Original Paper

Sequential Inhalational Tobramycin-Colistin-Combination in CF-Patients with Chronic *P. Aeruginosa* Colonization – an Observational Study

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Key Words

Cystic Fibrosis • Lung infection • *P. aeruginosa* • Inhaled tobramycin and colistin

Abstract

Background/Aims: In cystic fibrosis (CF), chronic microbial lung infections are difficult to treat and cause morbidity and increased mortality. **Methods:** In a multicentre, open-label, exploratory, non-interventional study, inhaled tobramycin (300 mg twice daily) and colistin (1 million I.U. twice daily) were sequentially combined with the aim to investigate the effect on 41 CF patients with chronic *P. aeruginosa* infections for six months (mean age 24 ± 10.8y). **Results:** Six patients had adverse events that were assessed as being related to treatment. Mucus production and coughing both decreased in 39%, whereas FEV₁ absolute and relative to baseline increased by 4.9% and 9.1%, respectively (p = 0.004) in 29 patients, who were definitely treated sequentially. Efficacy of the therapy was rated 'excellent' or 'good' by the physicians in 80.5% of the patients. **Conclusions:** The results indicate that treatment with inhaled antibiotics, sequentially combined, was very well tolerated by most patients and may have a beneficial effect, even if transitory on lung function and respiratory symptoms.

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Introduction

The genetic disorder cystic fibrosis (CF) caused by mutations in the gene CF Transmembrane Conductance Regulator (CFTR) [1, 2], affects approximately 80,000 Caucasian individuals in Europe and North America. In airways of CF-patients chronic microbial infection and inflammation is provoked [3] leading to excessive lung tissue destruction which is the most important cause of morbidity and mortality in these patients [4].

Inhaled tobramycin and/or colistin monotherapy are used as long-term medications for the treatment of *Pseudomonas aeruginosa* infection in patients with CF [3, 5, 6]. Inhaled antibiotic therapy, shortly initiated after colonization of the airways by *P. aeruginosa*, has led to high eradication rates [3]. However, once *P. aeruginosa* lung infections become chronic, antibiotics do not eradicate the pathogens in the CF airways due to bacterial biofilm formation [7], development of mutator strains [8] and drug adsorption to sputum components [9]. In clinical practice, several inhaled and systemic antibiotics are combined to treat CF-associated lung infections at this stage. The tolerability and efficacy of these combinations have not yet been systematically investigated [10]. If chronic *P. aeruginosa* infection is persistent and mucoid *P. aeruginosa* strains are present, systemic antibiotics, e.g. ciprofloxacin, clarithromycin, fosfomycin or azithromycin, are used as an add-on therapy to inhaled tobramycin or colistin monotherapy [11]. Recently, a combination of inhaled antibiotics, i.e. colistin and tobramycin, was studied in a pilot study [12].

Increasingly, sequential therapy of inhaled tobramycin followed by inhalation of either colistin or aztreonam during the off cycle of tobramycin, is occurring among CF patients.

Here we report on the feasibility, safety and efficacy of an open-labelled, non-interventional study of inhaled tobramycin and colistin sequential therapy in 41 CF patients treated for 6 months.

Materials and Methods

Study design and patients

Pediatric and adult patients with CF, enrolled in twelve Cystic Fibrosis Centers in Germany (Tuebingen, n = 7; Stuttgart, n = 3; Freiburg, n = 8; Hamburg, n = 5; Oldenburg, n = 5; Neubrandenburg, n = 1; Aachen, n = 2; Berlin, n = 1; Mainz, n = 5; Erlangen, n = 2; Frankfurt, n = 1 and Essen, n = 1), were eligible to be included in the study: 1. diagnosis of cystic fibrosis was verified, 2. 6 years or older, 3. chronic *P. aeruginosa* lung infection (≥ 6 months) at screening, 4. susceptibility of *P. aeruginosa* to tobramycin and/or colistin, 5. normal kidney function and normal hearing ability, 7. a treatment escalation due to aggravated symptoms or deterioration of lung function was present. Patients were not included in case of: 1. hypersensitivity to colistin, polymyxin B, or aminoglycosides were present, 2. Myasthenia gravis was present and 3. were pregnant or breast feeding.

