

Sinonasal inhalation of tobramycin vibrating aerosol in cystic fibrosis patients with upper airway *Pseudomonas aeruginosa* colonization: results of a randomized, double-blind, placebo-controlled pilot study

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Rationale: In cystic fibrosis (CF), the paranasal sinuses are sites of first and persistent colonization by pathogens such as *Pseudomonas aeruginosa*. Pathogens subsequently descend to the lower airways, with *P. aeruginosa* remaining the primary cause of premature death in patients with the inherited disease. Unlike conventional aerosols, vibrating aerosols applied with the PARI Sinus™ nebulizer deposit drugs into the paranasal sinuses. This trial assessed the effects of vibrating sinonasal inhalation of the antibiotic tobramycin in CF patients positive for *P. aeruginosa* in nasal lavage.

Objectives: To evaluate the effects of sinonasal inhalation of tobramycin on *P. aeruginosa* quantification in nasal lavage; and on patient quality of life, measured with the Sino-Nasal Outcome Test (SNOT-20), and otologic and renal safety and tolerability.

Methods: Patients were randomized to inhalation of tobramycin (80 mg/2 mL) or placebo (2 mL isotonic saline) once daily (4 minutes/nostril) with the PARI Sinus™ nebulizer over 28 days, with all patients eligible for a subsequent course of open-label inhalation of tobramycin for 28 days. Nasal lavage was obtained before starting and 2 days after the end of each treatment period by rinsing each nostril with 10 mL of isotonic saline.

Results: Nine patients participated, six initially receiving tobramycin and three placebo. Sinonasal inhalation was well tolerated, with serum tobramycin <0.5 mg/L and stable creatinine. *P. aeruginosa* quantity decreased in four of six (67%) patients given tobramycin, compared with zero of three given placebo (non-significant). SNOT-20 scores were significantly lower in the tobramycin than in the placebo group ($P=0.033$).

Conclusion: Sinonasal inhalation of vibrating antibiotic aerosols appears promising for reducing pathogen colonization of paranasal sinuses and for control of symptoms in patients with CF.

Keywords: PARI Sinus, nasal lavage, SNOT-20, cystic fibrosis, *Pseudomonas aeruginosa*, sinonasal, upper airways

Introduction

Cystic fibrosis (CF) is the most frequent life-threatening inherited disease in Caucasians, caused by defective ion transport which causes impaired mucociliary airway clearance. The life expectancy of CF patients has increased markedly in recent decades, with patients who would not have reached school age about 50 years ago now surviving to the fifth decade of life.¹ Improved prognosis in these patients is due primarily to aggressive treatment with systemically applied and bronchially inhaled antibiotics.² Nevertheless, pulmonary

manifestations associated with airway colonization by pathogens such as *Pseudomonas aeruginosa* account for up to 90% of fatal outcomes in patients with CF.³

The upper airways (UAW) and paranasal sinuses (PNS) have been shown to be involved in CF pathogen colonization. This respiratory compartment, with the same epithelial ion transport defect as the lower airways (LAW), has been associated with frequent rhinosinusitic symptoms in patients with CF, including nasal obstruction, smelling deficiencies, impaired sleep, chronic pain, and abundant secretions.⁴ Most importantly, the sinonasal niche has been identified as a site of initial and persistent airway colonization by *P. aeruginosa* and other pathogens.^{5,6} Bacteria in the PNS have been shown to adapt to host systems, diversify, and spread into the LAW.⁷ Over time, 96% of CF patients with *P. aeruginosa* colonization harbor identical clones in both airway levels UAW and LAW.⁸

Besides impaired mucociliary clearance, anatomic and immunologic factors appear to facilitate the persistence of pathogens in the sinonasal area.^{9–11} Anatomically, the ostiomeatal complex, with narrow orifices into the PNS, can be easily obstructed by inflamed mucosa, mucus, crusts, and nasal polyps.^{4,12} Immunologically, the sinonasal area is distinguished by predominantly immunoglobulin A responses to bacterial colonization, which are less effective than neutrophil-dominant host responses to bacteria in the LAW associated with oxidative burst.^{7,10} In addition, the deposition of systemically applied antibiotics in the PNS, as well as in abscesses and other hollow structures filled with mucus, pus, or mucocoeles, appears to be limited.^{5,6}

We recently described a juvenile CF patient with isolated first sinonasal *P. aeruginosa* colonization.⁵ The pathogen was detected by nasal lavage (NL) with 10 mL of isotonic saline per nostril, as part of our multicentric study program for the assessment of pathogen colonization in CF UAW and LAW.⁸ After 2 weeks of treatment with intravenous antibiotics, *P. aeruginosa* was detected again only in the patient's NL. Since the current standards of care for CF do not include sampling of the UAW, the pathogen would not have been detected if we had followed the standards. The failure of systemic antibiotics to eradicate *P. aeruginosa* from the sinonasal compartment of our patient prompted a discussion of further therapeutic options.

