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# INHALATION OF TOBRAMYCIN IN PATIENTS WITH CYSTIC FIBROSIS: COMPARISON OF TWO METHODS

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Inhalant tobramycin is established in the treatment of cystic fibrosis patients. Conventional nebulizers require a large amount of the expensive compound, because only a small fraction is deposited in the targeted lung region. In contrast, techniques based on controlled inhalation allow a high and reproducible deposition of the drug in specific lung regions. In our study we compared the efficiency of two techniques based on conventional and controlled inhalation in 16 cystic fibrosis patients aged 13-39 years. Inhalations with the doses of tobramycin of 300 mg and 150 mg were performed twice daily for three days. The efficiency of the drug deposition was measured by the determination of its serum concentration 1 h after the end of the inhalation. The mean $FEV_1$ value in our patients was 61% of predicted, range 36%-116%. There were no differences in tobramycin serum concentrations among the three study days in both methods (controlled inhalation: 0.983 ±0.381(±SD) mg/l, 1.119 ±0.448 mg/l, 1.194 ±0.568 mg/l; conventional inhalation: 1.075 ±0.798 mg/l, 1.294 ±0.839 mg/l and 1.269 ±0.767 mg/l, on Day 1, Day 2, and Day 3, respectively). Even though the drug amount was double in the conventional technique, there was no significant difference in its overall serum concentration from the three study days (conventional inhalation: 1.210 ±0.783 mg/l, controlled inhalation: 1.092 ±0.461 mg/l). In addition, the coefficient of variation and the required inhalation time were shorter in controlled inhalation than in conventional inhalation (42% *vs*. 65% and 7-8 min *vs*. 20 min, respectively). Our data suggest that controlled inhalation can significantly reduce the amount of a drug required for therapy, the inhalation time required for drug deposition, and the variability of pulmonary dosage. It seems probable that controlled inhalation can improve the antibiotic prevention of pulmonary infection.

K e y   w o r d s: *aerosol, cystic fibrosis, inhalation therapy, tobramycin*

INTRODUCTION

Patients with cystic fibrosis (CF) typically suffer from dyscrinia with increased viscosity of their bronchial secretions (1, 2). They develop chronic, recurrent pulmonary infections in early childhood (2, 3). Colonization of the respiratory tract varies as a function of age in these patients. At an early age, *Staphylococcus aureus* and *Haemophilus influenzae* can be detected (3), and *Pseudomonas aeruginosa* comes in later on. The frequency of samples positive for *P. aeruginosa* increases up to 80% after the age of 5 (3-5). A number of studies have shown that infections with *P. aeruginosa* play a crucial role for the further course and the long-time prognosis of the pulmonary disease in CF patients (3,4). Early pulmonary infections of the patients are caused by environmental strains because the isolated strains of *P. aeruginosa* show a high genetic variability and a good susceptibility in antibiotic treatment (3, 4, 6). In contrast, *P. aeruginosa* strains in chronic infections are characterized by a mucoid phenotype (up to 60 %) which is resistant to antibiotic treatment (5-9). Frequent exacerbations of pulmonary infections are caused by pulmonary reinfections and not by infections from the environment (10). At a later stage of the disease, the respiratory tract is colonized by other bacteria (e.g., *Burkholderia cepacia*, *Stenotrophomonas maltophilia* and *Achromobacter xylosoxidans*) and fungi (e.g., *Candida ssp.*, *Aspergillus ssp.* (frequently: *A. fumigatus*), *Scedosporium apiospermum*, *Exophiala dermatitidis* and *Penicillium emersonii*), some of which serve as relevant pathogens (3). Due to chronic infections and their frequent exacerbations, CF patients develop morphological changes in the respiratory tract which promote further infections. This vicious circle results in an ongoing lung destruction, changes of lung ventilation, and an impairment of lung function (11-13). In the end, these patients may develop respiratory failure and require lung transplantation (14, 15).