Criteria for termination of treatment were not prospectively defined and protocol violations were not determined because of the non-interventional character of the study. The decision for a therapy with inhaled tobramycin and colistin had to be made by the physician prior to the inclusion of a patient into the study, but after inclusion some patients or patient's parents decided not to be treated analogue the study outline.

All patients were analyzed for safety and efficacy. Efficacy analyses were also performed in a subgroup, which was defined as patients who had combined tobramycin/colistin treatment and had no acute pulmonary exacerbations at the beginning or at the end of the observational period. Pulmonary exacerbations were defined as two or more of the following criteria: change in sputum volume, color or increased cough or increased malaise, fatigue, lethargy or anorexia, weight loss or decrease in pulmonary function (FEV₁) by 10% or more, or radiographic changes or increased dyspnoea. In addition to the study protocol, data on acute pulmonary exacerbations were collected.

In the case report forms all patients were anonymized by means of a patient number to be identified via the patient identification list only. The patient identification list was kept exclusively with the investigating physicians in the investigator site file. Monitoring for this study was provided by the sponsor.

As requested by law, the study was reported to the Federal Institute for Drugs and Medical Devices (Bundesinstitut für Arzneimittel und Medizinprodukte, BfArM). The study was performed in accordance with the Declaration of Helsinki and under the regulations of Good Clinical Practice. The study was approved by the ethics committee at the medical faculty of the Eberhard-Karls-University and the University Hospital Tübingen and written informed consent was obtained from all patients.

Study Procedures

Patients have been foreseen to receive 300 mg of tobramycin (Bramitob®) inhaled twice daily (morning and evening, 12-hour interval recommended) for 28 days followed by Colistin (1 million I.U.) twice daily for 28 days, given during the therapy free intervals of tobramycin treatment ('off cycles'), over an overall period of 6 months, because of known chronic *Pseudomonas* colonization. Both drugs were inhaled via individual used inhalator devices (PARI GmbH, Starnberg, Germany). Patients were screened prior to treatment and treated as outpatients for 6 months. Details on previous therapies for *P. aeruginosa* infection within the 6 months prior to the first visit were documented. Concomitant medications for the treatment of diseases/medical conditions other than chronic *P. aeruginosa* infection were not documented. Data were documented at three visits: first visit at baseline, second visit after 84 ± 14 days and the final visit after 180 ± 28 days analogous to common visits at sites.

Study-related tests and examinations included the assessment of medical history data, previous treatment regimens, reasons for change from monotherapy to sequential therapy, pulmonary function testing, *Pseudomonas* antibiogram, respiratory symptoms, assessment of compliance by questionnaire and adverse events at all visits. At the final visit, physicians and patients evaluated the tolerability of the combination therapy on a 5-point scale (1 = very good, 2 = good, 3 = satisfactory, 4 = insufficient, 5 = not assessable).

Aged-matched efficacy measurements included assessment of lung function (forced vital capacity (FVC); forced expiratory volume in 1 second (FEV₁); maximal expiratory flow - MEF₂₅ and MEF₂₅₋₇₅) at each visit performed according ATS criteria, respiratory symptoms (e.g. cough, dyspnoea, sputum production) at each visit, frequency of *P. aeruginosa*, modified revised Cystic Fibrosis Questionnaire (CFQ-R) on 4-point scale and antibiograms in sputa testing the susceptibility of *P. aeruginosa* and/or other bacterial strains to tobramycin and colistin at each visit. Clinical laboratory investigations such as molecular determination or counting of *Pseudomonas* strains were not done due to the character of the study.

Outcomes

The primary endpoint was safety and tolerability which was assessed by numbering of side effects and adverse and serious adverse events.

Secondary endpoints (efficacy data) included the change in FVC, FEV₁, MEF₂₅ and MEF₂₅₋₇₅ from baseline, change of pulmonary colonization, change of respiratory symptoms and assessment of efficacy and tolerability by physicians and patients at the final visit (after 6 months). The change of exacerbation and iv-treatment rates were retrospectively analyzed by questionnaire.

Finally, a post hoc analysis was done to evaluate the lung function trend observed in the 6 months prior to enrollment.

