Studies on antibiotic aerosols for inhalation in cystic fibrosis
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Dry powder inhalation of colistimethate sodium in cystic fibrosis patients using the Twincer® inhaler: pulmonary deposition after adapted conditions

Elsbeth M Westerman, Anne H de Boer, Paul PH Le Brun, Daan J Touw, Albert C Roldaan, Henderik W Frijlink, Harry GM Heijerman
Summary

The aim of this study was to investigate the effect of an adapted particle size distribution of a colistimethate sodium (colistin sulfomethate) dry powder dose inhaled at an intermediate inspiratory flow rate with an injection moulded, ready-to-use Twincer® inhaler in order to improve lung deposition.

Seven CF patients participated in an open, randomised, cross over, single dose pilot study. A 25 mg dry colistimethate sodium dry powder dose was compared with a 158 mg liquid dose administered with a Ventstream® nebuliser connected to a PortaNeb® compressor. The dry powder dose was well tolerated by the patients. $C_{\text{max}}$ and AUC-values after dry powder inhalation were significantly lower compared to jet nebulisation. However, relative bioavailability ($F_{\text{dpi}}/F_{\text{neb}}$) was 3.0 (nominal dose) and 1.4 (actual dose) respectively. The effect of the adapted inhalation conditions (larger median particle diameter of 2.1 μm and an inspiratory flow rate of 40-45 l/min) on lung deposition could not be established with the pharmacokinetic method used and gamma scintigraphy may be of additional value in future studies. The results support further research on dry powder inhalation of drugs in CF using the Twincer® inhaler.
Introduction

Drug targeting to the lungs of CF patients, especially relevant to antibiotics, receives increasing attention nowadays. Administration of antibiotics by inhalation improves lung function and reduces the number of exacerbations and hospital admissions (Murphy et al., 2004; Flume et al., 2007). A majority of CF patients inhale anti-pseudomonal drugs on a daily basis. According to the US Cystic Fibrosis Foundation patient registry, 61.8% of the patients registered in 2006 were eligible for treatment with an anti-pseudomonal drug for inhalation (tobramycin; no data provided for colistimethate sodium (colistin sulfomethate)), corresponding with over 15000 patients who inhale an anti-pseudomonal drug in the USA every day (Cystic Fibrosis Foundation, 2006). The median survival of CF patients has risen from approximately 25 years in 1985 (Davis 2006) up to 36.9 years in 2006 (Cystic Fibrosis Foundation, 2006). It is likely that inhaled antibiotics will have contributed considerably to this (Frederiksen et al., 1996), given the fact that survival is related to (relative) lung function (FEV₁) (Kerem et al., 1992) and anti-pseudomonal drugs have proven to slow down pulmonary deterioration (Ryan et al., 2003). However, little is known on the optimal dose (Brochet et al., 2007), optimal particle size and the optimal inhalation method in relation to patient characteristics (e.g. age, disease progression) for these antibiotics. Furthermore, patient adherence to inhalation therapy is known to be poor (Arias Llorente et al., 2008), mainly because of the time and effort needed, twice daily, to aerosolize a drug dose and to clean the nebuliser. Therefore, optimisation of, and particularly time saving in inhalation therapy for CF patients is likely to further improve current treatment results.

Dry powder inhalation of anti-pseudomonal drugs is expected to have several advantages over conventional jet nebulisation techniques: patient adherence to inhalation therapy is expected to improve whereas the risk of contamination can be eliminated when using a disposable inhaler (this study). Several publications on dry powder inhalation of antibiotics in CF have been released recently (Geller et al., 2007; Pilcer et al., 2008). Tobramycin dry powder has been inhaled by 90 (Geller et al., 2007) and 9 CF patients (Pilcer et al., 2008) with promising results. Dry powder inhalation of the anti-pseudomonal drug colistimethate sodium has been described in a study with healthy volunteers and CF patients (Westerman et al., 2007a, 2007b). The dry powder drug configuration was well tolerated and appreciated by the CF patients but the pharmacokinetic parameters $C_{\text{max}}$ and AUC after powder inhalation of the 25 mg dose of colistimethate sodium from the Twincer® were lower than observed after a 158 mg dose of colistimethate sodium using the Ventstream® nebuliser and PortaNeb compressor. However, the relative bioavailability of the DPI was 2.7 times higher with reference to the nominal dose.

