inner ear. Clinical studies in patients without CF have demonstrated equal clinical efficacy and equal or better tolerability after once-daily dosing compared with the traditional regimens.

In our population of adult CF patients tobramycin therapy in combination with a β-lactam antibiotic was highly effective with a significant improvement of all investigated clinical outcome parameters. We have also shown that once-daily tobramycin has at least equivalent clinical efficacy to three-times daily treatment. These results are absolutely comparable to those observed in other clinical trials with CF patients (children and adults). The same is also true for the safety of treatment with tobramycin once a day. In our study no nephro- or ototoxic events were detected. Therefore, we can conclude an at least equally good tolerability of once-daily administration compared with the traditionally thrice-daily dosing.

Another approach to determine the optimal dosing regimen is the use of 'MIC-based PK/PD indices' for correlation with clinical outcome. It is well known that aminoglycosides exert their killing effect in a concentration-dependent manner and for antibiotics with this kill pattern two different PK/PD indices ($C_{\text{max}}$/MIC versus AUC$_{24}$/MIC) exist. However, there has been no agreement which of these two indices is more appropriate.

In our comparative study with two tobramycin treatment regimens we found reasonable correlations between $C_{\text{max}}$/MIC and AUC$_{24}$/MIC versus lung function of CF patients in both patient groups. However, predictions based on AUC$_{24}$/MIC were dependent on the dosing regimen (Q24 > Q8). For the same AUC$_{24}$/MIC, the once-daily treatment with higher $C_{\text{max}}$ values consistently performed better. From this, it could be concluded that the most important PK/PD parameter for prediction of clinical outcome in CF patients is the $C_{\text{max}}$/MIC ratio. Hence, once-daily administration of total tobramycin daily dose is better for the therapeutic success in CF patients with chronic P. aeruginosa infections, compared with traditional thrice-daily dosing. In another recently published study in the same patient collective, all three known PK/PD parameters ($C_{\text{max}}$/MIC, AUC$_{24}$/MIC, $t >$ MIC) correlated significantly with clinical efficacy (increase in FEV$_1$ and FVC).

The correlation was highest for AUC$_{24}$/MIC. However, from in vitro and animal studies, it is well known that $t >$ MIC is not a good predictor of aminoglycoside activity. The reason for the observed correlation between $t >$ MIC and FEV$_1$ found in the present study is the interdependence of the PK/PD indices when only one dosing regimen is studied. Therefore, also the other results of the study have to be interpreted with this limitation in mind.

In our study no correlation was found between $C_{\text{max}}$/MIC or AUC$_{24}$/MIC and change in other outcome parameters such as C-reactive protein and leucocyte count. These outcomes may be influenced by several factors, for example the progression of the disease. In CF patients, the primary endpoints for therapeutical interventions are well defined. The FEV$_1$, the exacerbation rate and the quality of life have been used successfully in various clinical trials.

Another potential interference is the influence of additionally given β-lactam antibiotics on the observed PK/PD relationships. In the present study, assignment of patients to the co-administered combination drugs (ceftazidime or meropenem) was equal and combination therapy did not affect the pharmacokinetics of tobramycin. Therefore, combination therapy did not seem to have any effect on observed PK/PD relationships.

Population pharmacokinetic parameters of tobramycin found in the present study were similar to previously published data in CF patients. A two-compartment disposition model best described the population observations. Among the estimated parameters, the volume of distribution (0.31 L/kg) was slightly higher than that reported for aminoglycosides (0.25 L/kg).

The estimated β half-life of 1.7 h is in agreement with previously reported values in CF patients. A surprising finding in our study was the significant resistance increase of P. aeruginosa after once-daily tobramycin administration, whereas MICs remained unchanged for thrice-daily dosing. These results are important because it is well known that patients who carry a multidrug-resistant strain of P. aeruginosa have a worse prognosis than those with susceptible strains of the bacterium. In general, decreased susceptibility of P. aeruginosa to antibiotics is a common consequence of repeated courses of antimicrobial therapy in chronically infected CF patients. A study by Mouton et al. showed that the long-term administration of antipseudomonal antibiotics to CF patients who are chronically colonized with the bacterium is associated with the
development of resistance to fluoroquinolones, aminoglycosides and β-lactam antibiotics. Another study confirmed these results. These authors observed increases of *P. aeruginosa* MICs especially against aminoglycosides and fluoroquinolones over a limited period of time. In other previously published clinical trials in CF patients for comparison of once- versus thrice-daily administration of tobramycin, the emergence of resistant *P. aeruginosa* isolates before and after treatment was not evaluated. Therefore, the effect of the two regimens on *P. aeruginosa* eradication and resistance development is unknown. The question arises: which is the best explanation for a higher resistance increase after once-daily dosing compared with administration thrice daily? Tobramycin resistance is often described as a transient adaptive resistance and characterized by a temporary down-regulation of drug uptake into the bacteria. However, our observation is in contradiction of the general opinion that once-daily dosing of aminoglycosides reduces adaptive resistance because longer dosing intervals are necessary for adaptive resistance to resolve. On the other hand, elimination of tobramycin in CF patients is rapid, resulting in a significant portion of the dosing interval with very low to undetectable serum concentrations that greatly exceed the reported post-antibiotic effect. This is the main reason for selection of multidrug-resistant bacteria. Another problem in chronically infected CF patients is the development of the so-called ‘biofilms’. These biofilms resist antibiotic treatment and contribute to bacterial persistence in chronic infections. A previously published study showed that subinhibitory concentrations of aminoglycoside antibiotics induce biofilm formation in *P. aeruginosa*. Here, the newly discovered ‘aminoglycoside response regulator’ gene (*arr* gene) was essential for this induction and contributed to biofilm-specific aminoglycoside resistance. Therefore, the increase of *P. aeruginosa* resistance after once-daily administration may be linked to a long dosing interval. A compromise for the prevention of *P. aeruginosa* resistance in CF patients may be the administration of aminoglycosides in two divided doses per day.

In conclusion, our study documents that the clinical outcome in CF patients after once- or thrice-daily dosing tobramycin is comparable, as has been shown in other efficacy studies. The most important PK/PD parameter for prediction of clinical outcome in CF patients is the $C_{\text{peak}}$/MIC ratio. Predictions based on AUC$_{24}$/MIC were dependent on the dosing regimen, indicating a better outcome for a once-daily dosing regimen. The increase of *P. aeruginosa* resistance after once-daily administration is linked to a long dosing interval. However, our study is limited in sample size. More and larger studies are needed to optimize the dosing regimen for maximum clinical outcome with minimum resistance development.

**Acknowledgements**

This work was presented in part at the Thirty-fourth Annual Meeting of the American College of Clinical Pharmacology, Rockville, MD, 11–13 September 2005, and at the Forty-fifth Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, DC, 16–19 December 2005. The study was an investigator-initiated trial. Each author disclosed all pertinent involvement in any organization with a direct financial interest in the subject of the manuscript.

**Transparency declarations**

None to declare.

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