Treatment of CF patients focuses on the pulmonary symptoms. Intensive physical therapy is supported by inhalation therapy for improvement of secretolysis (e.g., sodium chloride solution, acetyl cysteine, amiloride, recombinant DNAse) and local antibiotic treatment (e.g., colistin and tobramycin) (5, 16-18). However, the inhalant antibiotics have a number of limitations. These are the tolerance of patients (especially to aminoglycosides), their lung function, accessibility of pulmonary lesions to treatment, suitability of a nebulizer used, and susceptibility of bacterial strains which are subject of the treatment (3, 5, 19, 20). A large number of studies have been performed with the aim to optimize the inhalation of antibiotics. In many of those studies, tobramycin was nebulized (5, 6, 9,19, 21-23, ).

Despite all medical progress, CF patients still show a substantial reduction in lifespan and quality of life compared with healthy children (24). It is hoped that a further optimization of treatment, particularly concerning the inhalation of antibiotics for prevention of exacerbations of chronic pulmonary infections, will

be followed by a further improvement of CF patients. The aim of the present study was to investigate the feasibility of a novel inhalation method, based on a standardized breathing pattern, which allows an improved targeting of predefined lung areas.

## MATERIAL AND METHODS

Our monocentric, open label, and cross-over study was approved by the Ethical Committee of the Landesaerztekammer Baden-Württemberg. We included 16 patients with cystic fibrosis; 7 M and 9 F, aged 21.8 ±7.1SD years. Their anthropometric and lung function characteristic are presented in *Table 1*.

Spirometry and body plethysmography were performed before and after each treatment period, using a Jäger-Masterlab (Erich Jaeger GmbH, Würzburg, Germany). Two lung function parameters were measured: forced vital capacity (FVC) and forced expiratory volume in one second ($FEV_1$), and were normalized to the reference values proposed by the European Community for Coal and Steel (39).

Inhalation of tobramycin (Tobi®, Chiron, Emeryville, USA) was performed by means of a conventional inhalation system, the Pari-LC plus nebulizer (Pari Boy N compressor, Starnberg, Germany) and a controlled breathing maneuver using an AKITA® system combined with a Pari LC plus nebulizer (Activaero, Gemünden, Germany). For conventional inhalation, the LC plus nebulizer was filled with one ampoule Tobi® (5 ml = 300 mg tobramycin) and inhalation was performed with tidal breathing until the nebulizer started sputtering, which corresponds to a nebulized volume of 4 ml (=240 mg tobramycin) (*Table 2*).

*Table 1*. Anthropometric data and baseline values of lung function parameters of the study participants. Values are means; n=16.

|  | Mean ±SD | Range |
|---|---|---|
| Age (yr) | 21.8 ±7.1 | 13-39 |
| Height, (cm) | 163.0 ±11.4 | 143-183 |
| Weight (kg) | 51.9±11.4 | 43-76 |
| $FEV_1$ (l) | 1.89±0.71 | 1.00-3.40 |
| $FEV_1$ (%) | 61.0±24.1 | 35.0-116.0 |
| FVC (l/s) | 2.59±0.71 | 1.60-4.00 |
| FVC (%) | 70.8±18.7 | 51.0-116.0 |

*Table 2*. Data for the inhalation regimen of controlled inhalation (AKITA®) and conventional inhalation (PARI LC-Plus).

|  | AKITA® | LC-Plus |
|---|---|---|
| Filling dose | 2.5 ml/150 mg | 5 ml/300 mg |
| Nebulized dose | 1 ml/60mg | 4 ml/240 mg |
| Time to nebulize | 7-8 min | 20 min |