Statistical Analysis

The safety population included all patients treated with inhaled tobramycin or colistin for whom any data were documented. In case no entry on dosage was made, but other data were available, it was assumed that the patient received treatment for *P. aeruginosa* infection and the patient was included in the safety population. Patients with sequential therapy (tobramycin and colistin) and full available data were included in the subgroup.

The Student's t-test or Wilcoxon was applied where appropriate. Continuous variables were summarized by number, mean, standard deviation, minimum, lower quartile, median, upper quartile, and maximum. Categorical variables were presented in frequency distribution tables with number and percentage. Statistical analyses were performed using the statistical software package SAS (SAS Institute Inc.) version 9.1.3. The contract research organization Syneed Medidata GmbH, Konstanz, Germany was responsible for data management and monitoring. Adverse events were coded using the Medical Dictionary for Regulatory Activities (MedDRA) coding system (version 16.0). Medications were analyzed on the basis

of international nonproprietary names. Data analysis was done by Syneed Medidata GmbH.

No formal sample size determination was performed, because no prior systematic studies on the use of combined antibiotics for the treatment of CF-associated *P. aeruginosa* infection had been performed upon which to base effect calculations.

Role of the funding source

The funding sources had a role in study design, but not in data collection or analysis, report writing, or the decision to submit this paper for publication. All authors had full access to the data and participated in the decision to submit for publication.

Results

The prospective, multicentric, open label, observational study lasted for 24 months (4/2011 - 4/2013). A total of 41 patients were included in the study and in the safety population (Table 1). Protocol deviations were not analyzed due to the non-interventional character of the study. No patient was excluded from analyses of efficacy and safety. Efficacy data were available for 40 patients at the second visit and for 38 patients at the final visit. Thirty-six of all patients (88%) received sequential therapy with tobramycin and colistin. Six of the patients (14.6%) received an alternative treatment regimen with inhaled tobramycin

Table 1. Demographic characteristics, diagnosis of CF and respiratory tract infections

Characteristic	Total (N = 41)	
Age [years]	n	41
	Median (range)	24.0 (6, 50)
Height [cm]	Median (range)	163.0 (122, 192)
Weight [kg]	Median (range)	54.0 (21, 92)
Sex [n (%)]	Male	13 (31.7)
	Female	28 (68.3)
Duration of CF, time since first diagnosis [years], n=40	Median (range)	18.5 (2, 42)
Time since first detection of <i>P. aeruginosa</i> in respiratory tract [years], n=39	Median (range)	7 (1, 28)
<i>P. aeruginosa</i> strains [n (%)], n=40		44 (69.8)
Mucoid <i>P. aeruginosa</i> [n (%)], n=18 of 40		19 (30.2)
Time since latest detection of <i>P. aeruginosa</i> in respiratory tract [n (%)]	< 1 months	13 (31.7)
	1-2 months	8 (19.5)
	3-4 months	10 (24.4)
	5-6 months	1 (2.4)
	> 6 months	3 (7.3)
	Missing data	6 (14.6)
Detection of additional germs within the last 3 months [n (%)]	No	4 (9.8)
	Yes	37 (90.2)
Kind of detected germ	Staphylococcus	22 (53.7)
	Candida	22 (53.7)
	Aspergillus	18 (43.9)
	Haemophilus	2 (4.9)
	Streptococcus	1 (2.4)
	Other	5 (12.2)
Antibiotic treatment regimens		
Tobramycin (300mg BID) and Colistin (1Mio BID)		33 (80.5)
Tobramycin (150-300mg OD) and Colistin (1Mio BID)		3 (7.3)
Tobramycin (150-300mg OD) or Colistin (1 Mio OD)		3 (7.3)
Tobramycin (300mg BID) and Aztreonam (75mg trice) or Colistin (1Mio BID) and Aztreonam (75mg trice)		2 (5.0)

(150-300mg tobramycin once daily-OD), and three of them received colistin in the off-cycles. Two patients (5%) received sequential therapy with aztreonam and tobramycin or aztreonam and colistin, two patients (5%) received monotherapy with tobramycin and one patient (2%) received monotherapy with colistin. Previous monotherapy over a period of at least 6 months with inhaled tobramycin (on/off cycle) was reported for 36.6% (N = 15) of the study population. Previous monotherapy with inhaled colistin was reported for 61.0% (N = 25) of the study population. 17 patients (41.5%) had previously been treated with intravenous (i.v.) antibiotics; 15 of these patients had received more than one i.v. antibiotic treatment in 6 months.