The clinical pilot study described here is a sequel to the previous patient study in which a prototype (machined) inhaler was used under suboptimal conditions for (peripheral) lung deposition with respect to powder formulation and inhalation manoeuvre (Westerman et al., 2007b). The goal of the present study was to investigate lung deposition of colistimethate
sodium with an adapted particle size distribution administered with a ready-to-use Twincer® at an intermediate inspiratory flow rate. The patients were asked for a breath hold of approximately 10 seconds following inhalation and lung deposition from dry powder inhalation was compared to that from liquid nebulisation (Darquenne et al., 2000; Usmani et al., 2005).

Materials and Methods

Study population

After having given written informed consent, seven CF patients visiting the outpatient clinic and on long term treatment with aerosolized colistimethate sodium or tobramycin participated in an open, randomised, cross over, single dose pilot study. Demographics of the patients studied are presented in Table 1. As this study was intended as a pilot study, applying an inhaler under development, no special requirements were made on the inclusion (e.g. disease state, FEV1) of the participants. Each participant was its own control. Patients were asked to stop their regular nebulisation of colistimethate sodium 3 days prior to each study visit. On two separate days, the patients inhaled a single dose of colistimethate sodium in a dry powder formulation or nebulised the colistimethate sodium solution. The order of treatment was determined by randomisation. A minimum of 3 days and a maximum of 10 days were allowed between visits 1 and 2.

The study was approved of by the regional ethical review board and was performed according to the Helsinki declaration. Inclusion criteria were age ≥18 years, clinical diagnosis of CF and a positive sweat test or two CF gen mutations, FEV1 >25% of predicted values, routine use of nebulised colistimethate sodium, normal kidney function (estimated creatinin clearance >50 ml/min), normal liver function (liver enzymes within normal range) and informed consent. Exclusion criteria were exacerbation of pulmonary infection (according to criteria by Fuchs et al., 1994), intravenous use of colistimethate sodium, colistimethate sodium hypersensitivity,

Table 1: Patient characteristics

<table>
<thead>
<tr>
<th>Patient</th>
<th>sex (M/F)</th>
<th>age (y)</th>
<th>height (cm)</th>
<th>weight (kg)</th>
<th>Body Mass Index (BMI)</th>
<th>FEV1 (% pred. baseline)*</th>
<th>FVC (% pred. baseline)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>F</td>
<td>29</td>
<td>164</td>
<td>55</td>
<td>20.4</td>
<td>58.1</td>
<td>78.9</td>
</tr>
<tr>
<td>P2</td>
<td>M</td>
<td>29</td>
<td>170</td>
<td>70</td>
<td>24.2</td>
<td>28.2</td>
<td>58.9</td>
</tr>
<tr>
<td>P3</td>
<td>F</td>
<td>37</td>
<td>181</td>
<td>83</td>
<td>25.3</td>
<td>34.2</td>
<td>69.5</td>
</tr>
<tr>
<td>P4</td>
<td>F</td>
<td>26</td>
<td>162</td>
<td>58</td>
<td>22.1</td>
<td>47.1</td>
<td>82.1</td>
</tr>
<tr>
<td>P5</td>
<td>F</td>
<td>20</td>
<td>154</td>
<td>59</td>
<td>24.8</td>
<td>99.1</td>
<td>104.6</td>
</tr>
<tr>
<td>P6</td>
<td>F</td>
<td>29</td>
<td>160</td>
<td>55</td>
<td>21.4</td>
<td>59.8</td>
<td>72.9</td>
</tr>
<tr>
<td>P7</td>
<td>M</td>
<td>24</td>
<td>173</td>
<td>58</td>
<td>19.3</td>
<td>56.8</td>
<td>78.7</td>
</tr>
<tr>
<td>Median</td>
<td></td>
<td>29</td>
<td>164</td>
<td>58</td>
<td>22.1</td>
<td>56.8</td>
<td>78.7</td>
</tr>
<tr>
<td>(range)</td>
<td></td>
<td>(20-37)</td>
<td>(154-181)</td>
<td>(55-83)</td>
<td>(19.3-25.3)</td>
<td>(28.8-99.1)</td>
<td>(58.9-104.6)</td>
</tr>
</tbody>
</table>

*Average of baseline values on day 1 and 2
pregnancy, suspected pregnancy, breast feeding or any other condition which in the opinion of the clinician would make the subject unsuitable for enrolment and treatment with an investigational drug within a month prior to enrolment. Each patient inhaled a dry powder dose of colistimethate sodium with the Twincer® (University of Groningen, Groningen, The Netherlands) and a wet aerosol of colistimethate sodium with the Ventstream® inhaler driven by the PortaNeb® compressor (Medic Aid, Romedic, Meerssen, The Netherlands).