Based on the results of prior studies demonstrating a significant sparing effect of the controlled breathing maneuver with AKITA® only 150 mg tobramycin were filled into the Pari LC plus jet nebulizer and administered by means of the AKITA® inhalator. This resulted in a filled dose of 2.5 ml Tobi® (1/2 ampoule = 150 mg tobramycin). Only about 1 ml of this filled volume was nebulized, to reach a similar lung dose compared to the conventional nebulizer. Inhalation was performed with an air flow of 200 cm³/s. The inhalation volume (tidal volume, $V_T$) was normalized to the inhalation capacity ($V_{IC}$) of the patient according to the following equation:

$$V_I = 1.2 \cdot e^{\frac{-1.3 \cdot L}{V_{IC}}} \cdot L + 0.23 \cdot L$$

To reduce extrathoracic depositon, 150 cm³ clean air was inhaled after each aerosol inhalation. According to the individual inhalation capacity of the subject, on average, 65 breaths were inhaled leading to a nebulized drug solution of about 1 ml (= 60 mg tobramycin) (*Table 2*). After a pretreatment washout period of three days, tobramycin was inhaled twice daily in a cross-over design with two treatment blocks of three days each. The randomly assigned patient groups inhaled the study medication in filled doses of 300 mg (conventional inhalation) and 150 mg (controlled inhalation). Between the first and second treatment period, a washout phase of three days was kept. The efficiency of the inhalation was measured by determination of the serum tobramycin concentration 1 h after the start of the first administration in the morning of every study day (three determinations per method on Day 1, Day 2, and Day 3 by means of the Cobas Integra test Roche Diagnostics, Germany), based on fluorescence polarization.

Differences between serum concentrations of tobramycin were tested for statistical significance using a *t*-test (SAS 9.1.3 for Windows XP). Differences were considered significant at P<0.05.

RESULTS

Lung function values prior to inhalation were reduced, which predominantly concerned the expiratory flow rates (*Table 1*). On all study days, inhalation of tobramycin caused no evident changes in the clinical behavior of the participating individuals. The time required for aerosol inhalation was much shorter for controlled inhalation than for conventional inhalation (7-8 min *vs*. 20 min). Serum concentrations of tobramycin determined 1 h after the start of the inhalation on Day 1, Day 2, and Day 3 showed no differences between the conventional inhalation method and the controlled breathing method (*Fig. 1 and Fig. 2*). The mean values after inhalation of 150 mg tobramycin by controlled inhalation were 0.963 mg/l, 1.119 mg/l, and 1.194 mg/l with interindividual coefficients of variation (CV-values) of 39.6%, 40.0%, and 47.6 % on Day 1, Day 2, and Day 3, respectively. The respective serum values for conventional inhalation of 300 mg tobramycin were 1.075 mg/l, 1.294 mg/l and 1.269 with the CV-values of 74.3%, 64.8%, and 60.4% (*Table 3*). However, there was a relevant variability of the tobramycin serum concentrations. In some measurements, all after conventional inhalation, tobramycin concentrations higher than 2 mg/l were found. Specifically, on Day 1 one subject in the group with conventional inhalations showed a serum level of 3.5 mg/l (corresponding value after controlled inhalation was 1.8 mg/l). On Day 2,
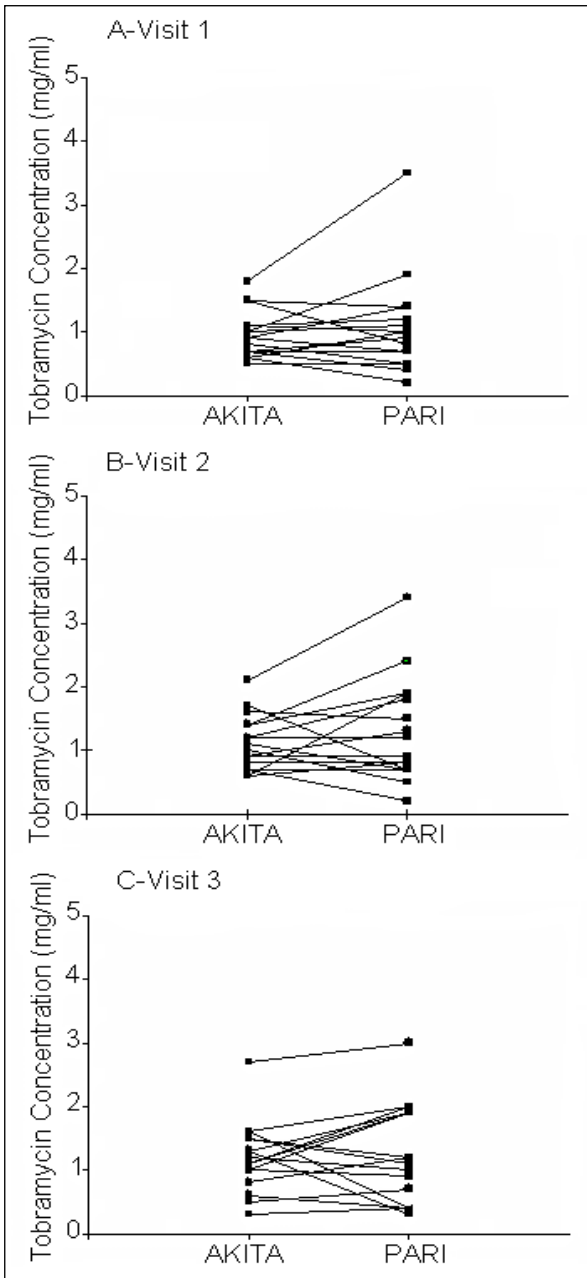
*Fig. 1.* Individual serum concentrations after nebulization of 60 mg tobramycin by controlled inhalation (AKITA®) and 240 mg tobramycin by conventional inhalation (PARI LC-Plus jet nebulizer) in 16 individuals on Day 1, Day 2, and Day 3 (top, middle, and bottom panel, respectively).