Safety

During the observation period, a total of 9 patients showed intolerance symptoms (including hoarseness, cough, and bronchial obstruction) after inhalation of tobramycin and/or colistin. Four of these patients had shown intolerance reactions to inhaled tobramycin and/or colistin prior to the start of the study. The number of patients with intolerances were lower at the final visit (N = 5), than at the second visit (N = 9). Intolerance symptoms to inhaled tobramycin or sequential therapy including tobramycin were also reported as adverse events.

Eighteen adverse events were reported in 11 patients (26.8%). Sixteen adverse events reported in 10 patients were assessed as being non-serious; two adverse events reported in 1 patient were assessed as being serious (hemoptysis, bronchial obstruction) and by the opinion of the investigator not related to the treatment. The most frequently reported adverse event was cough (9.8%, N = 4), followed by bronchial obstruction (7.3%, N = 3), dysphonia (7.3%, N = 3), oropharyngeal pain (4.9%, N = 2) all related to tobramycin treatment and viral infection (4.9%, N = 2). Haemoptysis, throat irritation, abdominal pain, and weight increase (in the opinion of an investigator declared as an AE) each occurred in 2.4% (N = 1) of all patients. A 30 year old female patient experienced bronchial obstruction after inhalation of tobramycin and severe haemoptysis. The therapy of the patient was changed to colistin monotherapy due to the bronchial obstruction.

Efficacy Results

Treatment Compliance

According to the assessment by the physicians at the end of the study (visit 3), treatment compliance was unchanged compared to the compliance of the previous therapy in the majority of the patients, i.e. in 73.2% (N = 30). Compliance had improved in 14.6% (N = 6) and decreased in 2.4% (N = 1) of the study population. Data were missing for 9.8% (N = 4).

Changes from baseline in respiratory parameters

According to the physicians' assessment, there were 22 patients with an improvement compared to baseline in at least one of the assessed respiratory parameters (mucus production, coughing, and FEV₁) at the final visit. Ten of these patients showed an improvement in all three assessed parameters. At the final visit, mucus production was unchanged in 51.2% (N = 21) and decreased in 39.0% (N = 16) of all patients. Increased mucus production was not reported for any of the patients at the final visit. Data on mucus production were missing for 9.8% (N = 4). Coughing had not changed in 46.3% (N = 19), had decreased in 39.0% (N = 16), and had increased in 7.3% (N = 3) of the study population at the final visit; data were missing for 7.3% (N = 3).

Determination of *P. aeruginosa*

In 40 patients 44 non-mucoid and 19 mucoid *P. aeruginosa* strains could be found at study start, whereas in 36 patients a sputum analysis was possible after 6 months of treatment (Table 1). In these 36 patients the 37 non-mucoid strains were 12 times found to be resistant

Table 2. Lung function – time course and change from baseline to the end of the study (patients with complete data).^a Change from baseline. FVC = forced vital capacity, FEV₁ = forced expiratory volume in 1 second, MEF₂₅ / MEF₂₅₋₇₅ = maximal expiratory flow at 25% or between 25 and 75% of vital capacity. Means ± SD are given

Characteristic		Visit 1 (Baseline)	Visit 2 (after 3 m)	Visit 3 (after 6 m)	p-value
FVC [% predicted]	N = 37	79.5 ± 21.8	81.4 ± 22.4	82.4 ± 23.5	0.12
Absolute change ^a				2.9 ± 14.6	
% (relative) change ^a				5.1 ± 24.1	
FEV ₁ [% predicted]	N = 38	68.8 ± 26.3	70.4 ± 27.2	70.9 ± 25.6	0.14
Absolute change ^a				2.1 ± 11.5	
% (relative) change ^a				5.8 ± 18.8	
MEF ₂₅ [% predicted]	N = 36	33.9 ± 26.9	33.7 ± 26.7	32.4 ± 25.6	0.33
Absolute change ^a				-1.5 ± 20.1	
% (relative) change ^a				21.3 ± 112.4	
MEF ₂₅₋₇₅ [% predicted]	N = 34	43.6 ± 32.0	47.5 ± 34.8	46.0 ± 32.3	0.21
Absolute change ^a				2.4 ± 17.6	
% (relative) change ^a				16.8 ± 45.6	

against tobramycin and the 15 mucoid strains were not resistant against tobramycin and not against colistin. After 6 months of treatment the 32 non-mucoid strains ($p = 0.081$) were 14 times ($p = 0.089$) found to be resistant against tobramycin and the 16 mucoid ($p = 0.17$) strains were 2 times found resistant against tobramycin and non against colistin. A non-detection of *P. aeruginosa* was reported for 12.2% ($N = 5$) of the study population at the final visit.

