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Research paper

Dry powder inhalation of antibiotics in cystic fibrosis therapy: part 2 Inhalation of a novel colistin dry powder formulation: a feasibility study in healthy volunteers and patients

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Abstract

The aim of the present study was to perform a proof of principle study with a new colistin dry powder inhalation system in six healthy volunteers and five patients with cystic fibrosis. All subjects were asked to inhale 25 mg colistin sulfate dry powder. The patients were also asked to nebulize 160 mg colistin sulfomethate as a solution. Colistin serum concentrations were determined as an indirect parameter to compare both forms of administration. Pulmonary function tests were performed. Peak serum colistin concentrations ranged from 14 to 59 μg/l in volunteers after inhalation of 25 mg as dry powder. In patients, peak concentrations ranged from 18 to 64 μg/l after nebulization of 160 mg colistin sulfomethate solution and from 77 to 159 μg/l after inhalation of 25 mg colistin sulfate dry powder. Pulmonary function tests were not significantly different after inhalation of the dry powder by the volunteers nor after nebulization of the solution by the patients. In some patients a decrease in pulmonary function and moderate to severe cough was observed after inhalation of the dry powder. The new colistin inhaler provides an attractive alternative for nebulized colistin and was highly appreciated by the patients. The decrease in pulmonary function and cough in patients is a drawback, which may be overcome by dose reduction and a further improvement of the new dosage form.

Keywords: Clinical use; Colistin sulfate; Colistin sulfomethate; Cystic fibrosis; Dry powder inhaler; Feasibility study

1. Introduction

Patients with cystic fibrosis (CF) and chronic pulmonary infection with *Pseudomonas aeruginosa* may benefit from the inhalation of antibiotics [1,2]. In addition, inhalation is also recommended in the European Consensus document on antibiotic therapy against *P. aeruginosa* in CF [3]. Several antibiotics have been investigated for administration by inhalation and, at present, nebulized tobramycin and colistin are used in daily practice by CF patients in many countries.

Dry powder inhalation may provide a suitable alternative for drug nebulization. Few studies regarding the inhalation of micronized antibiotics have already been reported. In one study, the inhalation of 180 mg micronized gentamicin sulfate with carrier lactose, using the Glaxo Rotahaler®, was compared with the inhalation of 160 mg of the same drug from nebulization [4]. Similar concentrations of gentamicin were detected in bronchoalveolar lavage fluid, but the authors reported cough in half of the patients during dry powder inhalation as a serious drawback. In a second study, in vitro depositions for tobramycin from nebulization (160 mg dose) and dry powder aerosol generation (22 mg dose) were compared at a flow rate of 60 l/min [5].

In a more recent study [6], a much higher sputum concentration (97.2 μg/g) was found after nebulization of 160 mg gentamicin than after dry powder inhalation of the same dose (13.1 μg/g). Nevertheless, both concentrations appeared to be equally efficient in decreasing the sputum *Pseudomonas* concentration.

Colistin is nebulized as the sulfomethate salt. In vivo the

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colistin sulfomethate is converted back to colistin sulfate by spontaneous hydrolysis. In vitro assessment of the bactericidal activity in terms of minimal bactericidal concentration (MBC) and minimal inhibitory concentration (MIC) shows a much higher activity for colistin sulfate than for colistin sulfomethate. Furthermore, the colistin sulfomethate activity was delayed, probably because of the requirement of hydrolytic activation [7]. This means that a lower amount of the sulfate may be necessary for an equivalent antimicrobial effect.

de Boer et al. [8] described in part 1 of this study the development of a colistin sulfate dry powder formulation for inhalation. The formulation was tested in vitro for deposition behavior using a test inhaler. An in vitro deposition efficiency of at least 40% was reached with this formulation and the test inhaler. The test inhaler was improved with a dose system for use in the in vivo study discussed in this paper. With the new formulation and inhaler the time to administer the dose of colistin will decrease from at least 20 min to less than a minute. Furthermore, the dry powder inhaler is relatively small and does not need an external power source. It is expected that patient compliance will benefit from this increase in comfort. Furthermore, more frequent dosing may be possible.

The aim of this study was to assess the suitability of the new dry powder formulation in healthy volunteers and patients. This first in vivo study has to be considered as a proof of principle for the newly developed dry powder inhaler.