the same subject showed a concentration of 3.4 mg/l during conventional inhalation and a second subject showed 2.4 mg/l (corresponding concentrations after controlled inhalations were : 2.1 mg/l and 1.4 mg/l, respectively). On Day
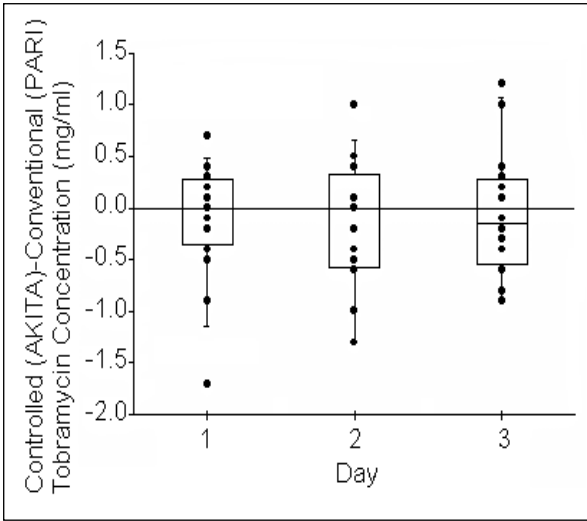
*Fig. 2.* Individual differences in serum concentrations after nebulization of 60 mg tobramycin by controlled inhalation (AKITA®) and 240 mg tobramycin by conventional inhalation (PARI LC-Plus jet nebulizer) in 16 patients on Day 1, Day 2, and Day 3.

*Table 3.* Serum concentrations after nebulization of 60 mg tobramycin by controlled inhalation (AKITA®) and 240 mg tobramycin by conventional inhalation (PARI LC-Plus) on study Day 1, Day 2, and Day 3.

|  | AKITA® | AKITA® | AKITA® | LC-Plus | LC-Plus | LC-Plus |
|---|---|---|---|---|---|---|
|  | Mean ±SD | Median | CV-value | Mean ±SD | Median | CV-value |
|  | (mg/l) | (mg/l) | (%) | (mg/l) | (mg/l) | (%) |
| Day 1 | 0.963 ±0.381 | 0.900 | 39.6 | 1.075 ±0.798 | 0.950 | 74.3 |
| Day 2 | 1.119 ±0.448 | 1.050 | 40.0 | 1.294 ±0.839 | 1.050 | 64.8 |
| Day 3 | 1.194 ±0.568 | 1.150 | 47.6 | 1.269 ±0.767 | 1.150 | 60.4 |
| Total | 1.092 ±0.461 | 1.000 | 42.2 | 1.213 ±0.783 | 1.000 | 64.6 |