In treating CF patients with LAW involvement, bronchial inhalation of antibiotics was found to achieve the highest drug concentrations in sputum, preventing or reducing pulmonary destruction in *P. aeruginosa* colonized lungs.¹³ Therefore, it has become the gold standard of treatment in patients with CF.

Conventional aerosols nebulized nasally were shown not to reach the PNS with their narrow orifices. Only after surgical widening of the sinus ostia could common aerosols or medication applied by NL be deposited within the PNS.¹⁴

In contrast, vibrating aerosols applied with the PARI Sinus™ nebulizer (PARI Corp, Starnberg, Germany) have been shown scintigraphically to reach the PNS in sufficient amounts.^{15,16} We recently performed the first clinical trial with this device, applying dornase alfa, a recombinant DNase approved as a mucolytic agent for CF patients with chronic rhinosinusitis and LAW involvement. A crossover, double-blind, placebo-controlled pilot trial in five patients found that sinonasal inhalation of dornase alfa significantly reduced symptoms ($P=0.019$), as assessed by the Sino-Nasal Outcome Test 20 (SNOT-20).¹⁷ These results were recently confirmed in a larger trial (unpublished data, JG Mainz, 2013).

We utilized a new approach to eliminate pathogens from the above-described patient with isolated primary sinonasal colonization by *P. aeruginosa* that persisted after 14 days of intravenous antibiotic treatment.⁵ This patient was treated with 80 mg sinonasally inhaled tobramycin once a day over 28 days as a vibrating aerosol using the PARI Sinus™ device.¹⁶ Since October 2006, this patient has remained negative for *P. aeruginosa* in the UAW and LAW (>50 samples each) and negative for serum *P. aeruginosa* antibodies.

We recently reported on the prevention of pulmonary colonization by *P. aeruginosa* in CF patients undergoing lung transplantation (LTx).⁶ Two CF patients showed sinonasal persistence of genetically identical *P. aeruginosa* clones after LTx. One of them showed colonization of the transplanted lungs by identical clones within weeks after LTx, accompanied by a critical course and transplant rejection. The second patient was treated with inhaled colistin 1 Mio IU sinonasally and 2×1 Mio IU bronchially every day for more than 52 months. She remains free of *P. aeruginosa* in sputum and bronchoalveolar lavage since transplantation and her lung function is stable, without rejection/bronchiolitis obliterans syndrome, which has been reported to be enhanced by *P. aeruginosa* colonization of transplanted lungs.¹⁸

These case reports describing off-label sinonasal nebulization of antibiotics with the novel device have shown promising results. However, prospective controlled trials are required to assess safety, tolerability, and efficacy of this new approach. To our knowledge, our double-blind, placebo-controlled pilot trial of sinonasal inhalation with tobramycin (Infectopharm, Arnz. und Consilium, Heppenheim, Germany) is the first controlled trial on sinonasal vibrating inhalation of antibiotics. The primary endpoint was the change in numbers

of *P. aeruginosa* colonies in NL after sinonasal inhalation of tobramycin or placebo for 28 days. The secondary endpoints included effects on SNOT-20 scores, rhinoscopic findings, and pulmonary colonization and function, as well as drug safety and tolerability.

Materials and methods

Study design

This bicentric, double-blind, placebo-controlled, randomized prospective parallel group study, followed by facultative open-label antibiotic therapy during a second treatment period, was the pilot for a planned efficacy trial of the same design.

Study subjects

Patients were included if they had been diagnosed with CF, as confirmed by two positive sweat tests and/or genetic analysis, and showed chronic *P. aeruginosa* colonization of the LAW,¹⁹ with the pathogen detected in NL⁸ within 28 days prior to inclusion. Further inclusion criteria were age ≥ 7 years, ability to utilize the PARI SinusTM for nasal inhalation, and the ability to perform NLs.