2. Materials and methods

2.1. Volunteers

Six healthy volunteers were recruited by advertisement. Only non-smoking adults (age 22.8 ± 2.4 years, height 173.2 ± 6.0 cm and weight 69.2 ± 6.2 kg) with no history of a chronic pulmonary disease were included. The volunteers were asked to inhale 25 mg of colistin sulfate dry powder mixture. The study was carried out according to the Helsinki Declaration and was approved by the ethical review board of the hospital. Volunteers were fully informed by the investigator and written consent was obtained from every volunteer.

2.2. Patients

Five stable CF patients (age 33.8 ± 6.3 years, height 175.2 ± 9.8 cm and weight 65.8 ± 18.8 kg), admitted for regular check up to the outpatient clinic of the Adult Cystic Fibrosis Center at the Leyenburg Hospital in The Hague, The Netherlands, were enrolled in the study. CF was diagnosed by clinical symptoms and confirmed by pathological sweat tests or DNA analysis. All patients were using nebulized colistin daily for a period of more than 6 months.

The patients were asked to stop the daily nebulization of colistin 3 days prior to the visit of the outpatient clinic. They were asked to nebulize 160 mg colistin with a Porta-Neb Ventstream® nebulizer compressor combination on day 4 during the visit to the clinic. Patients were discharged after the collection of blood samples. During a second visit to the outpatient clinic, the patients were asked to inhale 25 mg of colistin sulfate dry powder mixture, again after a 3 day interruption of the daily nebulization of colistin. After blood sample collection the patients were discharged and the usual colistin therapy was continued. The study was carried out according to the Helsinki Declaration and was approved by the ethical review board of the hospital. Patients were fully informed by the investigator and written consent was obtained from every patient.

2.3. Blood sample collection

Five serial blood samples were drawn before (to check wash out) and at 0.5, 1, 2, 3 and 4 h after the inhalation (or nebulization) of colistin by the volunteers and the patients.

2.4. Pulmonary function

Pulmonary function tests were performed before, immediately after and 3 h after inhalation (or nebulization) both in the volunteers and the patients. Inspiratory vital capacity (IVC), forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1) were measured in all subjects using a calibrated Masterlab pneumotachograph (Jaeger, Würzburg, Germany). Pulmonary functions were related to expected baseline values [9].

2.5. Nebulization of colistin

Colistin sulfomethate (Colymicine®, Rhône-Poulenc Rorer, Amstelveen, The Netherlands) was supplied by the hospital pharmacy. An amount of 160 mg colistin was dissolved in 6 ml 0.9% aqueous sodium chloride solution. The pH of this solution was approximately 7.2 and osmolality was 362 mosmol/kg.

The solution was aerosolized with a combination of a Porta-Neb® compressor and a Ventstream® jet nebulizer (Medic Aid, Romedic, Meerssen, The Netherlands). The patients were instructed to operate the device until the complete dose was released. The devices were weighed before and after use. It was not possible to sample the remaining solution after nebulization for concentration measurements. Therefore, the actual dose administered was calculated from weight measurements before and after aerosolization only.

The performance of the nebulizer/compressor/drug solution combination was evaluated in vitro by particle size distribution measurement. The particle size distribution of the aerosol cloud from the nebulizers was measured with a Sympatec HELOS Compact laser diffraction analyzer, model KA (Sympatec GmbH, Clausthal-Zellerfeld, Germany) [10]. The particle size distribution, depicted as

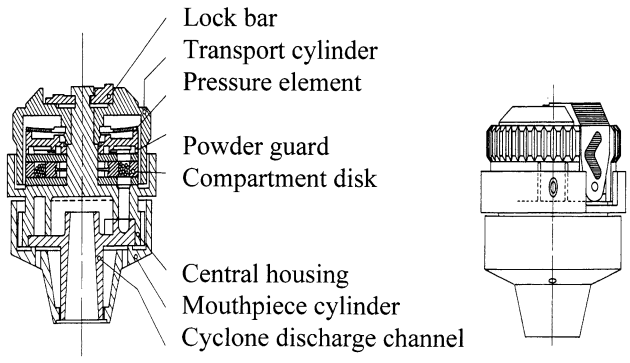

Fig. 1. The improved test inhaler with the dose system.