General clinical improvement

According to the physicians' assessment, a general clinical improvement was seen in 56.1% ($N = 23$) of all patients at the final visit. At visits 2 and 3, no general improvement was observed in 34.1% ($N = 14$) of the study population. Data were missing for 9.8% ($N = 4$) at the final visit.

Changes in intravenous antibiotic therapy and exacerbation rates since baseline

The frequency of i.v. antibiotic therapy in 39 patients had changed from 37 treatments (mean 0.95 ± 1.17) 6 months before the start of the study to 27 treatments (0.69 ± 1.0) after 6 months of therapy (difference of 27%, $p = 0.02$). Exacerbation rates similarly decreased from mean 39 (1.0 ± 1.56) 6 months before to 30 (0.77 ± 0.96) at the final visit (difference of 23%; $p = 0.156$).

Lung Function

The results with regard to the lung function variables FVC, FEV₁, MEF₂₅, and MEF₂₅₋₇₅ are summarized in Table 2 for all patients having complete data available. The mean relative improvement in FVC % predicted compared to baseline was 5.1% at the final visit ($p = 0.12$). The mean relative improvement in FEV₁ % predicted compared to baseline was 5.8% at the final visit ($p = 0.14$). The inter-patient variability with regard to the MEF₂₅ values and the change in MEF₂₅ from baseline was high. The mean relative improvement in MEF₂₅ % predicted compared to baseline was $21.3 \pm 112.4\%$ at the final visit, whereas the median relative improvement was -1.5% ($p = 0.33$). The mean relative improvement in MEF₂₅₋₇₅ % predicted compared to baseline was 16.8% at the final visit ($p = 0.21$).

Furthermore, FEV₁ was determined by spirometry in 36 CF patients 6 and 3 months before and 3 and 6 months after sequentially combined inhalational therapy. We found significant differences between 6 ($71.8 \pm 29.8\%$, $p = 0.024$) and 3 months ($71.3 \pm 29.3\%$, $p = 0.037$) before the start compared to baseline ($68.8 \pm 26.3\%$) of the trial and not significant differences after 6 months $70.9 \pm 25.6\%$, $p = 0.12$) of treatment.

Table 3. Lung function – time course and change from baseline to the end of the study (sub group). ^a Change from baseline. FVC = forced vital capacity, FEV₁ = forced expiratory volume in 1 second, MEF₂₅ / MEF₂₅₋₇₅ = maximal expiratory flow at 25% or between 25 and 75% of vital capacity. Means ± SD are given

Characteristic		Visit 1 (Baseline)	Visit 2 (after 3 m)	Visit 3 (after 6 m)	p-value
FVC [% predicted]	N = 29	79.9 ± 19.3	82.5 ± 32.3	85.1 ± 22.0	0.035
Absolute change ^a				5.3 ± 15.0	
% (relative) change ^a				6.2 ± 18.0	
FEV ₁ [% predicted]	N = 29	69.4 ± 24.2	71.5 ± 27.0	74.4 ± 24.4	0.004
Absolute change ^a				4.9 ± 9.6	
% (relative) change ^a				9.1 ± 17.2	
MEF ₂₅ [% predicted]	N = 28	32.4 ± 26.7	35.2 ± 27.5	31.6 ± 25.6	0.33
Absolute change ^a				1.6 ± 19.1	
% (relative) change ^a				20.0 ± 62.2	
MEF ₂₅₋₇₅ [% predicted]	N = 29	47.4 ± 32.3	50.4 ± 35.3	46.0 ± 32.3	0.11
Absolute change ^a				4.2 ± 17.8	
% (relative) change ^a				16.5 ± 46.4	

Fig. 1. Sequential treatment with tobramycin and colistin increases lung function in 29 cystic fibrosis (CF) patients in a subgroup. Forced expiratory volume in 1 sec % predicted (FEV₁) was determined by spirometry 6 and 3 months before, at start, after 3 months and 6 months after sequentially combined inhalational therapy. Means ± SD are given. * p ≤ 0.05; ** p ≤ 0.005, student's T-test.

