Studies on antibiotic aerosols for inhalation in cystic fibrosis
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Concluding remarks and future perspectives
Introduction

Inhalation of antibiotics has become a cornerstone in the treatment of pulmonary *Pseudomonas aeruginosa* infections in cystic fibrosis (CF) patients, either in preventing or stabilising chronic infections, sometimes combined with intravenous therapy. As this approach has proven to be successful in contributing to an improved life expectancy, extensive attention is now given to the technical aspects of inhalation. The focus is on innovating drug delivery devices, sometimes combined with specific drug formulations, which allow for the administration of large doses in a short time frame and in a reproducible way. The majority of CF patients are confronted with extensive and time-consuming drug administration, several times a day. Nebulised drugs are a major contributor to this and have a major impact on the daily life of a cystic fibrosis patient. Therefore it is worthwhile to investigate every possible option to improve this life-long routine. The ultimate aim is to prevent, limit and treat lung damage in order to maintain and improve clinical well-being and quality of life.

In general, drugs are administered by inhalation to exert a clinical response, either by direct action on lung tissue or after systemic absorption. Pulmonary administration of drugs has been investigated in treating several diseases in recent years. Next to COPD and asthma, for which inhalation therapy has been the golden standard for years, insulin, antibiotic, antifungal, immunosuppressant and chemotherapeutic agents have been subject of clinical trials. The objectives for inhalation of the drug are either to make administration easier (insulin), to reduce systemic adverse effects and to obtain higher local concentrations which result in increased efficacy (antibiotics, antifungal agents, immunosuppressants, chemotherapeutic agents). Inhaling an antibiotic agent in CF treatment aims at all three objectives, and offers the patient the opportunity of preventive anti-pseudomonal therapy at home, with intravenous treatment as the only alternative route for administration of aminoglycosides or colistimethate sodium.

High local concentrations are not only favourable with respect to efficacy, but will also reduce the risk of the development of bacterial resistance due to suboptimal local concentrations of antibiotics. Furthermore, a possible role is reserved for drug-device combinations which are able to produce high local concentrations in treating CF patients who suffer from antibiotic-resistant strains of bacteria. In extreme circumstances, it is imaginable that high local drug concentrations will be the last treatment option for CF patients with extremely resistant strains of bacteria who do not respond sufficiently to various intravenously administered antibiotics. Furthermore, treatment of acute pulmonary exacerbations may be possible if local antibiotic concentration can reach sufficiently high levels; this hypothesis deserves further study.

The current challenge in inhalation therapy in CF is to reach the most peripheral parts of the lung to combat *Pseudomonas aeruginosa* and other pathogens with antibiotics like colistimethate sodium and tobramycin, as CF disease starts from there (Tiddens 2002). This area of the lung is characterised by a large airway surface area and a thin barrier to the systemic circulation and is
the most difficult part of the lung to be reached with aerosols. While targeting at the periphery, other, more central parts of the lung which are colonised with the pathogens, will be in contact with the antibiotic also. Unfortunately, the relation between the deposition of a high dose in the peripheral parts of the lung and the alleged improved clinical response has not been proven yet, nor is information available on how high this dose should be. The lack of knowledge on the optimal lung dose of antibiotics, and the inter-device and inter-individual variability between patients makes it impossible to predict successful inhalation treatment beforehand. Several factors add to the variability found in inhalation treatment:

- Nebulisers and inhalers have variable efficiencies which may result in significant variations in the lung dose.
- Physical properties of the drug may affect aerosolization results; with the obtained particle size of the aerosol and the output rate as most prominent variables.
- Each patient introduces several variabilities to treatment outcome: their disease state and inhalation technique are only two examples.

All these factors contribute to a highly variable pulmonary drug deposition.

As at present inhalation of antibiotics in CF has become mainstay, it is a challenge to investigate whether individualised inhalation regimens can be established. This implies that the basic mechanisms of the inhalation process in CF patients and the influence of disease progression, airway anatomy and the ‘compatibility’ of a patient with the various inhalers should be further explored. If this approach turns out to be successful, perhaps in future inhalation therapy will be initiated based on the optimal match between the patient’s and aerosol generation device profiles.