X_{50}, was found to be within the range of 1–5 μm (mean 2.51 μm).

2.6. Inhalation of colistin dry powder

The design and development of the colistin dry powder formulation and the performance of the inhaler were previously described by our group [8]. The improved test inhaler with the dose system is presented in Fig. 1. The dose system of the inhaler can be loaded with three doses of 8–12 mg colistin sulfate each. The in vitro experiments demonstrated that the improved inhaler had comparable deposition behavior to the test inhaler described previously [8]. The particle size distribution of the colistin aerosol cloud was found to be independent of the flow rate between 30 and 60 l/min. This means that the performance of the inhaler is stable over a wide range of inhalation flows.

The three holes of the dose compartment disk of the inhaler were filled with colistin sulfate dry powder mixture by the hospital pharmacy. The mixture contained 83.3% colistin sulfate and 16.7% of a lactose fraction 106–150 μm. The mean particle size diameter of the colistin sulfate was 2.14 μm. A lactose size fraction of 106–150 μm derived from Pharmatose 100 M (DMV International, Veghel, The Netherlands) was used as excipient. The volunteers and patients were first instructed with a placebo inhaler. Subsequently three doses were inhaled.

2.7. Analytical method

Colistin serum concentrations were determined by a recently described chromatographic analysis [11]. A calibration curve in the concentration range of 20–200 μg/l was used. The lower limit of quantitation (LLQ) was assessed as the concentration of the analyte in the matrix of interest for which the confidence interval at the 95% probability level does not overlap with the confidence interval of the matrix blank standard according to the method described by Kucharczyk [12]. The calculated LLQ was 16.8 μg/l for the samples of the volunteer study and 19.6 μg/l for the samples of the patients' study.

2.8. Pharmacokinetic analysis

Serum concentrations were fitted using the KINFIT module of the MW/Pharm software (Version 3.18; MediWare, Groningen, The Netherlands) [13].

In this pilot study, the structural model used to describe the data was an open one-compartment model with first-order elimination and extravascular absorption without a lag time. The serum concentration-time curves were analyzed by nonlinear regression analysis using a weighted least-squares simplex algorithm.

The individual pharmacokinetic parameter values were calculated also using the KINFIT module of the MW/Pharm software. Parameters were calculated by standard pharmacokinetic equations [14]. Pharmacokinetic estimates were used as (indirect) parameters to compare the different administrations of colistin.

2.9. Statistical analysis

The *t*-test was used to compare the pharmacokinetic data of the patients and the volunteers. A probability value (*P*) of less than 0.05 was considered significant.

Table 1
Pulmonary function of six volunteers before and after inhalation of 25 mg colistin dry powder mixture[a]

Volunteer	Dose (mg)	IVC_{before} (l) (%)	ΔIVC (Δ%, 30 min)	ΔIVC (Δ%, 3.5 h)	$FEV_{1before}$ (l) (%)	ΔFEV_1 (Δ%, 30 min)	ΔFEV_1 (Δ%, 3.5 h)	Cough
V1	26.5*	5.07 (121)	−4.3	−5.7	4.07 (113)	−4.2	−3.4	+
V2	23.0*	5.06 (128)	−3.0	−0.6	4.25 (128)	−1.4	−1.6	+
V3	23.8*	3.28 (89)	−4.0	−0.3	3.22 (101)	−1.2	−8.0	+
V4	24.7*	4.55 (104)	+3.7	+2.9	3.43 (91)	+2.3	−0.9	+
V5	24.7*	5.24 (121)	0	+1.9	4.17 (112)	−1.4	+0.2	+
V6	24.7*	4.75 (106)	−0.2	−2.9	3.95 (103)	−3.5	−3.0	+
Mean	24.6	4.66 (112)			3.85 (108)			
(SD)	(1.2)	(0.72) (14)			(0.42) (13)			

[a] Dose, colistin dose; *, three inhalations within 1 min; IVC_{before}, vital capacity before inhalation of colistin; (%), percentage predicted value; ΔIVC, relative change (percentage; Δ%) in vital capacity 30 min and 3.5 h after inhalation of colistin compared to baseline; $FEV_{1before}$, forced expiratory volume before inhalation of colistin; ΔFEV_1, relative change (percentage; Δ%) in forced expiratory volume 30 min and 3.5 h after inhalation of colistin compared to baseline; Cough, mild (+), moderate (++), severe (+++), none (−).