Subgroup analyses

29 patients were analyzed in the subgroup analyses (SG) and complete data are summarized in Table 3. Significant differences of 5.3 and 6.2 (absolute and relative, p = 0.036) in FVC % predicted between baseline and final visit and FEV₁ % of predicted of 4.9 and 9.1% (absolute and relative, p = 0.004) between baseline and final visit were observed. Furthermore the frequency of i.v. antibiotic therapy in the SG population had changed from a mean of 1.0 ± 1.25 at study start to 0.66 ± 1.01 at the final visit (difference of 34%, p = 0.015). Exacerbation rates similarly decreased from a mean of 1.1 ± 1.72 at study start to 0.83 ± 0.97 at the final visit (difference of 25%, p = 0.174).

Furthermore, FEV₁ was determined by spirometry in 29 CF patients 6 and 3 months before and 3 and 6 months after sequentially combined inhalational therapy. We found significant differences (p = 0.02) between 6 and 3 months before the start of the trial and significant differences (p = 0.0045) after 6 months of treatment (Fig. 1).

Physician's Final Assessment of Efficacy

At the final visit, the efficacy of the therapy was rated 'excellent' or 'good' by the physicians in 80.5% (N = 33) of patients (excellent: 31.7%, N = 13; good: 48.8%, N = 20). Efficacy was rated 'satisfactory' in 12.2% (N = 5) and 'insufficient' in 2.4% (N = 1) of patients. The final assessment of efficacy was missing for 4.9% (N = 2) of patients.

Table 4. Results of the modified CFQ-R (patients with complete data) in 32 patients

Characteristic [n (%)]		Visit 1 (Baseline)		Visit 2 (after 3 m)		Visit 3 (after 6 m)	
1. Have your airways been congested?	A great deal	6	(18.8)	1	(3.1)	0	
	Somewhat	7	(21.9)	11	(34.4)	10	(31.3)
	A little	15	(46.9)	15	(46.9)	13	(40.6)
	Not at all	5	(12.5)	5	(15.6)	9	(28.1)
2. Have you been coughing during the day?	A great deal	5	(15.6)	1	(3.1)	0	
	Somewhat	11	(34.4)	13	(40.6)	11	(34.4)
	A little	11	(34.4)	13	(40.6)	15	(46.9)
	Not at all	4	(15.6)	5	(15.6)	6	(18.8)
3. Have you had to cough up mucus?	A great deal	5	(15.6)	0		0	
	Somewhat	8	(25.0)	12	(37.5)	9	(28.1)
	A little	13	(40.6)	11	(34.4)	16	(50.0)
	Not at all	6	(18.8)	9	(28.1)	7	(21.9)
4. Has your mucus mostly been:	Clear	1	(4.8)	3	(14.3)	2	(9.5)
	Light to yellow	9	(42.9)	11	(52.4)	12	(57.1)
	Dark to greenish	10	(47.6)	6	(28.6)	7	(33.3)
	Bloody	0		0		0	
	Don't know	1	(4.8)	1	(4.8)	0	
5. Have you been wheezing?	Daily	2	(6.3)	1	(3.1)	0	
	Often	3	(9.4)	1	(3.1)	0	
	Sometimes	15	(46.9)	12	(37.5)	11	(34.4)
	Never	12	(37.5)	18	(56.3)	21	(65.6)
6. Have you had trouble breathing?	Daily	5	(15.6)	1	(3.1)	0	
	Often	3	(9.4)	1	(3.1)	1	(3.1)
	Sometimes	8	(25.0)	11	(34.4)	12	(37.5)
	Never	16	(50.0)	19	(59.4)	19	(59.4)
7. Have you woken up during the night because you were coughing?	Daily	3	(9.4)	2	(6.3)	0	
	Often	4	(12.5)	1	(3.1)	1	(3.1)
	Sometimes	10	(31.3)	9	(28.1)	9	(28.1)
	Never	15	(46.9)	20	(62.5)	22	(68.8)