*Table 4.* Differences (Δ) of the serum concentrations after nebulization of 60 mg tobramycin by controlled inhalation (AKITA®) and 240 mg tobramycin by conventional inhalation (LC-Plus) on study Day 1, Day 2, and Day 3.

|  | Δ AKITA® - LC-Plus | Δ AKITA® - LC-Plus |
|---|---|---|
|  | Mean ±SD | Median |
|  | (mg/l) | (mg/l) |
| Day 1 | -0.113±0.569 | -0.050 |
| Day 2 | -0.175±0.652 | 0.000 |
| Day 3 | -0.075±0.620 | 0.000 |

3, the same subject showed again a concentration of 3.0 mg/l during conventional inhalation (concentration after controlled inhalation was 2.7 mg/l). Even though, there were some intraindividual differences of the serum

concentrations determined after conventional and controlled inhalation, the mean values of the differences (controlled inhalation – conventional inhalation; AKITA® - LC-Plus®) after inhalation of 150 mg and 300 mg tobramycin were -0.113±0.569 mg/l, -0.175±0.652, and -0.075±0.620 mg/l on Day 1, Day 2, and Day 3, respectively, which did not assume significance (*Table 4*). Comparison of the interindividual CV-values of the serum concentrations after inhalation of 150 mg and 300 mg tobramycin (filled dose) revealed higher CV-values after inhalation by means of the conventional inhalation method, even though the serum concentrations were similar (*Table 3*).

## DISCUSSION

The frequently exacerbating chronic pulmonary infection with different bacteria and fungi is the underlying cause of ongoing pulmonary destruction and progressive loss of lung function in patients with CF. Lung transplantation may be a necessity at the end of the clinical course of this progressive disease (11-15). The most relevant pathogen for lung destruction in CF patients is *P. aeruginosa* (3, 4). In consequence, prevention of lung destruction is based on early detection and rapid and sufficient treatment of infections with this bacterial strain.

In cases of acute exacerbations patients are frequently treated with systemically administered antibiotics; the treatment being optimized by isolation and susceptibility tests of bacteria (3, 6, 7, 29). However, this treatment has a number of disadvantages: a/ bacteria may develop secondary resistance against these substances; b/ antibiotics may have systemic toxicity (nephrotoxicity and ototoxicity), and c/ antibiotics must diffuse into the respiratory tract, where their concentration should be higher than the minimum inhibitory concentration (MIC) for the treated strain. For example, *P. aeruginosa* may develop an appreciable secondary resistance against tobramycin ("small colony" strains without cell wall which are able to survive even in granulocytes and as mucoid strains) making a frequent susceptibility testing necessary for optimization of therapy (3, 5, 7-9, 20). Furthermore, the aminoglycoside tobramycin is nephrotoxic and ototoxic, particularly after intravenous administration (3, 7, 19). The same holds true for the polymyxin colistin which also is neurotoxic (7, 19). Only a small proportion of tobramycin is transported into the sputum after intravenous administration, where its concentration is only between 12% and 20% of the maximum serum concentration (7, 19). Furthermore, aminoglycosides are inactivated by binding to various sputum components (e.g., bivalent cations and DNA) with the consequence that the sputum concentration required for antibiotic treatment must be 10-25 times higher than the MIC determined in vitro (3, 19, 30). Finally, the mucoid phenotype of *P. aeruginosa*, which is frequently found in chronic pulmonary infections of CF patients, causes a further reduction of the antibiotic effect; in case of tobramycin a reduction is of 50 % (49).