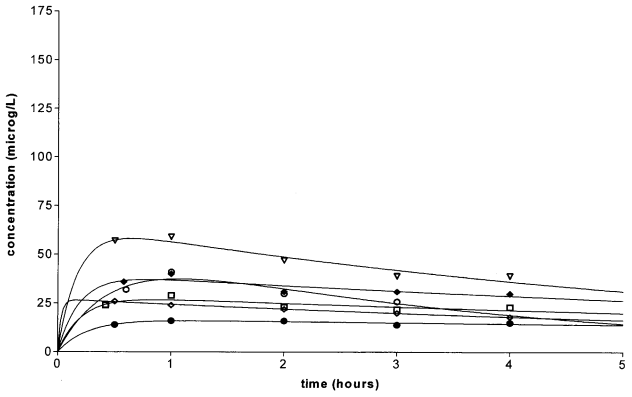

Fig. 2. The individual fitted serum concentrations versus time of six volunteers after inhalation of 25 mg colistin dry powder mixture.

3. Results

3.1. Volunteers

The pulmonary function test results of the volunteers after inhalation of colistin dry powder are presented in Table 1. No significant changes were found after inhalation.

The colistin serum concentration-time profiles after inhalation of the colistin dry powder mixture by the volunteers are shown in Fig. 2.

3.2. Patients

The pulmonary function test results of the patients after nebulization of colistin solution are presented in Table 2. No significant changes were found after inhalation.

The colistin serum concentration-time profiles after nebulization of colistin solution are presented in Fig. 3a–e.

The pulmonary function test results of the patients after inhalation of colistin dry powder are presented in Table 3. Changes in pulmonary function and moderate to severe cough were observed in some patients after inhalation. Supportive care with salbutamol and oxygen was given to patient 2 afterwards. Cough was not expected as a side effect. Observations were evaluated by an experienced lung physician.

The colistin serum concentration-time profiles after inhalation of colistin dry powder are also presented in Fig. 3a–e.

The calculated pharmacokinetic parameter estimates are summarized in Table 4.

4. Discussion

The clinical observations and the pharmacokinetic results found in the pilot study with volunteers were promising. Serum concentrations of colistin after inhalation of the dry powder were of the same order of magnitude as those from nebulization of a higher dose of colistin sulfomethate [10]. Except for mild cough, no side effects were observed. However, the current dose results in an unacceptable change in lung function and cough in some patients with CF.

A significantly higher amount of colistin, reflected by a higher AUC and higher C_{max}, was absorbed by the patients after inhalation of the dry powder as compared to the volunteers. This observation suggests different pharmacokinetics after inhalation of the powder in CF patients.

The C_{max} was significantly higher and the AUC up to 4 h tends to be higher after inhalation of the powder as compared to the nebulization, in spite of the four-times lower dose. The 25 mg dry powder dose, calculated beforehand, can be lowered to reach similar serum concentrations and therefore comparable exposure as after nebulization of 160 mg colistin. Comparing the nebulization and the powder inhalation in patients, T_{max} is reached significantly faster with the dry powder indicating higher concentrations in the pulmonary fluids. Furthermore, in the CF patients, the half life time of colistin is significantly longer and the quotient of clearance and bioavailability is higher after nebulization.

There are several possible explanations for these observations. First of all, two different forms of colistin were used. The nebulized colistin sulfomethate is a prodrug. It is converted back to colistin sulfate in vivo. This hydrolysis takes time [7] which may be an explanation for later the T_{max}

Table 2
Pulmonary function of five CF patients before and after nebulization of 160 mg colistin[a]

Patient	Dose (mg)	IVC_{before} (l) (%)	ΔIVC (Δ%, 30 min)	ΔIVC (Δ%, 3.5 h)	$FEV_{1before}$ (l) (%)	ΔFEV_1 (Δ%, 30 min)	ΔFEV_1 (Δ%, 3.5 h)	Cough
P1	112	6.47 (116)	+0.3	+0.5	4.63 (105)	+0.9	+1.7	−
P2	67	6.09 (111)	+3.6	+1.6	3.86 (89)	−7.3	−3.1	−
P3	99	2.31 (65)	+0.4	+3.5	1.77 (58)	+0.6	+2.3	−
P4	96	3.83 (96)	+0.3	−3.7	2.71 (79)	−4.4	0	−
P5	101	3.38 (66)	+2.1	+8.0	1.38 (34)	−0.7	+3.6	−
Mean	95	4.42 (91)			2.87 (73)			
(SD)	(17)	(1.79) (24)			(1.37) (28)			