**Drug and devices**

One dry powder dose consisted of 25 mg colistimethate sodium (Ph. Eur quality, sterile, Alpharma, Copenhagen, Denmark; 13,200 units/mg), micronised in a jet mill (50 AS, Alpine, Augsburg, Germany), plus 4 mg of lactose sweeper crystals (fraction 150–200 μm from Pharmatose 100 M, DMV International, Veghel, The Netherlands). One dose was administered by a single inhalation. The (volume) median diameter ($X_{50}$) of the colistimethate sodium used in this study was 2.1 μm ($X_{10} = 0.9 \mu m$, $X_{90} = 3.8 \mu m$), which is 1.3 times larger than that of the powder used in the first colistin DPI study ($X_{10} = 0.7 \mu m$, $X_{50} = 1.6 \mu m$, $X_{90} = 3.1 \mu m$)(Westerman et al., 2007b).

Injection moulded Twincer® inhalers were used, slightly adapted compared to the devices used in the previous study in the sense that they were designed with one dose compartment in which a blister was placed. PVC-coated aluminium blisters were supplied by Tommy Nielsen, Esbjerg, Denmark and sealed with a peelable lid after filling one dose for inhalation with a Universal 301 FS blister machine (Tommy Nielsen). The dry powder doses were weighed in by hand into the blisters according to good manufacturing practice (GMP) guidelines and subsequently the inhaler parts were assembled using an ultrasonic welding machine (Rinco Ultrasonic, Switzerland).

Patients were instructed to inhale at a moderate flow rate (40-45 L/min) and to hold their breath subsequently for 10 seconds with a minimum of 7 seconds. Breath hold periods were timed by the investigator using a stopwatch. Prior to inhalation, patients were given the opportunity to practise the inhalation manoeuvre by inhaling through an instrumented dummy-Twincer®. All inhalation flow curves were recorded and as soon as a series of consistent manoeuvres was obtained, the colistimethate sodium dry powder inhaler was given to the patient for administration of the drug. In case of a visually incomplete discharge of the dose from the blister, the patient was asked to repeat the inhalation procedure. Patients were observed and asked for adverse effects after inhalation and during the study day. From the consistent flow recordings the (mean) peak, average flow rate and the inhaled volume were computed.

A dose of 2 million units or 158 mg of colistimethate sodium (Ph. Eur quality, sterile, Alpharma, Copenhagen, Denmark; approx. 13,200 units/mg) was dissolved in 6 ml of sterile isotonic saline by the hospital pharmacy shortly before nebulisation and was transferred into the Ventstream® nebuliser cup in two portions. An expiration filter was connected to the nebuliser. Patients were instructed to continue nebulisation until the inhaler started sputtering.
The drug output (actual dose) of the inhalation device was calculated by subtracting the retained mass of drug in the device from the nominal dose originally put in the device.

Drug retention was determined by weighing the Twincer® before and after inhalation (analytical balance, Mettler-Toledo, The Netherlands) and by using a modified Lowry protein assay for the Ventstream® nebuliser (Lowry et al., 1951).

**Study parameters**

Pulmonary function tests (FEV₁, FVC) were performed before, 5 and 30 min after inhalation or nebulisation, using a calibrated Masterlab pneumotachograph (Jaeger, Würzburg, Germany). The patients received instructions to carry out lung function tests. Measured lung function parameters were normalized to the reference values proposed by the European Community for Coal and Steel (Quanjer et al., 1993) and the results were related to the predicted baseline values for each patient. Any change (%) in lung function after drug inhalation was expressed relative to the patient’s actual baseline values, and is therefore not related to the predicted baseline values. A reduction in FEV₁ of 10% or more was considered to be a clinically relevant proof of airway reactivity.

Venous blood sampling, storage and sample analysis of serum sampling was performed identically to the former study (Westerman et al., 2007b). Similarly, no interference of the assay by co-medication of the patients was observed. Individual pharmacokinetic profiles were calculated based on the output/actual dose for each patient, using the method described earlier (Westerman et al., 2007b) while a new population pharmacokinetic model was made based upon data of the 7 patients. The results have been used as an indirect reflection of peripheral pulmonary deposition.