Final Assessment of Tolerability

The final assessment of tolerability (treatment regimen was not specified) was slightly more positive according to the physicians' assessment than according to the patients' assessment. The tolerability was rated 'excellent' or 'good' in 82.9% (N = 34) of the study population by the physicians and in 68.3% (N = 28) by the patients. Tolerability was rated 'satisfactory' in 9.8% (N = 4) by the physicians and in 17.1% (N = 7) by the patients. Tolerability was 'insufficient' in 2.4% (N = 1), according to the physicians' as well as the patients' assessments. A final assessment of tolerability by the physician was missing for 4.9% (N = 2) and by the patient for 12.2% (N = 5) of all patients.

Patients' Assessment of Respiratory Symptoms (Modified CFQ-R)

Improvements in respiratory symptoms by means of the modified 7-item CFQ-R in the course of the study compared to baseline were indicated by the decrease in the number of patients with severe/frequent symptoms and the increase in the number of patients with mild/no symptoms (Table 4). The proportion of patients with severely ('a great deal') congested airways decreased, whereas the proportion of patients whose airways were not congested increased at the final visit. The proportion of patients with severe coughing during the day and patients who had to cough up mucus a great deal decreased, too. Throughout

the study, the proportion of patients who never had wheezing increased, the proportion of patients who never had trouble breathing increased and patients who never woke up during the night due to coughing increased.

Of the 32 patients with CFQ-R data available at all visits, 21 patients completed item 4 (colour of mucus) at all three visits. Most patients who did not answer this question at one or more visits had not had to cough up mucus. Based on the results of the 21 patients with complete data, there was a tendency towards a lighter/clearer color of the mucus throughout the course of the study.

Discussion

This multicentre, open-label, exploratory, non-interventional study was performed to collect clinical data on the therapy with inhaled tobramycin and/or colistin in CF patients with chronic *P. aeruginosa* infection of the lung under the conditions of daily practice. The tolerability of alternating therapy with inhaled antibiotics, the clinical effects of the treatment with inhaled tobramycin and/or colistin, and the treatment compliance were of interest. Our study is the first study to investigate the safety and efficacy of sequentially inhaled tobramycin and colistin treatment in CF patients [13]. We noted that the novel treatment regimen is safe and led to a significant increase in lung function in subgroup analyses.

Although the planned patient number of approximately 100 was not achieved due to difficulties in study centre recruitment, 41 patients was considered sufficient to draw some general conclusions from the descriptive analysis on clinical effects and tolerability of treatment with inhaled tobramycin and colistin. The majority of the patients had previously been treated with inhaled antibiotic monotherapy. At the start of the study, 93% of the patients received sequential combination therapy with inhaled antibiotics. The most frequently named reason for starting sequential therapy was an aggravation of clinical symptoms. A decrease in FEV₁ by more than 5% during the last year had been observed in almost half of the patients (48.8%, N = 20).

During the observation period, a total of 9 patients showed intolerance symptoms (including hoarseness/dysphonia, cough, and bronchial obstruction) after inhalation of tobramycin and/or colistin. Cough and hoarseness/dysphonia are adverse reactions which have commonly been observed in clinical studies with inhaled tobramycin and colistin [14-17], but less frequent in our study; oropharyngeal pain has occasionally been observed. Post-marketing studies have shown that bronchoconstriction (bronchial obstruction) occurs rarely after inhalation of tobramycin [16]. However, in this study, there were 4 patients (9.8%) with reported bronchial obstruction – all received sequential therapy with tobramycin and colistin. In 3 of the patients, bronchial obstruction was reported as adverse event (i.e. as related or probably related to tobramycin in 2 patients) and in one patient bronchial obstruction was reported as intolerance to inhaled colistin.