Patients who had undergone sinonasal surgery within 3 months were excluded, as were patients with: nasal bleeding; ear drum perforation; severe damage to the eighth cranial nerve (nervus vestibulocochlearis), including impaired hearing or vertigo; or advanced renal insufficiency. Further exclusion criteria were critical general condition, forced expiratory volume in 1 second (FEV₁) $< 30\%$, pulse oximetry $< 93\%$ without O₂-substitution, exacerbation at randomization with need for new systemic antibiotics, treatment with systemic (oral or intravenous) antibiotics for *P. aeruginosa* infection 14 days before randomization or during the study, history of clinical relevant adverse reactions to tobramycin or placebo, pregnancy or breastfeeding, ongoing participation in another therapeutic clinical study, or participation in an investigational drug study within 30 days prior to baseline. Females of child-bearing potential needed to use effective methods of contraception.

For safety reasons within the trial, patients with serum tobramycin concentrations > 2.0 mg/L 1 hour after sinonasal inhalation on day 1 or 30 was required to be excluded from the trial. However, there were no such patients.

Written informed consent was obtained from all patients and from the parents or guardians of children, where appropriate. The study was approved by the local Ethics Committee and the Federal Institute for Drugs and Medical

Devices (BfArM) and has been registered with the US National Institutes of Health (NCT00265434).

Preparation and administration of study medication

Patients with nasal obstruction were allowed decongestants before sinonasal inhalation, and secretions or crusts in nasal airways could be removed by therapeutic NL with 125 mL sterile isotonic saline per side. Both treatments were documented in patients' diaries.

Sinonasal inhalation was performed using the PARI SinusTM compressor together with a PARI LC SPRINT STARTM nebulizer (PARI Corp) as previously described.¹⁷ In brief, the study drug was aerosolized into one nostril, while the contralateral nostril was occluded and the soft palate elevated as recommended for NL.^{15–17}

During the double-blind, placebo-controlled part of the study, subjects were randomized to sinonasal inhalation of 80 mg tobramycin (Gernebcin[®]) or placebo (isotonic saline) once daily for 28 days with the PARI SinusTM nebulizer; drug was administered to each nostril for 4 minutes, with the maximum volume per nostril of 1 mL. During this part of the trial, we had planned to treat equal numbers of patients with tobramycin and placebo, with patients randomized in blocks of four patients each and stratified by age (< 18 and ≥ 18 years) and by pulmonary function ($<$ and \geq FEV₁ medians of the German CF patients, quality register).

Following the double-blinded treatment period, all patients with *P. aeruginosa* in NL were offered open-label facultative sinonasal treatment with tobramycin for 28 days. Subjects were examined at baseline and on days 30 and 60. The primary outcome was change in numbers of *P. aeruginosa* colonies in NL fluid (method of sampling: see below), measured before the start of sinonasal treatment and 2–3 days after the end of treatment. The delay of 2–3 days from active treatment to measurement was selected to prevent interference by any antimicrobial activity in NL fluid.

Secondary outcome parameters included: changes in ENT (ear, nose, and throat)-related quality of life (QoL), as assessed by the German-adapted version of the SNOT-20 (SNOT-20-GAV); changes in pulmonary function, as assessed by forced vital capacity (FVC), FEV₁, and forced expiratory flow at 75%–25% of FVC (FEF_{75–25}); changes in rhinoscopic appearance of the mucosa, and assessments of secretions, crusts, and polyps, as determined by bilateral nasal endoscopy; and tolerance of the inhalation procedure and the medication, as assessed by high-tonal sensorineural impairment of hearing, serum tobramycin levels 1 hour after

sinonasal inhalation, and differences in serum creatinine concentration before and after sinonasal inhalation.

Quantification of *P. aeruginosa* in NL

Nasal secretions were collected in our outpatient clinic on days 1, 30, and 60 as described previously.⁸ Briefly, each nostril was flushed with 10 mL isotonic saline during head reclamation, and the fluid was collected into a sterile beaker after 10 seconds by anteflection.

NL fluid and sputum from the head of each patient were processed according to current microbiological standards for Gram-positive and -negative bacteria and fungi. The numbers of *P. aeruginosa* colonies were quantified in NL fluid by plating 0.1 mL aliquots of NL fluid onto different media. After incubation, *P. aeruginosa* was quantified in classes by assessing the numbers of colony forming units (CFUs) and multiplying by factor 10:

<10¹ CFU/mL

10² CFU/mL

10³ CFU/mL

10⁴ CFU/mL

>10⁵ CFU/mL.

Pathogens in sputum were assessed qualitatively without quantification.