[a] Dose, colistin dose, inhaled within 10 min; IVC_{before}, vital capacity before inhalation of colistin; (%), percentage predicted value; ΔIVC, relative change (percentage; Δ%) in vital capacity 30 min and 3.5 h after inhalation of colistin compared to baseline; $FEV_{1before}$, forced expiratory volume before inhalation of colistin; ΔFEV_1, relative change (percentage; Δ%) in forced expiratory volume 30 min and 3.5 h after inhalation of colistin compared to baseline; Cough, mild (+), moderate (++), severe (+++), none (−).

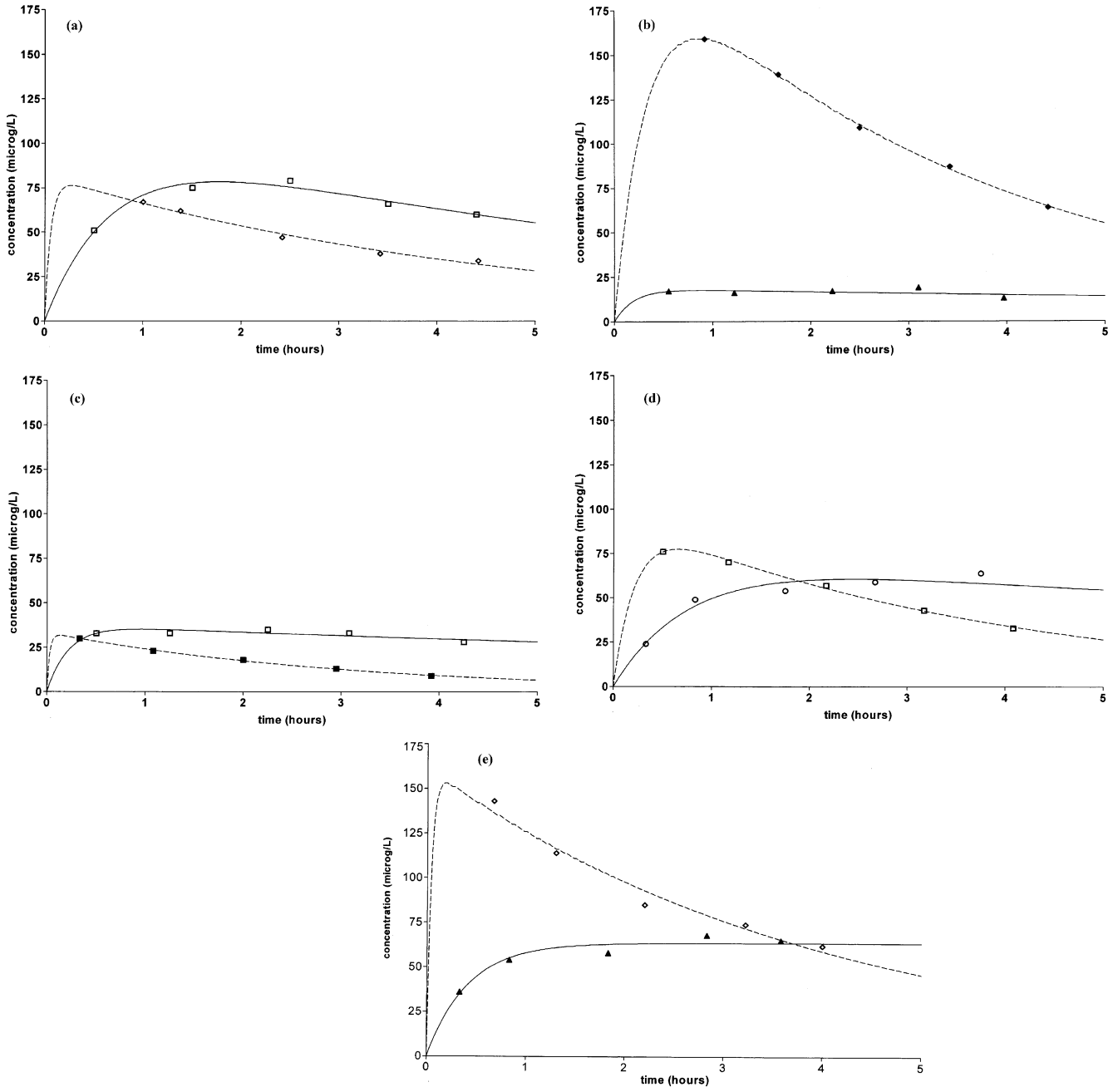

Fig. 3. (a–e) The individual fitted serum concentrations versus time of the respective five CF patients after inhalation 25 mg (except for patient 3 who inhaled 8 mg) colistin dry powder mixture (dotted lines) and after inhalation 160 mg of colistin solution (straight lines).

and the longer apparent half life after nebulization. Another explanation for the prolonged half life after nebulization may be the observed cough after inhalation of the powder.

Due to cough, part of the inhaled dose may have been removed. In the volunteer study and after nebulization by the patients only mild cough was observed. If not removed by cough, a prolonged absorption can take place suggesting a flip-flop absorption model. Prolonged absorption after nebulization was also observed in two previous studies with aerosolized tobramycin [15,16].

From our data, it is not possible to elucidate the most likely explanation for the difference in the determined pharmacokinetics. Furthermore, the data are limited and have to be considered as a first estimate to observe the pharmacokinetics of colistin after inhalation. However, based on the presented data it can be concluded that the dry powder dose can be reduced. This may lead to a better tolerance by the patients in clinical practice.