In clinical practice, several inhaled and systemic antibiotics are combined to treat CF-associated lung infections at this stage, but tolerability and efficacy of these combinations have not yet been systematically investigated [10, 11]. To our knowledge this is the first study presenting data for a prospective sequential inhalation treatment. *In vitro* and *in vivo* combination treatment presented by our own group might not be as effective as it was presented first line [12]. Preliminary data of an ongoing cohort study (not published) combining tobramycin and colistin compared to colistin monotherapy reveal not the huge decrease of *Pseudomonas* colony forming units (cfu) usually seen in patients who are naïve to new formulations either to tobramycin or colistin [14, 17]. Although not significant, the well-known increasing resistance against tobramycin was also shown in our study, but nevertheless we found an obviously beneficial clinical effect. However, further clinical trials with laboratory investigations such as molecular determination and counting of *Pseudomonas* strains or microbiome determination of pulmonary colonization should be done. Furthermore, susceptibility tests of combined antibiotics must be routinely implemented by microbiologists to verify synergistic effects in treatment of *P. aeruginosa*.

During the observation period, improvements in lung function were observed. In this study a mean relative improvement in FEV_1 % predicted of 5.8% was noted in 38 patients. This is slightly lower than the 6.7% value observed by Hodson et al. after 4 weeks of treatment with inhaled tobramycin in a clinical study in patients with CF and *P. aeruginosa* infection (N = 50, median age: 20 years) [5]. The mean relative improvement in FVC % predicted was 5.1%. The inter-patient variability in the relative changes in the maximum expiratory flow variables was very high. The median relative improvement in MEF_{25-75} was 16.8% but with a broad range. There was a mean relative improvement in MEF_{25} of 21.3% again with a broad range of individual values (Table 3). Since in this study a sample size calculation was not done for the primary endpoint “lung function”, no significance was expected for lung function parameters, Astonishingly the subgroup analysis showed a significant mean relative improvement in FEV_1 % predicted of 9.1% and mean relative improvement in FVC % predicted of 6.2% in 29 patients (Table 3 and Fig. 1). These are remarkable results considering that patients were not naïve to either tobramycin or colistin.

According to the physicians' assessment at the final visit, a general clinical improvement was seen in 23 patients (56.1%). In line with this, according to the physicians' assessment of respiratory symptoms, there were 22 patients with an improvement compared to baseline in at least one of the assessed respiratory symptoms (mucus production, coughing, and FEV_1) at the final visit. Ten of these patients showed an improvement in all three assessed parameters. All three symptoms were improved with the same frequency, i.e. each symptom was improved in 16 patients (39.0%). Improvements in respiratory symptoms in the course of the study were also seen according to the patients' assessment of respiratory symptoms by means of the modified CFQ-R.

The physicians' assessment of treatment efficacy at the final visit was positive in approximately 93% of the patients, i.e. efficacy was rated ‘excellent’ in 13 patients, ‘good’ in 20 patients, and ‘satisfactory’ in 5 patients.

At the end of the study, the patients' compliance to treatment was rated ‘good’ (>80%) in 61.0% (N = 25) of all patients. Compared to the compliance to the previous therapy, compliance had improved in 14.6% (N = 6) of the patients. Increased compliance may have contributed to the improvements in lung function and respiratory symptoms. The final assessment of tolerability was slightly more positive according to the physicians' assessment than according to the patients' assessment. Possible reasons for this may be an underreporting of adverse events by the patients or that patients probably perceive their symptoms as being more severe than the physicians.

Since this trial was a non-interventional study the main limitation of the study is a missing control group and the small patient numbers. However, the observed period of six months before starting sequential therapy showed a significant decrease of lung function parameters in all patients, which might function as control data. Another limitation was that *Pseudomonas species* had not been described adequately in this study by molecular determination. Therefore, a post/pre comparison of different *Pseudomonas* lines could not be carried out satisfactorily. Moreover, the results need to be verified in a larger patient population.

Taken together, the results of this non-interventional, exploratory study in patients with CF and chronic *P. aeruginosa* infection of the lung indicated that, under the conditions of daily practice, treatment with inhaled antibiotics in sequential combination with tobramycin and colistin may have a beneficial effect on lung function and respiratory symptoms due to its ability to reduce exacerbation and intravenous treatment rates.

Sequential treatment with inhaled tobramycin and colistin was very well tolerated by most patients. However, physicians should be aware that bronchoconstriction may occur after inhalation of tobramycin or colistin.

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Disclosure Statement

No author has a conflict of interest in regards of drugs or assays discussed in this manuscript.

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