Due to the above mentioned limitations of systemic antibiotic treatment and the requirement for a preventive treatment, a number of studies has recently been performed (predominantly with tobramycin) in which the feasibility and safety of inhalant antibiotic treatment in CF patients is investigated (3, 5, 6, 19, 21-23, 32). However, inhalation treatment depends on a number of prerequisites to ensure an optimal effect. These include properties of the substance affecting their antibiotic effect (no relevant primary or secondary resistance of the bacterial strains, results of susceptibility testing), their nebulization (chemical and physical stability, solubility in aqueous solutions, surface properties (e.g., foaming)), pulmonary tolerance in case of inhalant administration (e.g., local irritation), characteristics of the nebulizer (size and mass distribution of the produced particles, quantity of thoracic and intrapulmonary particle deposition, time required for inhalation of the required dose), and characteristics of patients (compliance, pulmonary morphology and function, breathing maneuver (e.g., inspiratory volume, end-inspiratory breath-hold, inspiratory and expiratory flow rates)) which are described in detail in the literature (33-36).

Although, benefit of inhalant antibiotic therapy for prevention of pulmonary infections in CF patients has been demonstrated by a reduction of pulmonary *P. aeruginosa* colonization up to eradication and a decrease in the number of patient hospitalizations (5, 21-23, 37, 38) and by an improvement of lung function (21-23, 30, 37-39), there are a number of controversial issues. These issues regard the administered substance (tobramycin, colistin, or others), the physical characteristics of the aerosol (powder *vs.* solution), the administered doses and frequency of inhalation, the efficiency of therapy for the long-time prevention of infections because of a risk for the development of a secondary bacterial resistance, the safety of inhalation therapy because of a risk of local and systemic side effects, the type of a nebuliser, and the breathing maneuver chosen (40, 41). However, our study was designed only to optimize the efficiency of aerosol administration for a solution developed and approved for inhalative administration (TOBI®). Other questions were not addressed (type of the substance, long-time effect and safety, dose and frequency of inhalation) or can only partially be answered (short-term safety).

In our study, we compared the inhalation of tobramycin filling doses of 150 mg and 300 mg by means of a controlled breathing maneuver with an AKITA® inhalation system and a conventional jet nebulizer with standard breathing maneuver. The difference of the administered doses was chosen, because prior studies from our group have demonstrated a high intrathoracic deposition of aerosols administered by controlled inhalation compared with conventional inhalation technique (26-28). We determined the serum concentration of tobramycin one hour after the start of the first inhalation at every study day rather than the sputum concentration. Although, sputum concentration seems to be more relevant for assessing the local antibiotic effect, because it allows for a direct comparison of a local concentration in comparison with the MIC, the variability

of the probe sampling made us take the serum concentration. Prior studies have demonstrated that inhaled tobramycin is reabsorbed from the respiratory tract showing maximum serum concentrations about 1 hour after the end of inhalation and can also be detected in urine (19, 21, 30, 41-45). In addition, measurement of serum concentration may provide information regarding the risk for systemic side effects which correlates with the serum concentration of a substance after inhalant administration.

As expected, there was a large interindividual variability of the concentrations, reflecting individual pulmonary deposition, absorption, and pharmacokinetics. Based on the results of these measurements, the tobramycin serum concentration can be used as a surrogate marker for assessing pulmonary deposition of the inhaled antibiotic, although small amounts are also absorbed from the intestine after extrapulmonary deposition (46).

In our study we observed similar serum concentrations after inhalation of 150 mg and 300 mg by means of controlled inhalation and conventional inhalation, respectively. The observed serum concentrations of tobramycin after inhalation were much lower than after intravenous administration, and the results we obtained were within concentration ranges described in other pertinent studies (19, 41-45). The lack of a difference in the serum concentration demonstrates that inhalation of 150 mg (60 mg nebulized dose) by controlled breathing is equivalent to the administration of 300 mg (240 mg nebulized dose) by conventional administration and stands in full agreement with prior results of our group (26-28). This can be explained by two facts: a) an extremely high proportion of intrathoracically deposited aerosol particles of about 85 % and b) the aerosol is produced exclusively during the inspiration of a patient. We further found a significantly lower interindividual variability of tobramycin serum concentrations after controlled breathing inhalation (39.6%, 40.0%, and 47.6%) compared with conventional inhalation (74.3%, 64.8%, 60.4%). Because of the cross-over design of our study, the most likely cause of this difference is a corresponding variability of the amount deposited in the lungs. The observation of a smaller interindividual variability also confirms prior data using the controlled breathing technique in different patient groups (26, 27, 28).