SNOT-20

The SNOT-20 is a disease-specific, health-related, 20-item QoL measure for patients with rhinosinusitis that focuses on both rhinologic and general complaints.^{20,21} SNOT-20 scores range between 0 and 5 for each item, with higher scores indicating a greater rhinosinusitis-related health burden. The validated SNOT-20-GAV used here²⁰ includes four specific sections:

1. SNOT overall score, including all 20 items;
2. Primary nasal symptoms, including “nasal obstruction”, “sneezing”, “runny nose”, “thick nasal discharge”, and “reduced sense of smell”;
3. Secondary nasal symptoms, including “postnasal discharge”, “need to clear one’s throat or sore throat”, “dry throat”, “cough”, “ear fullness”, “ear pain”, and “facial pain”; and
4. General quality of life, including “dizziness”, “difficulty falling asleep”, “waking up at night”, “waking up feeling tired”, “reduced productivity”, “reduced concentration”, “frustrated/restless/irritable”, “a feeling of sadness”, and “embarrassment”.

Pulmonary function testing

Pulmonary function testing was performed with the Master Screen Body/Spirometer (Viasys Corp; Jaeger/Toennies/

CareFusion, Hoechberg, Germany) following the American Thoracic Society recommendations.²²

Nasal endoscopy

Anterior rhinoscopy was performed by flexible or rigid endoscopy after decongestion. Staging of nasal polyps on each side was defined as Stage 0 (no polypoid mucosa around the middle nasal concha); Stage 1 (nasal polyps limited to the middle nasal meatus); Stage 2 (nasal polyps growing or hypertrophic to the inferior nasal meatus); and Stage 3 (complete obturation of the nose through polyps).

Tolerance of therapy

Hearing tests

Pure tone audiometry was performed on days 1, 30, and 60 to assess potential ototoxicity according to current standards.²³

Serum analysis

Blood samples were obtained on day 1, 1 hour after the first inhalation, and on day 30. Blood samples were also obtained on day 60 from the patients who participated in the open-label part of the trial. Serum concentrations of tobramycin and creatinine were measured.

Biometry

Data were analyzed statistically using SPSS (IBM Corporation, Armonk, NY, USA) software, versions 18.0 and 19.0. Normal distribution was assessed by the Kolmogorov–Smirnov test. Differences in SNOT scores and pulmonary function in the treatment groups were analyzed by Mann–Whitney *U*-tests, and differences in numbers of *P. aeruginosa* colonies were assessed by paired *t*-tests and repeated-measures analysis of variance. Significance level was defined as an alpha of 0.05.

Results

Demographic data

Nine patients, six males and three females, were included, seven from Jena and two from Tübingen. Patients ranged in age from 10.6 to 38.7 years (mean 22.4±7.6 years). Demographic data are presented in Table 1.

Changes in numbers of *P. aeruginosa* colonies after 28 days of blinded sinonasal inhalation of tobramycin or isotonic saline

Figure 1 and Table 2 compare the effects of sinonasal inhalation of tobramycin and placebo (isotonic saline)

Table 1 Characterization of all randomized cystic fibrosis patients

Patient number	Age (years) ^a	Genotype	Sex	S.a. LAW ^b	S.a. UAW ^b	FEV ₁ %	FEF ₇₅₋₂₅ %	Preceding ENT-surgery
1	22.9	dF508/405+ IG>A	M	–	–	109.0	91.3	–
2	21.8	dF508/R347P	M	+	+	85.4	53.5	–
3	23.1	dF508/dF508	F	–	–	66.3	28.8	Polypectomy (1995)
4	38.7	dF508/unknown	F	–	–	64.3	35.5	–
5	21.6	dF508/dF508	M	+	+	71.6	38.8	–
6	24.5	dF508/dF508	M	+	+	47.0	17.4	FESS (before 2010)
7	22.8	dF508/dF508	M	–	–	102.5	85.6	–
8	15.3	dF508/E92X	F	Unknown	Unknown	86.0	58.0	–
9	10.6	dF508/dF508	M	Unknown	Unknown	83.0	53.0	FESS (2007 and 2008)

Notes: ^aAge at inclusion; ^b± detected/not detected, respectively.