The same questionnaire that has been used previously (Westerman et al., 2007b) was applied to get an impression of the patient’s experience with the dry powder inhaler compared to wet nebulisation at home. Answers were given on a 6 scale scoring system (none (1) - severe (6) or very bad (1) – excellent (6)).

**Statistical analysis**

The data were tested for a normal distribution using the Kolmogorov-Smirnov test. The Student’s paired t-test for changes within groups was applied to compare both inhalation methods. Data are expressed by means and confidence intervals. The significance level was set at p=0.05.

**Results**

**Pulmonary function tests**

Both colistimethate sodium doses (either from Twincer® or from Ventstream®) were well tolerated by all patients. Patient 1 suppressed a cough reflex during inhalation of the dry powder which is likely to have negatively influenced the output of the inhaler (15.2 mg).
Lung function tests after dry powder inhalation showed no abnormalities. See Table 2. Patient 2 and 4 experienced chest tightness both after dry powder inhalation and nebulisation (see results Questionnaire). No airway narrowing (fall in FEV₁ > 10%) was observed after dry powder inhalation. Nebulisation caused a fall in FEV₁ of 13.5% in patient 7 shortly after nebulisation, which improved to a decrease of 9.7% at t = 30 min. Patients 3 and 4 had a post-nebulisation fall in FVC of 10.5% and 14.8% respectively, which after 30 minutes was unchanged for patient 3 (12.5%) but improved for patient 4. Mean inspiratory flow rate was 35.3 L/min (range 33.7-37.3 L/min, C.V. 4%) and peak inspiratory flow rate 43.9 L/min (range 40.6-48.8 L/min, C.V. 6%), corresponding with a calculated inhaled volume of 1.8 L (range 0.9-2.4 L, C.V. 28%). No correlations were found between mean inspiratory flow rate, peak inspiratory flow rate, inhaled volume and AUC or Cmax. All patients were able to hold their breath for at least 7 seconds after inhaling the dry powder.

**Colistimethate sodium output and pharmacokinetics**

The retained drug mass in the DPI, calculated with reference to the nominal dose, was 25.5% (range 20.2-38.0%) which is a substantial increase compared to the 7.6% (range 3.0-22.0%) in the previous study. The residual volume in the Ventstream® after nebulisation was 33.2% (range 20.3-47.5%) which is lower than the approximately 40% with the same nebuliser-compressor combination observed in other studies (Le Brun et al., 2002; Westerman et al., 2007). However,