The patients selected for this study were used to nebulizing a colistin solution. The nebulization was well tolerated as was confirmed by the individual pulmonary function tests. The dry powder inhalation was welcomed by all

Table 3
Pulmonary function of five CF patients before and after inhalation of 25 mg colistin[a]

Patient	Dose (mg)	IVC_{before} (l) (%)	ΔIVC (Δ%, 30 min)	ΔIVC (Δ%, 1.5 h)	ΔIVC (Δ%, 3.5 h)	$FEV_{1before}$ (l) (%)	ΔFEV_1 (Δ%, 30 min)	ΔFEV_1 (Δ%, 1.5 h)	ΔFEV_1 (Δ%, 3.5 h)	Cough
P1	22.5*	6.57 (117)	−5.3	NM	−2.1	4.61 (105)	−5.6	NM	+0.9	+
P2	23.3*	6.29 (115)	−13.7	−7.4	−4.3	3.88 (90)	−15.5	−13.4	−10.1	+++
P3	8.0***	2.30 (65)	+3.5	+3.9	+1.3	1.77 (59)	+2.8	+1.1	−6.2	++
P4	22.5**	3.74 (94)	−7.8	−1.6	−2.4	2.69 (78)	−17.1	−11.2	−16.3	+
P5	23.3**	3.60 (70)	−4.7	−6.9	−4.7	1.40 (35)	0	−4.2	−1.4	+
Mean	19.9	4.50 (92)				2.87 (73)				
(SD)	(6.7)	(1.85) (24)				(1.36) (27)				

[a] Dose, colistin dose; *, three inhalations within 1 min; **, three inhalations within 10 min; ***, one inhalation only; IVC_{before}, vital capacity before inhalation of colistin; (%), percentage predicted value; ΔIVC, relative change (percentage; Δ%) in vital capacity 30 min, 1.5 h and 3.5 h after inhalation of colistin compared to baseline; $FEV_{1before}$, forced expiratory volume before inhalation of colistin; ΔFEV_1, relative change (percentage; Δ%) in forced expiratory volume 30 min, 1.5 h and 3.5 h after inhalation of colistin compared to baseline; Cough, mild (+), moderate (++), severe (+++), none (−); NM, not measured.