Considerations on study design and inhaler development

Pharmacokinetic and scintigraphic studies

The clinical research described in this thesis applied a pharmacokinetic methodology to assess lung deposition. Two or three dimensional imaging technologies are also used to study pulmonary deposition in inhalation treatment. Pharmacokinetic methods are useful for determining the total lung dose after inhalation, as has been proven for some drugs or drug-device combinations (Newnham et al., 1993). Urinary concentration measurements after inhaling tobramycin can be used as well (Asmus et al., 2002). These methods work well for comparing relative lung deposition, but do not provide information on regional deposition in the lung, whereas two dimensional (gamma scintigraphy) or three dimensional (single photon emission computed tomography (SPECT) or positron emission tomography (PET)) (Newman et al., 2003, Eberl et al., 2006) methods provide both. Furthermore, these techniques may be useful in studies regarding dose-response relationships. However, these scintigraphic techniques require a reformulation of the original dry powder drug because of the radiolabeling process which can influence the
particle size distribution and thereby the clinical results. This study approach is obviously more complex and more expensive to perform.

Therefore it was decided that the first clinical study in the development of the Twincer® colistin dry powder inhaler, described in this thesis, would start with a pharmacokinetic analysis, with feasibility as a major topic. Now that satisfactory phase I results have been obtained, two or three dimensional imaging studies can possibly provide new, valuable information on regional deposition of the drug. Moreover, these studies could deepen our understanding on the relationship between physical characteristics of the generated aerosol and lung deposition. Obtaining data on regional deposition of the dry powder particles is desirable, especially if these data can be related to clinical efficacy parameters as local bioavailability is pertinent to reflect efficacy of drugs that act directly in the lung (Pilcer et al., 2008). Furthermore it may be worthwhile to study the relation between a clinical efficacious lung dose in the peripheral lung and pharmacokinetic parameters (AUC, C_{max}, t_{max}), as this might be used as a less invasive method for therapeutic drug monitoring in future.

It is, however, a challenge to radiolabel the colistimethate sodium dry powder particles without changing their aerodynamic behaviour and to use this material in a clinical inhalation study within the period of activity of the radionuclide. A method for labelling a tobramycin dry powder for inhalation has been described recently (Pilcer et al., 2008) but to our best knowledge no such experiment has been performed with colistimethate sodium yet. In the Pilcer study lung deposition, measured by gamma scintigraphy, was lower than the fine particle fraction measured in vitro, which was attributed to the nature of CF and its severity (Pilcer et al., 2008). This is in agreement with other data (Newman and Chan, 2008). A relationship between the data on lung deposition obtained by gamma scintigraphy and the pharmacokinetic method was described.

**Influences on lung pharmacokinetics**

In general, serum concentration levels obtained after drug inhalation are considered to reflect pulmonary drug deposition in the lung, provided that no gastro-intestinal absorption of the drug in question occurs, as is the case with colistimethate sodium and aminoglycosides. This is an indirect or surrogate method to determine pulmonary drug deposition. Drug concentrations in sputum are used as a measure of pulmonary drug deposition too, but the highly variable results, likely to be caused by patient- and disease specific factors, make sputum PK analysis less valuable compared to serum PK analysis (Geller et al., 2007; Laube et al., 1989). Moreover, sputum concentrations are merely a reflection of drug deposition in the upper airways and provide little information on the deposition in the deeper airways or alveoli.

Research on lung deposition using pharmacokinetic methods is complicated by the fact that little is known of fundamental pharmacokinetics and pharmacodynamics of inhaled drugs in CF patients.
Only a few studies have incorporated pharmacokinetic methods in their data analysis, whereas most clinical (efficacy) studies have either omitted to perform a proper pharmacokinetic analysis on their data or have not measured serum levels at all.