Abbreviations: S.a., *Staphylococcus aureus*; LAW, lower airways; UAW, upper airways; FEV₁, forced expiratory volume in 1 second; FEF₇₅₋₂₅, forced expiratory flow at 75%–25% of forced vital capacity; ENT, ear, nose, and throat; M, male; F, female; FESS, functional endoscopic sinus surgery.

on numbers of *P. aeruginosa* colonies in NL. Of the nine subjects randomized, six were randomized to tobramycin and three to isotonic saline. This unequal distribution occurred randomly and because of screening failure. The numbers of *P. aeruginosa* colonies decreased in four of the six (67%) tobramycin-treated patients, remaining unchanged in one patient (16.7%) and increasing in one (16.7%). In contrast, none of the patients inhaling isotonic saline experienced a reduction in *P. aeruginosa* colonies in NL; rather, the number of colonies remained unchanged in one patient (33.3%) and increased in the other two (66.7%). Due to the small numbers of patients in this pilot study, the between-group differences did not reach statistical significance.

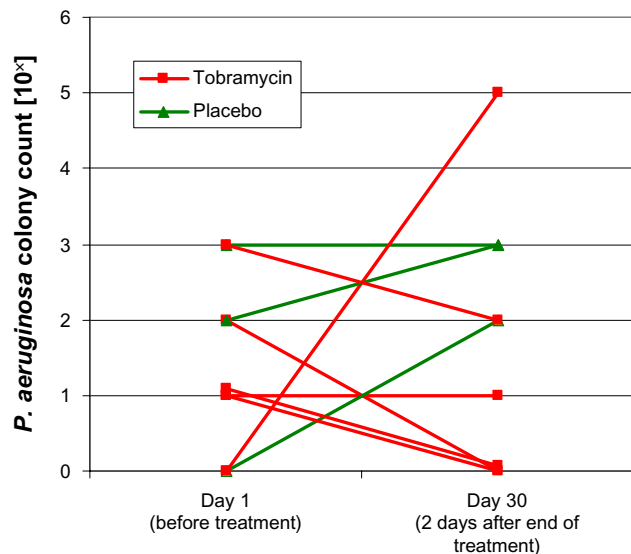


Figure 1 Logarithmic changes in *Pseudomonas aeruginosa* colony count in nasal lavage from day 1 (before first sinonasal inhalation) to day 30 (2 days after last sinonasal inhalation) within the double-blind, placebo-controlled phase (three patients inhaled isotonic saline; and six, tobramycin).

Effects of tobramycin and isotonic saline on SNOT-20 scores

Compared with isotonic saline, sinonasal inhalation of tobramycin had significant positive effects on sinonasal symptom-related QoL, by total SNOT-20-GAV scores^{20,21} (Table 3 and Figure 2; $P=0.033$).

Subjects in the tobramycin group showed a 6.67 ± 4.71 point decrease (95% confidence interval 0.78–12.55 points) in sinonasal symptom score, whereas subjects in the control group showed an increase of 3.34 ± 2.12 points ($P=0.033$). Altogether, changes in SNOT-20-GAV-scores of ≥ 5 points were considered clinically relevant.

Table 2 Effects of isotonic saline or tobramycin on *Pseudomonas aeruginosa* colony count in NL within the blinded study period (V1–V2) and facultative open-label sinonasal antibiotic treatment (V2–V3), as offered to all participating patients

	<i>P. aeruginosa</i> colony count in NL (category, 10 ^x)		
	28 days of DBPC treatment	28 days of facultative open-label treatment with tobramycin	
	V1 (day 1)	V2 (day 30)	V3 (day 60)
Tob/†	10*	10*	†
Tob/tob	100	0	0
Tob/tob	0	100,000	0
Tob/tob	1,000	100	100
Tob/†	10*	0	†
Tob/†	10*	0	†
Iso sal/tob	1,000	1,000	1,000
Iso sal/tob	0	100	0
Iso sal/tob	100	1,000	1,000

Notes: **P. aeruginosa* detection in NL below quantifiable levels (taken as 10^x colony counts); †patients not taking the second open-label treatment with tobramycin.

Abbreviations: NL, nasal lavage; DBPC, double-blind, placebo-controlled; tob, tobramycin; iso sal, isotonic saline.

Table 3 Changes in quality of life assessed by SNOT-20-GAV overall scores: effects of tobramycin or isotonic saline treatment

	SNOT-20-GAV overall scores		
	28 days of DBPC treatment	28 days of facultative open-label treatment with tobramycin	
	V1 (day 1)	V2 (day 30)	V3 (day 60)
Tob/ [†]	10	6	8
Tob/tob	19	14	9
Tob/tob	7	4	11
Tob/tob	8	3	4
Tob/ [†]	18	0	nd
Tob/ [†]	35	30	nd
Iso sal/tob	13	24	24
Iso sal/tob	21	19	9
Iso sal/tob	9	7	8

Note: [†]Patients not taking the second open-label treatment with tobramycin.