Table 4
Colistin mean (SD) pharmacokinetic parameter values in six volunteers after inhalation of 25 mg colistin dry powder, in five CF patients after nebulization of 160 mg colistin with a jet nebulizer and in four CF patients after inhalation of 25 mg colistin dry powder[a]

Parameter	Volunteers (DPI 25 mg, $n = 6$)	CF patients (DPI 25 mg, $n = 4$)	CF patients (Neb 160 mg, $n = 5$)	P value Vol-CF_{inh}	P value CF_{inh}-CF_{neb}
AUC_{0-4} (h mg/l)	106.1 (41.9)	283.5 (87.2)	165.9 (76.5)	0.017	NS
C_{max} (μg/l)	33.6 (14.4)	115.0 (45.8)	51.0 (24.4)	0.003	0.028
T_{max} (h)	0.8 (0.4)	0.4 (0.3)	1.9 (1.2)	NS	0.047
K_a (h^{-1})	9.6 (12.9)	14.8 (10.3)	3.0 (1.8)	NS	NS
$T_{1/2}$ (h)	8.1 (5.1)	2.7 (0.3)	10.4 (3.6)	NS	0.006
Cl/F	0.3 (0.36)	0.06 (0.02)	0.27 (0.15)	NS	0.028

[a] AUC, area under the curve (trapezium, $t = 0$–4 h); $T_{1/2}$, terminal elimination half-life; C_{max}, maximum serum concentration; T_{max}, time to maximum serum concentration; K_a, absorption rate constant; SD, standard deviation; Vol-CF_{inh}, statistical analysis of the DPI inhalation of 25 mg colistin by the volunteers and by the patients; CF_{inh}-CF_{neb}, statistical analysis of the DPI inhalation of 25 mg colistin and the nebulization of 160 mg colistin by the patients; NS, not significant ($P > 0.05$); DPI, dry powder inhaler; Neb, nebulization; Cl/F, quotient of clearance and bioavailability.

patients because of its convenience. However, chest tightness reflected by moderate to severe cough and a decrease in FEV_1 and IVC were observed in four patients. This side effect is not acceptable for long-term maintenance treatment and it was a reason to change the inhalation protocol during the study. Cough was mild in patient 1 but severe in patient 2. Therefore, patients 3, 4 and 5 were instructed to wait for several minutes between the subsequent inhalations. Patients 4 and 5 completed all inhalations, whereas patient 3 stopped after one inhalation because of moderate cough.

It is not clear whether the chest tightness was caused by the inhaled amount of powder or by an irritating effect of colistin sulfate itself. Although not found in this pilot study, chest tightness is a common side effect after nebulization of colistin which leads to discontinuation of regular therapy in some patients [17,18]. Colistin causes mast cell degeneration in vitro which may account for the bronchoconstriction [17]. However, the exact mechanism has not been established. Cough was also reported in 50% of the patients after inhalation of 160 mg gentamicin dry powder, a relatively large amount, in a pilot study with 40 CF patients [4]. On the other hand, the inhalation of bronchodilating drugs as dry powder is generally accepted, probably due to the relatively small amount of inhaled dry powder or to the pharmacologic action of the drug itself.

Based on the data presented in this pilot study (part 1 and part 2) it can be concluded that the inhalation of colistin as dry powder with the new inhaler is a potential and attractive alternative to nebulization. The convenience of the inhaler is of major advantage and the dosage form was highly appreciated by the patients. It may improve patient compliance and allow for a higher dosing frequency. The observed decrease in pulmonary function and cough in patients is a serious drawback. However, several alternatives to prevent this problem exist. First of all, this study shows that the dose can be significantly reduced. Already a dose of 10 mg colistin sulfate is likely to be sufficient to mimic exposure of the currently used nebulization. The dosage form itself can be further optimized, e.g. by changing the particle size distribution of the drug substance. As a result specific regions of the airways may be targeted with greater accuracy. Finally, a lower dose of colistin dry powder or a lower dose in combination with a bronchodilator pretreatment may overcome the observed side effects. These alternative strategies will be subject to future studies.