Serum levels measured shortly after inhalation possibly reflect alveolar deposition as the alveolar surface area is by far the largest of all lung parts (Patton, 1996), but at the same time the amount of drug in the alveoli might be redistributed to the large airways by the pulmonary circulation (Chrystyn, 2001). The total lung dose is not influenced by this, but the concentration at the peripheral target site in CF probably is and may possibly influence therapeutic efficacy. Similarly, an identical total lung dose may be deposited more centrally for instance because severe disease progression prevents peripheral deposition (Mukhopadhyay et al., 1994). It is not clear if or how such differences in local lung distribution are reflected by pharmacokinetic data.

Furthermore, it has been argued for tobramycin that the physical state (solution versus dry powder) of the drug influences the extent of systemic absorption: in the study by Pilcer (Pilcer et al., 2008) a dry powder lung dose resulted in a lower systemic absorption, including a delay in t$_{\text{max}}$ and the authors suggested that this would be beneficial for the local antimicrobial treatment in the CF lung. These findings cannot be compared to the results described in Chapters 7 and 8, as no data are available on the obtained lung doses in both studies, albeit that the t$_{\text{max}}$ values after dry powder administration were shorter compared to wet nebulisation, indicating a faster absorption process. Further studies are required in order to be able to estimate the influence of the physical state of a drug on systemic absorption as well as on therapeutic efficacy. Colistimethate sodium has surface tension lowering properties, which may influence absorption characteristics as well.

Lung deposition data in pharmacokinetic inhalation studies are expressed as the maximum serum concentration (C$_{\text{max}}$) level and the area under the curve (AUC). Of these two, the AUC is a better descriptor in trials in which inhalation devices with different inhalation times and/or different absorption rates are being compared (chapter 3, Chrystyn, 2001). If the supposed relationship between (peripheral) lung deposition, clinical efficacy and AUC can be established in the future, this parameter might be used to optimise treatment on an individual basis and facilitate in vivo bridging studies. The absorption rate of a drug, reflected by the t$_{\text{max}}$ might be a descriptor in lung deposition studies as well. Different mechanisms by which (systemic) absorption takes place, have been described (Patton, 1996). The objective in antibiotic aerosol treatment is obviously a high local peripheral concentration in the lung, but perhaps the pharmacokinetic parameters, based on serum levels, remain useful for describing lung deposition in future. For example it would be interesting to learn whether a shorter t$_{\text{max}}$ found in the Chapters 3, 7 and 8, indeed corresponds with a larger fine particle fraction, as has been suggested in Chapter 3. In these three clinical pilot-studies, drug aerosol inhalation using the PARI-LC Plus®-CR60® nebuliser-compressor combination (tobramycin) and the Twincer® dry
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Inhaled antibiotics and safety

Although the AUC might be the preferred parameter to reflect lung deposition, it should be noted that, especially for the aminoglycosides (tobramycin, gentamicin, amikacin), knowledge on $C_{\text{max}}$ and $C_{\text{trough}}$ is valuable too. For this class of antibiotics, efficacy with the lowest risk of toxicity (nephro- and ototoxicity) in intravenous therapy is related to high peak and low trough serum levels, preferably in an extended dose interval regime (Touw et al., 2007). In contrast with the extensive knowledge available on the risk of toxicity with intravenous use of aminoglycosides, only sparse, anecdotal information is available on the toxicity risk of inhaled tobramycin on a daily basis for longer periods of time, as is the case in CF treatment. In long term term trials, no nephro- or ototoxicity were observed, but these studies were performed with a nominal dose of 80 mg tobramycin (Steinkamp et al., 1989, MacLusky et al., 1989). A shorter, 12-week study, with 600 mg nebulised tobramycin three times daily, revealed no toxicity. Generally, a compromised renal function can result in increased toxicity in aminoglycoside therapy, for which high trough serum levels (> 1 mg/L) are a marker (Hoffmann et al., 2002, Edson et al., 2004, Kahler et al., 2003). Extrapolation of toxic serum levels in intravenous therapy to estimate toxicity for aerosolized aminoglycosides cannot be done without due consideration however, as first of all no data are available on possible toxic effects of high concentrations of aminoglycosides within the lung and secondly no relationship has been established between intrapulmonary concentrations and serum drug levels as an indirect parameter of deposition. Therefore it is difficult to estimate aminoglycoside toxicity in inhalation treatment. Until more data are available, we are confined to extrapolating knowledge from i.v. treatment to aerosolisation in CF for estimation of toxicity. Therefore, pragmatic toxic limits ($C_{\text{trough}} > 1 \text{ mg/L}, \ C_{\text{max}} > 4 \text{ mg/L}$) have been introduced in chapter 3. Fortunately, although only sparse information is available on risk for toxicity on the inner ear during inhalation treatment, short term nebulisation up to 4 weeks and 24 weeks appears to have no negative effects on auditory function (Mukhopadhyay et al., 1993, Ramsey et al., 1999, Lenoir et al., 2007, Chuchalin et al., 2007). It is nevertheless advisable to collect information on possible toxicity by performing audiometric (especially high tone) measurements during long term inhalation treatment (> 6 months continuously or monthly on/off) with aminoglycosides, as these data are lacking for the CF population.