Abbreviations: SNOT-20-GAV, German-adapted version of the Sino-Nasal Outcome Test; DBPC, double-blind, placebo-controlled; nd, not done; tob, tobramycin; iso sal, isotonic saline.

Effects of tobramycin or isotonic saline on pulmonary function

No significant changes in FVC, FEV₁, and FEF₇₅₋₂₅ occurred in either the tobramycin (6) or isotonic saline (3) groups during the first 28 days of treatment (Table 4).

Rhinoscopy

Mucosal status, polyps, and secretions remained unchanged from day 1 to day 30, as assessed by rhinoscopy, in

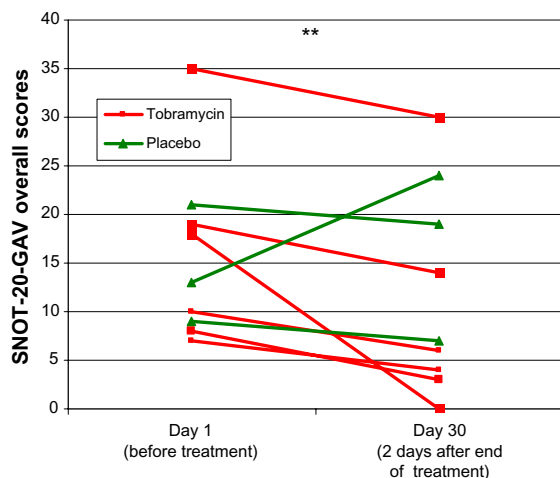


Figure 2 Effects of tobramycin or isotonic saline (placebo) treatment on quality of life assessed by SNOT-20-GAV overall scores.

Notes: **Significant changes for tobramycin, compared with isotonic saline; $P=0.033$.

Abbreviation: SNOT-20-GAV, German-adapted version of the Sino-Nasal Outcome Test.

Table 4 Individual changes in pulmonary function (FEV₁ in % predicted) after 28 days of sinonasal therapy with tobramycin respective isotonic saline

	Pulmonary function (FEV ₁)		
	28 days of DBPC treatment	28 days of facultative open-label treatment with tobramycin	
	V1 (day 1)	V2 (day 30)	V3 (day 60)
Tob/ [†]	109.0	92.1	nd
Tob/tob	64.3	63.1	62.0
Tob/tob	71.6	72.1	69.7
Tob/tob	102.5	98.5	101.2
Tob/ [†]	86.0	93.8	†
Tob/ [†]	83.0	80.0	†
Iso sal/tob	85.4	82.8	80.2
Iso sal/tob	66.3	64.2	66.7
Iso sal/tob	47.0	42.6	40.6

Note: [†]Patients not taking the second open-label treatment with tobramycin.

Abbreviations: FEV₁, forced expiratory volume in 1 second; DBPC, double-blind, placebo-controlled; nd, not done; tob, tobramycin; iso sal, isotonic saline.

four patients in the tobramycin group and two in the placebo group.

Drug safety and tolerability

During the trial, serum creatinine concentrations were not elevated after sinonasal inhalation of tobramycin (Table 5), suggesting that sinonasal inhalation of 80 mg tobramycin per day did not affect renal function. In contrast, systemic application of the aminoglycoside has been shown to be potentially nephrotoxic. Serum concentrations of tobramycin 1 hour after sinonasal inhalation of antibiotic did not exceed 0.5 mg/L in all included patients.

Hearing tests were performed before and after both treatment periods. After the open treatment period, one patient showed impaired hearing with a typical pattern of noise damage. She had been at a hard-rock concert the day before, where she stood in front of an amplifier. No hearing abnormalities were observed in any other patients.

Adverse events

Overall, topical sinonasal inhalation of tobramycin was well tolerated. Two patients were admitted for scheduled intravenous antibiotic therapy, both during the second, open-label period, with tobramycin omitted. One patient showed bilateral hearing impairment at high frequencies on day 55 of tobramycin inhalation; this impairment was probably caused by an acute acoustic trauma (see above). No serious adverse events were observed.