<table>
<thead>
<tr>
<th>FEV₁</th>
<th>DPI at t=0 min, (% predicted)</th>
<th>DPI at t=5 min, relative to baseline (%)</th>
<th>DPI at t=30 min, relative to baseline (%)</th>
<th>Neb. at t=0 min, (% predicted)</th>
<th>Neb. at t=5 min, relative to baseline (%)</th>
<th>Neb. at t=30 min, relative to baseline (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>patient 1</td>
<td>1.84 (58)</td>
<td>-2.7%</td>
<td>-0.5%</td>
<td>1.83 (58)</td>
<td>-7.1%</td>
<td>-5.5%</td>
</tr>
<tr>
<td>patient 2</td>
<td>1.11 (28)</td>
<td>-8.1%</td>
<td>-9.0%</td>
<td>1.14 (29)</td>
<td>-6.1%</td>
<td>-2.6%</td>
</tr>
<tr>
<td>patient 3</td>
<td>1.18 (33)</td>
<td>3.4%</td>
<td>5.9%</td>
<td>1.30 (36)</td>
<td>-6.2%</td>
<td>-5.4%</td>
</tr>
<tr>
<td>patient 4</td>
<td>1.47 (47)</td>
<td>-6.8%</td>
<td>-6.8%</td>
<td>1.48 (47)</td>
<td>-0.7%</td>
<td>4.7%</td>
</tr>
<tr>
<td>patient 5</td>
<td>2.85 (100)</td>
<td>3.2%</td>
<td>3.9%</td>
<td>2.81 (98)</td>
<td>-6.1%</td>
<td>2.1%</td>
</tr>
<tr>
<td>patient 6</td>
<td>1.74 (58)</td>
<td>-3.5%</td>
<td>-5.2%</td>
<td>1.84 (61)</td>
<td>-2.2%</td>
<td>-2.2%</td>
</tr>
<tr>
<td>patient 7</td>
<td>2.42 (57)</td>
<td>-3.3%</td>
<td>-1.7%</td>
<td>2.38 (56)</td>
<td>-13.5%</td>
<td>-9.7%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>FVC</th>
<th>DPI at t=0 min, (% predicted)</th>
<th>DPI at t=5 min, relative to baseline (%)</th>
<th>DPI at t=30 min, relative to baseline (%)</th>
<th>Neb. at t=0 min, (% predicted)</th>
<th>Neb. at t=5 min, relative to baseline (%)</th>
<th>Neb. at t=30 min, relative to baseline (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>patient 1</td>
<td>2.87 (79)</td>
<td>-1.4%</td>
<td>1.1%</td>
<td>2.84 (79)</td>
<td>-0.70%</td>
<td>0.0%</td>
</tr>
<tr>
<td>patient 2</td>
<td>2.79 (59)</td>
<td>-5.7%</td>
<td>-6.5%</td>
<td>2.74 (58)</td>
<td>-9.9%</td>
<td>-7.7%</td>
</tr>
<tr>
<td>patient 3</td>
<td>2.92 (70)</td>
<td>1.4%</td>
<td>-1.0%</td>
<td>2.87 (69)</td>
<td>-10.5%</td>
<td>-12.5%</td>
</tr>
<tr>
<td>patient 4</td>
<td>2.79 (78)</td>
<td>-4.7%</td>
<td>1.8%</td>
<td>3.10 (86)</td>
<td>-14.8%</td>
<td>-4.5%</td>
</tr>
<tr>
<td>patient 5</td>
<td>3.44 (105)</td>
<td>2.9%</td>
<td>1.2%</td>
<td>3.42 (104)</td>
<td>-6.1%</td>
<td>-1.5%</td>
</tr>
<tr>
<td>patient 6</td>
<td>2.49 (72)</td>
<td>-2.4%</td>
<td>-2.0%</td>
<td>2.53 (73)</td>
<td>-2.8%</td>
<td>-0.8%</td>
</tr>
<tr>
<td>patient 7</td>
<td>3.92 (79)</td>
<td>-0.5%</td>
<td>-2.3%</td>
<td>3.91 (79)</td>
<td>-9.5%</td>
<td>-7.7%</td>
</tr>
</tbody>
</table>

Baseline values in liters (% predicted)
Percentages are given relative to baseline
data from colistimethate sodium measurements in the expiration filter (data not shown) indicated a higher collected amount in this study compared to the previous study. As a result, the estimated inhaled fraction will have been lower which is an explanation for the lower \( C_{\text{max}} \) and AUC found. Despite this, the relative bioavailability based on the output (actual dose) is comparable to that calculated in the previous study (\( F_{\text{DPI}}/F_{\text{Neb}} \) is 1.4), whereas based on the nominal dose it is even higher (\( F_{\text{DPI}}/F_{\text{Neb}} \) is 3.0). These and other pharmacokinetic parameters are displayed in Table 3. Also in this study the differences between the \( \text{AUC}_{0-4} \), \( C_{\text{max}} \) and \( t_{\text{max}} \) between the two inhalations were statistically significant, while \( t_{1/2} \) and \( \text{CL/F} \), obviously, were not. Variation in PK parameters, expressed in C.V., was large. Figure 1 displays the individual fitted serum concentration time curves after inhalation.

**Questionnaire**

Four patients were very positive about the inhaler concept (score 6), because of the ease of use, including not having to prepare the drug prior to administration and to clean the inhaler afterwards, better hygiene and an expected improvement in adherence to inhalation treatment. One patient was positive but with a lower score (score 5) because she thought it difficult to inhale at the prescribed inhalation flow and two patients were only moderately content due to powder particle retention in the mouth and a strong taste or a cough reflex during inhalation. These patients therefore scored 3 with respect to adverse effects. One patient scored 2 as an adverse effect because of a dry, itching throat and two other patients experienced some chest