Colistimethate sodium toxicity is generally limited to reports of airway narrowing and chest tightness, whereas nephrotoxicity and neurotoxicity have been observed after intravenous treatment (Falagas and Kasiakou, 2005). Colistin is the most potent (Bergen et al., 2006) but also more toxic compound (Chapter 4) to which a fatality has been attributed recently (McCoy, 2007). Apparently hydrolysis of colistimethate sodium into colistin prior to intravenous or inhaled administration exerts toxic effects, while this process appears to be safe once it occurs within the human body.
Next to intrinsic drug toxicity, clinicians are responsible for monitoring patients that are susceptible for toxicity, e.g. reduced renal function, nephrotoxic co-administered drugs and auditory effects, among which high tone loss (Scheenstra et al., 2007). Obviously, this includes extra awareness during concomitant treatment of inhaled and intravenous aminoglycosides.

**How to obtain and study clinical efficacy?**

The lack of knowledge on the relationship between lung dose and drug serum levels, as discussed earlier, is a major drawback for the ability to estimate a clinical response after drug inhalation. It is expected that a high concentration of antibiotic in the peripheral lung will have the best antimicrobial effect and will therefore result in the best clinical response. Research on the relation between peripheral deposition and clinical effects, as has been done for inhaled beclometasone (Marshall et al., 2000), is needed. Once such a relationship has been established, further steps in exploring inhalation therapy in CF can be made, especially with respect to therapeutic drug monitoring.

Fundamental research on the interaction of deposited aerosolized particles, both liquid and dry powder particles, on lung tissue infected with bacterial colonies, will support our understanding of the mechanisms by which a clinical effect occurs. Furthermore, radiolabeled deposition studies, e.g. with polydisperse (droplets covering a range of sizes) and monodisperse (all droplets approximately the same size) particles will broaden our knowledge on particle size behaviour in relation to disease progression in the lung (Usmani et al., 2005). In studies with heterodisperse radioisotope-labeled particles, it has been shown that in patients with CF, compared to normal subjects, there is a marked heterogeneity in the pattern of deposition of particles in the lung (Alderson et al., 1974), due to e.g. obstructed and/or distorted airways and decreased regional ventilation.

In the meantime, comparing pharmacokinetic drug data after using two or more different inhalation systems is one of the simplest way of estimating (relative) lung deposition, albeit in an indirect manner. The clinical efficacy and safety of a nominal dose of 300 mg tobramycin (Ramsey et al., 1999, Bowman, 2002, Moss, 2002) and 160 mg colistimethate sodium (Frederiksen et al., 1997), administered with a jet nebuliser has been established over the years. This knowledge is the basis of current anti-pseudomonal inhalation treatment and is the gold standard to which new (including ‘me too’) preparations are being compared (Poli et al., 2007). Given the fact that only TOBI® comes with a recommendation on the nebuliser to be used, these doses are a remarkable ‘standard’, as it is well known that the ultimate lung dose is always highly variable due to various causes, as discussed previously.