Table 5 Drug safety and tolerability of sinonasal inhalation of tobramycin compared with isotonic saline

Patient number	Age at study entry (years)	Visit number	Therapy >blinded< open-label	Tobramycin serum level 1 hour after inhalation (mg/L)	Serum creatinine ($\mu\text{mol/L}$)	Audiometry	Facultative adjunct treatment
01-01*	22.9	1	>tobramycin<	<0.5	84.0	Normal	No
		2	nd	nd*	nd*	Normal	
		3		nd	nd	nd	
01-02	21.8	1	>isotonic saline<	<0.5	83.0	Normal	Yes
		2	tobramycin	0.2	76.0	Normal	
		3		<0.5	79.0	Normal	
01-03	23.1	1	>isotonic saline<	<0.5	61.0	Normal	Yes
		2	tobramycin	<0.5	62.0	Normal	
		3		0.3	56.0	Pathological***	
01-04	38.7	1	>tobramycin<	<0.5	66.0	Normal	Yes
		2	tobramycin	<0.5	66.0	Normal	
		3		<0.5	65.0	Normal	
01-05	21.6	1	>tobramycin<	<0.5	75.0	Normal	Yes
		2	tobramycin	0.3	72.0	Normal	
		3		<0.5	68.0	Normal	
01-06	24.5	1	>isotonic saline<	<0.5	70.0	Normal	Yes
		2	tobramycin	<0.5	72.0	Normal	
		3		<0.5	69.0	Normal	
01-07	22.8	1	>tobramycin<	<0.5	121.0**	Normal	Yes
		2	tobramycin	<0.5	124.0**	Normal	
		3		<0.5	120.0**	Normal	
02-08	15.3	1	>tobramycin<	0.18	53.0	Normal	No
		2	nd	0.28	61.9	Normal	
		3		nd	nd	nd	
02-09*	22.8	1	>tobramycin<	nd	nd	Normal	No
		2	nd	nd	nd	Normal	
		3		nd	nd	nd	

Notes: *Patients refused blood sampling; **elevated serum creatinine without clinical symptoms, not considered as reason for exclusion after discussion with nephrologists remaining at preceding level during therapy; ***bilateral high tonal sensorineural impairment of hearing was found in patient 01-03 typical for noise trauma. Blinded therapy phases (the first 30 days within the trial) are marked >in brackets<.

Abbreviation: nd, not done.

Discussion

Impaired mucociliary clearance in CF patients facilitates the persistence of pathogens such as *P. aeruginosa* in the UAW, allowing these pathogens to diversify, adapt to the host immune system, and proliferate to the lungs.⁷ *P. aeruginosa* is the most relevant causative agent of progressive lung destruction and premature death in patients with the inherited disease. However, pathomechanisms allowing pathogen nidation in CF UAW, as well as the prevention and treatment of infection, have not been determined.^{4,24,25} Identification of the sinonasal niche as the site of first and recurrent airway colonization, however, can lead to the development of additional therapeutic options.

The absence of vascularization within the PNS makes it unlikely that systemic antibiotic treatment⁵ will achieve clinically relevant therapeutic levels within the PNS,

especially those with retained purulent secretions or mucocèles. Therefore topical application of antibiotics may be of therapeutic interest. The highest concentration of antibiotic in sputum resulted from inhalation into the lungs.¹³ Bronchial inhalation of tobramycin, colomycin, and aztreonam lysine directly at the target site has become the gold standard of CF pulmonary therapy, contributing to considerable improvements in life expectancy in patients with the inherited disease.¹ In addition, topical antibiotics do not usually lead to critical blood levels and are therefore associated with fewer systemic side effects.

Conventional aerosols inhaled into the UAW do not migrate into the sinuses.¹⁴ Surgical enlargement of the sinus orifices is apparently necessary to deliver drugs administered with conventional nebulizers to the sinus mucosae.^{14,26,27} In contrast, scintigraphic deposition studies showed that

pressure gradients induced by vibrating air flow facilitate the deposition of significant amounts of aerosols into sinus cavities.^{15,16} This principle of vibrating aerosols was implemented in the PARI Sinus™ nebulizer, with a superimposed frequency of approximately 44.5 Hz.¹⁵ We performed the first double-blind, placebo controlled trial of this device, nebulizing the mucoactive recombinant dornase alfa into PNS of CF patients with chronic rhinosinusitis.¹⁷ Sinonasal symptoms, as assessed by SNOT-20-GAV scores, were significantly reduced in the dornase alfa group, but not in the placebo group, with results confirmed in a subsequent principal study. Presently, we are conducting a Germany-wide double-blind, placebo-controlled trial, using the same protocol, comparing sinonasal inhalation of hypertonic and isotonic saline (ClinicalTrials.gov identifier NCT01086839).

In addition to its use in administering mucolytic agents, this new method can be used to administer antibiotics, in order to eradicate and/or reduce PNS colonization by pathogens.^{8,28} We found that this method was successful in several CF patients and published first case reports on the topic.^{5,6} To our knowledge, the pilot study described here is the first prospective controlled trial on sinonasal inhalation of antibiotics as vibrating aerosols.