<table>
<thead>
<tr>
<th>Actual dose (mg)</th>
<th>Mean DPI</th>
<th>95% C.I.</th>
<th>C.V. (%)</th>
<th>Mean Neb</th>
<th>95% C.I.</th>
<th>C.V. (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC_{0-4} (h.( \mu \text{g/L} ))</td>
<td>182.5</td>
<td>117.7-247.3</td>
<td>48</td>
<td>380.3</td>
<td>334.5-426.1</td>
<td>43</td>
<td>0.02*</td>
</tr>
<tr>
<td>( C_{\text{max}} ) (( \mu \text{g/L} ))</td>
<td>62.7</td>
<td>38.4-86.9</td>
<td>52</td>
<td>122.5</td>
<td>107.7-137.3</td>
<td>43</td>
<td>0.02*</td>
</tr>
<tr>
<td>( t_{\text{max}} ) (h)</td>
<td>0.74</td>
<td>0.70-0.79</td>
<td>9</td>
<td>1.16</td>
<td>0.74-1.59</td>
<td>13</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>( t_{1/2,\text{el}} ) (h)</td>
<td>2.9</td>
<td>2.8-3.0</td>
<td>10</td>
<td>2.8</td>
<td>2.4-3.3</td>
<td>6</td>
<td>0.50</td>
</tr>
<tr>
<td>( \text{Cl/F} ) (L/h/kg)</td>
<td>1.1</td>
<td>1.0-1.2</td>
<td>34</td>
<td>1.5</td>
<td>1.4-1.7</td>
<td>42</td>
<td>0.09</td>
</tr>
</tbody>
</table>

\( F_{\text{DPI}}/F_{\text{Neb}} \) relative bioavailability, calculated on actual dose

| Actual dose | nominal dose minus remainder of colistin in inhalation device after inhalation.
| AUC_{0-4} | area under the curve from 0 to 4 h.
| \( C_{\text{max}} \) | maximum plasma concentration
| \( t_{\text{max}} \) | time to maximum concentration
| \( t_{1/2,\text{el}} \) | terminal half-life
| \( \text{Cl/F} \) | clearance following pulmonary administration
| \( F_{\text{DPI}}/F_{\text{Neb}} \) | relative bioavailability, calculated on actual dose
| C.I. | confidence interval
| C.V. | coefficient of variation
| * | statistically significant
tightness for which a rating 2 was given. Two patients did not experience any adverse effect at all. The adverse effects appeared within 10 minutes after inhalation with the DPI or nebulisation with the jet nebuliser. Patient 2 and 4 experienced chest tightness after dry powder inhalation, but their fall in FEV₁ was within 10% from the baseline. Five patients went through a period of chest tightness after jet nebulisation, of which patient 2 and 4 indicated that this effect was more severe than after dry powder inhalation. Four patients felt a cough reflex, three of them coughed during breath hold, which may possibly have had a negative influence on the lung dose. Two patients thought the cough reflex was generated by the air flow through the nebuliser. Four patients mentioned the taste of the dry powder, of which two patients disliked this taste.

**Discussion**

This study was conducted with the objective to improve lung deposition of inhaled colistimethate sodium with the Twincer® dry powder inhaler in CF patients. We have investigated the influence of a slightly larger median particle diameter in combination with a lower inspiratory flow rate compared to a previous study, expecting that this would yield a higher (peripheral) deposition. We indeed obtained a slightly higher relative bioavailability (Fdpi/Fneb) of 3.0 (compared to 2.7 in the previous study) based on the nominal dose and an equivalent relative bioavailability of 1.4 based on the actual dose (Westerman *et al.*, 2007). Obviously, the small number of participants makes it impossible to draw distinct conclusions from our results, but the data are useful in further dry powder development of drugs in CF treatment.

In this study four modifications to the previous study have been introduced: a dry powder formulation with a slightly larger median particle diameter, an intermediate inhalation peak flow rate, a longer breath-hold and an injection moulded Twincer® inhaler.

The median particle diameter in this study was 2.1 μm compared to 1.6 μm in the previous study. Although these proportions are indicative for the two powder formulations that have been used, it is actually the combination of the median particle diameter and particle size distribution.
that should be considered when estimating and calculating powder behaviour in the lung. However, these calculations are beyond the scope of this study. The larger median particle diameter was introduced to enlarge lung deposition primarily by improving sedimentation. The inhalation peak flow rate in this study was 43.9 l/min (was: 67.9 l/min). An intermediate flow rate is expected to reduce impaction of the aerosolized dry particles in the oropharynx and to result in an effective lung deposition at the same time. The longer breath hold was introduced to give the aerosol more time for deposition by sedimentation in the bronchiolar and alveolar regions of the lung. Finally, the injection moulded Twincer® inhaler is a ready-to-use device which is able to effectively disperse 25 mg in one inhalation manoeuvre. The Twincer® used in the previous study was a prototype which was filled and assembled by hand on each study day. This model was constructed with two dose compartments which contained 12.5 mg of colistimethate sodium each.