A consequence of the comparative study design method is that an equivalent dose (and not an optimal dose) is searched for, which is most likely to result in a comparable lung deposition. This dose is expected to result in a comparable therapeutic efficacy and can be obtained for example by performing a dose escalation study. It is believed that, by direct comparison, patient bound parameters that may influence the study results will be present in all study
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situations and may therefore be considered not to influence relative comparisons. The results can therefore directly be attributed to the device or drug presentations studied. This approach needs to be put in perspective however, as intra-patient variability may affect results, as well as inter-patient variability (Chrystyn et al., 1998). Mucus plugging and airway inflammation result in a reduction of the tidal volume, trapping of the drug in obstructed areas, a minimal airflow and thus a reduced peripheral deposition. It is likely that study outcomes will be influenced by heterogeneously composed patient groups, as peripheral deposition is inversely proportional to disease severity and correlated to the FEV\textsubscript{1} (Mukhopadhyay et al., 1994). This is especially relevant in studies with a small number of subjects. It is therefore important to employ well-designed cross-over designs and to search for new approaches (Simon and Chinchilli, 2007) in order to try to reduce the influence of the aforementioned variability.

Nevertheless, pharmacokinetic inhalation studies with a comparative design are feasible in CF patients, once one is aware of this variability. Ideally, the pharmacokinetic method is accompanied by a scintigraphic analysis and performed in a large number of study subjects, to compensate for the large variability described earlier.

**Inhaler development**

It is a challenge to aerosolize a dry powder dose in the over 10 milligram mass range, as this requires an inhaler which is capable to release and disperse large amounts of powder particles at a flow rate that is optimal for peripheral deposition in the lung. The urge for high dose administration of 20 mg sodium cromoglycate has led to the development of the first dry powder capsule inhaler in 1967 (Smith and Parry-Billings, 2003). Therefore, it is not surprising that several dry powder inhaler studies with antibiotics in CF have used capsule inhalers for administration of the drug as they can contain large amounts of dry powder. Although these inhalers are relatively easy to use for testing a new antibiotic dry powder principle in CF treatment, capsule inhalers have some major disadvantages. Poor efficiency of lung delivery (lung dose approximately 10%), patient related issues concerning reloading of a capsule prior to each dose (Smith and Parry-Billings, 2003) and drug/device related issues (brittleness of the capsule after long storage time, inhaler or capsule retention; Vidgren, 1988) have led to development of more efficient (multidose) dry powder inhalers for COPD and asthma treatment. Although understandable with respect to speed up commercial availability of a the dry powder device for antibiotics, it is unfortunate that much effort is put into the development of capsule inhalers for treating CF patients nowadays, while technically superior inhalers have been designed and are available.

The Twincer\textsuperscript{®} inhaler is, because of its technical features, able to release and disperse high powder doses in a range of 25-50 mg in one single inhalation. The 25 mg dose has been tested *in vivo* (this thesis), whereas *in vitro* results clearly indicate the feasibility of higher doses. An additional advantage of the Twincer\textsuperscript{®} design is the single use (disposable) concept, which reduces the risk of (re-)contamination of the user with (resistant) bacteria via the inhaler and of
a possible decrease in stability of the drug because of humid exhaled air into the device. Furthermore, the small size, the redundancy of an external power source and low production costs of the four-component device make this inhaler a promising design for future applications.

**Future perspectives and recommendations**

**Fundamental science**

Future research should continue to focus on fundamental aspects related to drug inhalation in CF. The influence of various factors like disease progression, aerosol behaviour in the lung and patient-related factors on the deposited dose should be mapped. The use of monodisperse particles for inhalation in a research environment with lung deposition imaged using scintigraphy, is expected to increase our understanding of critical parameters regarding inhaler and inhalation parameters in CF (Usmani et al., 2005). More knowledge is needed on the characteristics of the target region in the lung in order to be able to define the optimal clinical response in antibiotic inhalation treatment. This will correspond with an optimal lung dose, which equals an effective but safe drug concentration level both in the lung and in the systemic circulation with a minimal risk on adverse drug reactions.