During the initial double-blind, placebo controlled phase of the trial, CF patients with chronic pulmonary *P. aeruginosa* colonization and with pathogen detected in NL inhaled tobramycin or isotonic saline as vibrating aerosols for 28 days. Subjects were assessed before and 2 days after the end of treatment, in order to allow sufficient time to wash out the antimicrobial before quantifying *P. aeruginosa* colonies in NL. During this phase of the trial, six subjects received tobramycin and three placebo. The unequal distribution of patients treated with tobramycin and placebo resulted from separate randomizations at the two participating centers and because of screening failure.

The numbers of *P. aeruginosa* colonies were reduced in four of the six patients inhaling tobramycin sinonasally but in none of the patients inhaling placebo. Due to the small numbers of patients, this difference did not reach statistical difference. However, ENT-related QoL, as assessed by SNOT-20-GAV scores, differed significantly in the two groups ($P=0.033$). Moreover, sinonasal inhalation of tobramycin as a vibrating aerosol was well tolerated.

During the second unblinded phase of the trial, six of the nine patients elected to take open-label sinonasal tobramycin, three patients in the original tobramycin group and all three in the placebo group. During all 12 of the 28 day courses of treatment with tobramycin, none of these patients had serum tobramycin concentrations, measured 1 hour after

inhalation, exceeding 0.5 mg/L, and none showed elevations in renal parameters in serum. Mucosal tolerability was also good, with only one patient reporting bloody nasal secretion. Moreover, none of the patients inhaling isotonic saline developed epistaxis.

Patients were assessed by pure tone audiometry before treatment and after 30 and 60 days. One of the patients experienced hearing loss on day 60 after 28 days of sinonasal inhalation of tobramycin, but audiometry revealed hearing deficiencies typical of noise damage. The patient had attended a hard rock concert the evening before audiologic testing, where she had been exposed to loud noise standing in front of sound amplifiers for a long period of time. However, exposure to noise may have placed additional stress on a vulnerable inner ear. In general, our findings show that patients tolerated sinonasal inhalation of 80 mg/day tobramycin, as well as tolerating additional bronchial inhalation of 300 mg tobramycin bid.

We observed a trend towards a reduction in the number of *P. aeruginosa* colonies in NL in patients with chronic airway colonization by the pathogen. Mucoïd phenotypes of *P. aeruginosa* were observed in sinonasal colonies of CF patients. We did not expect, however, that antibody treatment would eradicate the pathogen after longstanding pulmonary colonization. Moreover, we would expect recolonization of the PNS from the LAW over time.^{8,28,29}

Remarkably, despite the small patient cohort and relatively short treatment period, we found that sinonasal antibiotic inhalation had significant effects on QoL, similar to findings in our pilot study of sinonasal inhalation with dornase alfa.¹⁷ In that crossover trial, patients were randomized to sinonasal inhalation of dornase alfa or isotonic saline for 28 days, followed by a 28-day washout period and a crossover to the other therapeutic arm for 28 days. Significant improvements in SNOT-20-GAV scores were observed in the dornase alfa relative to the placebo group, findings even more pronounced in a subsequent principal study that included 23 CF patients.

The results of our pilot study with tobramycin can be used to calculate sample sizes for a subsequent principal trial with an analogous design. Our findings on sinonasal colonization with *P. aeruginosa* strongly support the routine microbiological assessment of the UAW, as long as patients are not chronically colonized by the pathogen. NL appears to be the most sensitive noninvasive method of detecting pathogens in the UAW.⁸ Routine testing of NL may enable additional monitoring of the airways in CF patients and keep them free of the pathogen. A prospective, multicenter trial on first detection and subsequent eradication of *P. aeruginosa* in the UAW compartment may be useful in assessing measures for long-term prevention of infection.

Conclusion

Sinonasal inhalation of antibiotics applied as vibrating aerosols with adequate devices showed promise as a noninvasive method to reduce or even eradicate *P. aeruginosa* (and other pathogens) from susceptible airways. A tobramycin dosage of 80 mg per day was safe and well tolerated. Additional studies with larger patient cohorts are needed.

Disclosure

The study was supported by Infectopharm, Germany (grant and study supply) and PARI Corp, Germany (study supply). The support given by these companies did not influence the objectivity of the study and no other benefit was received. We confirm that none of the authors has any relevant commercial associations or financial interests.

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