The results, expressed as relative bioavailabilities, were similar to the results in the previous study and have been obtained by a pharmacokinetic analysis. Another, theoretical method for estimating the obtained lung dose from inhalers is to calculate the impaction parameter, that incorporates the particle aerodynamic diameter or geometric diameter and the average airflow rate and, depending on the equation used, the particle density (DeHaan and Finlay 2004). The impaction parameter is often used to predict drug loss in the oral cavity and oropharynx. Applying this equation on the results obtained in the previous and the current study results in comparable impaction parameters of 4346 and 4840 μm² cm³ s⁻¹, corresponding with a loss of approximately 9.1% and 10.1% in the oral cavity and oropharynx respectively. Therefore, the fraction of the 25 mg colistimethate sodium that was able to enter the lungs has been equal in both dry powder inhalation studies, and it is likely that inertial deposition in the upper respiratory tract will have been comparable as well. Theoretically, the advantage of the inhaler in the current study is therefore to be found in a higher sedimentation velocity in central and peripheral airways. It is to be expected that a higher lung deposition has been obtained in this study, as terminal velocity increases with a larger particle diameter and more time has been created for sedimentation in lung generations 11-23 because of an extended breath hold (Usmani et al., 2005). Unfortunately, no convincing evidence for this theoretical advantage has been found with the pharmacokinetic method used for determining lung deposition (relative bioavailability). Gamma scintigraphic methods can support further studies, as regional differences in deposition cannot be measured with PK method (Chrystyn 2001).

Among other (unknown) factors, interpatient variability will have influenced the results in this pilot study. This variability is well known in lung deposition studies in CF patients and is especially relevant in studies with a small number of subjects. This variability is made up by variation in inhalation technique and variation in lung deposition due to different stages of disease progression. In this study the coefficient of variation of \( C_{\text{max}} \) and AUC after dry powder inhalation (52% and 48% respectively) is larger compared to jet nebulisation (43% and 43% respectively). However, this variation is predominantly caused by patient 5. Excluding this patient results in a C.V. of 24% and 23% for \( C_{\text{max}} \) and AUC after dry powder inhalation respectively,
and this suggests that nebulisation is less reproducible than dry powder inhalation. Without the data of patient 5, the relative bioavailability changes slightly in favour of wet nebulisation, without changing the observed trends and significance in pharmacokinetic parameters.

The interpatient variability is a weak point in comparative study designs in CF as is jet nebulisation as reference treatment which is known for its variable results. It is a paradox, as clinically administration of colistimethate sodium by jet nebulisation is still the gold standard. The value of comparing relative bioavailabilities within this study or between the previous study and this study is therefore questionable, as the results are guided by patient variability.

The new inhaler was appreciated by the patients and the dry powder drug dose was well tolerated. Airway reactivity (FEV₁) was measured in one patient after wet nebulisation but not after dry powder inhalation. Post-nebulisation decrease in FVC values was observed in two patients, but not after dry powder inhalation. Similar results were obtained in the previous study, and further research is needed to elucidate whether these effects are a result of the physical form of the inhaled drug (aerosol in droplets versus powder particles), local high drug concentrations in the proximal lung and/or the absolute amount of drug entering the target area in the lung, in relation to the disease state of the particular patient.

The residual mass in the DPI was higher than previously observed, which can be attributed to the introduction of a large blister to the design of the injection moulded Twincer®. The blister used for this colistimethate sodium study was designed to contain 12.5 mg of spray-freeze dried powder which has a large volume due to its high porosity. The same blister may contain 60 mg of colistimethate sodium and as a result of only partially filling the blister (25 mg), considerable losses by adhesion onto the blister wall and blister seal occurred during transport and handling of the Twincer® devices. Without the blister seal in vitro data indicate only marginal drug loss within 1% of the nominal dose for payloads of 5-50 mg (data not shown).

In conclusion, colistimethate sodium dry powder inhalation with the Twincer® inhaler is efficient and well tolerated by CF patients. The influence of altered inhalation conditions on lung deposition could not be established with the pharmacokinetic method used. Gamma scintigraphy may be of additional value in future studies. The results support further research on dry powder inhalation of drugs in CF using the Twincer® inhaler.

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