Knowing the target area, the drug dose including particle size specifications, and the type of inhaler suitable for the individual patient will help in aiming at an optimal drug concentration at the target site and an optimal therapeutic effect. These data may help to establish a therapeutic index, which is the ratio between clinical efficacy and adverse drug reactions (including toxicity). The influence of the aerosol particle size (fine particle fraction (FPF), in vitro) and the particle size distribution within the FPF, on the therapeutic index, has been reviewed recently (Weda et al., 2008). The therapeutic index can be established for each antibiotic drug in combination with a specific inhaler, using information on the dose-efficacy response curve, the absorption pharmacokinetic profile of the drug from the lung into the systemic circulation and the dose-adverse drug reaction curve. Possibly this will support individual therapeutic drug monitoring, provided a sound dose-response relationship can be established in the future. If so, perhaps more information can be obtained on why some patients can be treated with inhaled antibiotics successfully and others cannot. If the use of a specific drug-inhaler combination for a patient results in a lung dose that is too low for effective treatment, perhaps a different inhaler will give improved results. If, however, the patient is not adherent to therapy, intensified guidance from CF healthcare professionals may be an alternative intervention.

**Adherence to therapy**

Adherence of CF patients to therapy with aerosolized drugs is poor, predominantly due to the time consuming drug administration and cleaning procedures (Arias Llorente et al., 2008). At the same time, adherence to treatment influences the outcome of aerosolized treatment (Kettler
et al., 2002). It is therefore of major importance that devices for administration of aerosolized drugs will be further developed, aiming at a short(er) administration time, a reduction in risk of device contamination, an user friendly size and preferably no dependence on an external battery or electricity, aiming at improving adherence to therapy and quality of life of CF patients. The Twincer® dry powder inhaler has all these features: inhalation can be performed within one minute; the inhaler is designed for single use, avoiding the risk of device contamination; it is the size of a credit card and uses the patient's inhalation flow to release the drug dose (see also Chapter 2). These advantages over regular jet- and ultrasonic nebulisers, combined with the Twincer®'s potential for effective regional drug deposition in the lung makes this inhaler a promising new development in CF treatment.

A specific topic that deserves attention is the potential difference between inhalation of dry and wet particles on the subjective well-being of patients. Several patients who took part in the studies described in this thesis, indicated to have missed their regular nebulisation sessions which they had to stop at least 24 hours prior to study days. While dry powder inhalation seems to have several advantages over wet nebulisation, including an improved adherence to therapy, it might well be that for certain patients wet nebulisation will remain daily routine in their drug administration to improve their subjective well-being. If so, it is imaginable that dose-critical drugs, like antibiotics, will be administered by dry powder inhalation, while ‘wetting’ of the lower airways is done by nebulisation of isotonic of hypertonic saline.

**Airway narrowing**

A complication of drug inhalation that is present in a substantial number of CF patients, is transient airway narrowing. This description is chosen deliberately, as the exact cause of a transient fall in lung function parameters (FEV₁, FVC) shortly after inhalation of drug in CF patients is not clear. Although constriction of the airways (bronchoconstriction) due to the tightening of smooth muscle is a likely cause, swelling of the airway lining or increased mucus amounts in the airways may also cause narrowing of the airways in CF patients.