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References

[1] D.J. Touw, R.W. Brimicombe, M.E. Hodson, H.G.M. Heijerman, W. Bakker, Inhalation of antibiotics in cystic fibrosis, Eur. Respir. J. 8 (1995) 1594–1604.
[2] S. Mukhopadhyay, M. Singh, J.I. Cater, S. Ogston, M. Franklin, R.E. Olver, Nebulised antipseudomonal antibiotic therapy in cystic fibrosis: a meta-analysis of benefits and risks, Thorax 51 (1996) 364–368.
[3] G. Doring, S.P. Conway, H.G.M. Heijerman, M.E. Hodson, N. Hoiby, A. Smyth, D.J. Touw, Antibiotic therapy against *Pseudomonas aeruginosa* in cystic fibrosis: a European consensus, Eur. Respir. J. 16 (2000) 749–767.
[4] J.M. Goldman, S.M. Bayston, S. O'Conner, R.E. Meigh, Inhaled micronised gentamicin powder: a new delivery system, Thorax 45 (1990) 939–940.
[5] D.J. Touw, A.H. de Boer, C.F. Lerk, Comparative evaluation of preliminary in vitro deposition results for tobramycin from WISTO Senior® nebulizer and two different dry powder inhalers, in: D.J. Touw (Ed.), Optimization of Tobramycin Treatment in Cystic Fibrosis. Thesis., University of Leiden, Leiden, 1996, pp. 179–188.
[6] R.N. Crowther Labiris, A.M. Holbrook, H. Crystyn, S.M. MacLeod, M.T. Newhouse, Dry powder versus intravenous and nebulized gentamicin in cystic fibrosis and bronchiectasis, Am. J. Respir. Crit. Care Med. 160 (1999) 1711–1716.
[7] R. Shawar, S. Anderson, L. Barker, L. Nguyen, D.R. VanDevanter, S.K. Tanaka, In vitro assessment of bactericidal activity and relevance to pharmacodynamics of aerosolized antibiotics, Neth. J. Med. 54 (Suppl.) (1999) S38–S39.
[8] A.H. de Boer, P.P.H. Le Brun, H.G. van der Woude, P. Hagedoorn, H.G. Heijerman, H.W. Frijlink, Dry powder inhalation of antibiotics

in cystic fibrosis therapy, part 1: design of a suitable powder formulation with colistin sulfate for a cyclone based test inhaler, Eur. J. Pharm. Biopharm. 54 (2002) 17–24.

[9] P.H. Quanjer, Standardized lung function testing, Eur. Respir. J. 6 (Suppl. 16) (1993) 1–95.

[10] A.R. Clark, The use of laser diffraction for the evaluation of the aerosol clouds generated by medical nebulizers, Int. J. Pharm. 115 (1995) 69–78.

[11] P.P.H. Le Brun, A.I. de Graaf, A.A.T.M.M. Vinks, A high performance liquid chromatographic method for the determination of colistin in serum, Ther. Drug Monit. 22 (2000) 589–593.

[12] N. Kucharczyk, Estimation of the lower limit of quantitation, a method detection performance parameter for biomedical assays, from calibration curves, J. Chromatogr. 612 (1993) 71–76.

[13] J.H. Proost, D.K.F. Meijer, MW/PHARM, an integrated software package for drug dosage regimen calculation and therapeutic drug monitoring, Comput. Biol. Med. 22 (1992) 155–163.

[14] M. Gibaldi, D. Perrier, Pharmacokinetics, in: J. Swarbrick (Ed.), 2nd Edition, Drugs and Pharmaceutical Sciences: Pharmacokinetics, 15, Marcel Dekker, New York, 1982.

[15] D.J. Touw, F.A.H. Jacobs, R.W. Brimicombe, H.G.M. Heijerman, W. Bakker, D.D. Breimer, Pharmacokinetics of aerosolized tobramycin in adult patients with cystic fibrosis, Antimicrob. Agents Chemother. 41 (1997) 184–187.

[16] P.P.H. Le Brun, A.A.T.M.M. Vinks, D.J. Touw, N. Hekelaar, G.P.M. Mannes, R.W. Brimicombe, H.W. Frijlink, H.G.M. Heijerman, Can tobramycin inhalation be improved with a jet nebulizer; a pharmacokinetic analysis, Ther. Drug Monit. 21 (1999) 618–624.

[17] J. Maddison, M.E. Dodd, A.K. Webb, Nebulized colistin causes chest tightness in adults with cystic fibrosis, Respir. Med. 88 (1994) 145–147.

[18] M.E. Dodd, J. Abbot, J. Maddison, A.J. Moorcroft, A.K. Webb, Effect of tonicity of nebulised colistin on chest tightness and pulmonary function in adults with cystic fibrosis, Thorax 52 (1997) 656–658.