In our study, serum concentrations of tobramycin were not significantly different during consecutive study days, demonstrating that there was no appreciable accumulation of the substance in the serum. Hence, our results confirm those of prior pharmacokinetic investigations (19, 41-45). However, drug accumulation cannot be definitely excluded if tobramycin is inhaled for a longer time. For example, such accumulation may occur if other substances are additionally administered which interfere in the metabolism, if renal excretion of tobramycin is impaired, or if other substances given at the same time have additional nephrotoxic properties (47-49). Even if there is no accumulation, serum concentrations of tobramycin may exceed the value of 2 mg/l, which serves as a borderline value for the risk of systemic side effects, because of the

variability of the deposited pulmonary drug dose after conventional inhalation (45). A severe allergic response has been described after inhalation of tobramycin, which seems to be dose-independent (50). However, the frequency of systemic adverse reaction after tobramycin inhalation is much lower than after intravenous treatment (3, 5, 19, 22, 23, 38). Therapeutic drug monitoring is usually performed in high-risk patients with intravenous tobramycin treatment and in those receiving inhalant therapy who are at an increased risk (e.g., additional treatment with ototoxic or nephrotoxic substances).

A typical side effect of tobramycin inhalation described in a number of studies is bronchoconstriction, due to a local irritant effect (19, 21, 41, 50-52). In our study we observed no appreciable symptoms of bronchoconstriction. However, this might be explained by a small number of participants, a low number of inhalations, and a short duration of the study.

Results of prior studies demonstrated that inhalation of tobramycin is followed by a rapid increase of its concentration in sputum which is largely higher than the MIC. The maximum concentration is achieved 10 min after inhalation. Then, it is rapidly decreasing with a median half-life time of about two hours (6, 21). It should be noted that in earlier studies only a small proportion of the administered drug is deposited in the lungs (sometimes less than 10% (53) and that the amount of the deposited drug showed a strong variability. Furthermore, it is not clear if the concentration of tobramycin in sputum also reflects the concentration in the lung which can be determined by measurement of the concentration in bronchoalveolar fluid. One study demonstrated that 30 min after inhalation, tobramycin concentrations in bronchoalveolar fluid were between 16 µg/ml and 204 µg/ml (mean value - 90 µg/ml) and ten times as high as the MIC for *P. aeruginosa* (44). The concentration rapidly decreases after the end of inhalation and after 1 h and 3 h only 40% and less than 10 % of the initial concentrations are detectable, respectively (5). Due to the high initial concentration, significant tobramycin concentrations were detected even 9 to 12 h after the end of inhalation (5). Therefore, it is likely that a sufficient concentration of tobramycin in sputum and bronchoalveolar fluid can be achieved by inhalation twice daily. Due to a rapid decrease of the tobramycin concentration in sputum and alveolar fluid, its pulmonary deposition should be optimized, e.g., by a breathing maneuver based on controlled breathing to ensure a concentration of the antibiotic much higher than the MIC for *P. aeruginosa*.

In summary, our study demonstrates the feasibility of the controlled-breathing method for tobramycin inhalation in cystic fibrosis patients. Compared with conventional inhalation techniques this method allows for a reduction of the pharmacon dose to 50%, which would reduce the daily costs of treatment. Furthermore, because of the reduction of the required nebulized dose, there also is a significant reduction of treatment time which will improve the compliance of the patients. Our study was performed in patients with cystic fibrosis only. However, inhalation of antibiotics, e.g., gentamycin, tobramycin, and colistin is a

therapeutic option in patients with other pulmonary diseases (e.g., bronchiectases, pneumonia and tracheobronchitis) (54-57).

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