Several mechanisms have been proposed to cause a post-inhalation decline in FEV₁ during or after drug inhalation. Some CF patients have a coexisting asthma or an inherent airway hyperreactivity, which may explain these post-inhalation observations. Drug related causes have been suggested, such as mast cell degeneration (hypertonic solutions (Alothman et al., 2005). Airway narrowing has furthermore been attributed to excipients, such as antioxidants and preservatives, and pH and osmolality. However, it has been found that solutions for inhalation both with or without excipients may cause airway responsiveness in susceptible patients (Alothman et al., 2002). Similarly, hypo-, iso- and hypertonic solutions provoked airway narrowing symptoms in CF patients (Dodd et al., 1997), suggesting that tonicity alone, within a reasonable range from isotonicity, is probably not responsible for airway narrowing. The small differences between pH-values of aerosolized antibiotics and normal saline makes the acidity a less likely cause of airway narrowing (Chua et al., 1990).
Generally, approximately 10-20% of CF patients react on a nebulised antibiotic dose with a fall in $\text{FEV}_{1,\text{predicted}} > 10\%$ (Hodson et al., 2002a, 200b; Geller et al., 2007), although higher numbers for inhaled colistimethate sodium have been described (Alothman et al., 2005, Cunningham et al., 2001). However, this clinical observation does not necessarily mean that the patient experiences chest tightness. It is worthwhile investigating the underlying mechanisms of this airway reactivity, as this might elucidate why subjective chest tightness is not always correlated with a fall in $\text{FEV}_{1,\text{predicted}} > 10\%$ (chapter 8), which is considered to be clinically relevant (MacLusky et al., 1986).

Furthermore, perhaps a consensus can be reached on which points in time lung function tests should be performed after drug inhalation in order to estimate airway reactivity. Most susceptible patients react during or shortly after the end of the nebulisation session and $\text{FEV}_1$ recovers within 30 minutes for the majority of patients. This is why in this thesis timepoints of 5 min and 30 min after inhalation were chosen to perform pulmonary function tests. Furthermore, the presence of airway narrowing after dry powder inhalation and wet nebulisation was shown to be different for each patient: no fall in $\text{FEV}_{1,\text{predicted}} > 10\%$ was observed after dry powder inhalation, but several patients reacted on wet nebulisation (this thesis). Whether dry powder inhalation has a lower potential for causing airway reactions or these observations are merely caused by a relatively lower inhaled dose, is not known at present and deserves further investigation.

Airway narrowing caused by a high concentration of drug particles in the bronchiolar area resulting in contraction of the bronchiolar muscles and a fall in $\text{FEV}_1$ in susceptible CF patients has been hypothesized (Chapter 3). This centrally deposited drug aerosol can be a result of an unfavorable particle size-flow rate combination and/or a reduced peripheral airway patency caused by disease progression. In contrast, a drug aerosol with optimal properties for peripheral airway deposition that is inhaled with a suitable flow rate and inhalation volume, may show a lower potential for airway narrowing due to a lower drug concentration in the central airways. This hypothesis may be studied in future.

**Dosing frequency and treatment of exacerbations**

While once daily intravenous dosing of aminoglycosides has been studied and applied in daily routine (Smyth et al., 2005), no data are available on once daily versus multiple-times daily dosing of aerosolized aminoglycosides in CF. Theoretically, once daily dosing might reduce the risk of toxicity and be advantageous with respect to adherence to inhalation treatment which may in turn increase efficacy. On the other hand, new treatment strategies with a higher daily dosing frequency than the current twice-a-day dosing schemes may be worthwhile exploring as well. Because of the availability of the new inhaler devices (mesh technology, dry powder inhalers e.g. the Twincer®) treatment times can drop considerably. This offers a perspective for a more frequent aerosol administration (up to 4 or 5 times daily), especially for dry powder inhalers, once clinical proof has been obtained that these drug-device combinations are effective in
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anti-pseudomonal treatment. It is worthwhile to investigate whether such dosing schemes will be superior to the current schemes.

Extrapolating intravenous therapy knowledge on aminoglycosides to inhalation treatment would mean that higher peak serum levels correspond with a higher lung deposition. High tobramycin doses of 600 mg and 1000 mg, administered with jet nebulisers, have been investigated (Le Brun 1999, Le Brun, 2001), corresponding with an estimated lung dose of 44 and 67 mg respectively, which, compared to an estimated lung dose of 27 mg after 300 mg tobramycin (Newman et al., 2001) is approximately 1.5-2.5 times higher. A dose of 1000 mg was considered at that time to be the highest possible dose that can be administered with a jet nebuliser within an acceptable amount of time (30 min.). However, more efficient nebulisers, including vibrating mesh nebulisers, are available nowadays which may deliver higher amounts of drug within a shorter time frame. Dry powder inhalers, like the Twincer® inhaler, may enable high dose pulmonary drug administration to an even further extent. This justifies renewed attention for the application of high dose inhaled antibiotics for treating pulmonary exacerbations (Le Brun et al., 2001), as a large dose may be administered within a reasonable time period, which may result in improved therapeutic results. Further investigations on the highest lung dose that can be reached with the newer devices within an acceptable time frame, are needed to estimate the potential added value in treating pulmonary exacerbations. Le Brun et al. (2001) estimated that a lung dose of 200 mg will result in a peak serum concentration of 9.2 mg/L, which is an acceptable result in case of intravenous treatment with tobramycin. In general, a local drug concentration of at least 4-5 times (beta-lactam antibiotics) to 10 times (aminoglycoside antibiotics) the minimal inhibitory concentration (MIC) of (susceptible) bacteria is needed for effective antibiotic treatment. In case of resistant bacteria and/or treatment of exacerbations in CF, the multiplication sum needed will be even higher. Obviously, the deposition pattern of the drug in the lung will have an additional influence on the ultimate effect. Furthermore, the optimal dosing frequency should be studied, as a prolonged elimination half life has been observed after inhalation of a high 1000 mg dose of tobramycin (Le Brun et al. 2001). Obviously thorough investigations, including therapeutic drug monitoring, risk on toxicity and collection of clinical data, are required before the value of this hypothesis can be estimated. Unfortunately no results from large clinical studies with aminoglycosides or colistimethate sodium administered with these new nebulisers and DPI’s are available to date, not to mention the limited amount of safety data (risk of toxicity) for normal and high dose administration of antibiotics.

**Bridging studies**

An inhaled drug should only be prescribed in combination with the inhaler device after (positive) results obtained from clinical studies with this combination in CF patients have become available (Chapter 2, CHMP, 2006). This is valid for new drugs and new inhalers but should also be applicable for older drugs that have been on the market for years. With respect to the numerous inhaler devices that are commercially available, it is impossible to study every existing device
with every existing drug. Therefore, bridging studies may be an answer to close the gap. In a bridging study the tested drug-device combination in CF patients serves as a reference to a drug-device combination not tested in this population. The comparison is made based on data from \textit{in vitro} (bench) studies. Currently sparse information is available on \textit{in vitro}–\textit{in vivo} correlations in inhalation therapy, but knowledge on this subject is evolving (Wedø \textit{et al.}, 2008, CHMP, 2006). The work described in this thesis is an example of combined \textit{in vitro} – \textit{in vivo} research: after \textit{in vitro} development studies on the drug and the devices, drug-device combinations have been tested \textit{in vivo}. The lack of information on local drug deposition within the lung in this work however makes it difficult to connect \textit{in vitro} and \textit{in vivo} data. Incorporating \textit{in vivo} scintigraphic studies in future development plans will improve information on this subject.

\textbf{Clinical efficacy from a different perspective}

As discussed previously, clinical efficacy of treatment with aerosolized antibiotics in CF is guided by a large variability in patients and drug-device combinations. Up till now, most research initiatives on this subject have focused either on clinical efficacy or drug-device efficacy. The need for combining these strategies has been explained in this thesis.

Another, new approach may prove to be useful in estimating the likelihood of a successful, effective aerosol treatment for an individual patient using experience from a large group of patients (population) in the past. This goal might be reached by using existing data for pattern recognition in the individual patient and to continuously improve and re-analyse this data set by adding information obtained from that particular patient. Support from statistical methodology is necessary to accomplish this, for example with Bayesian methodology. This methodology is frequently applied in pharmacokinetic calculations, also in CF research (chapters 6, 7 and 8 of this thesis). Furthermore, the methodology has been used in risk calculations in prenatal, carrier and neonatal screening in CF.

Bayesian methodology is a method of statistical inference which combines prior information (including a probability distribution) about a population parameter with the data of, for example, an individual patient. With the help of Bayes’s theorem, a posterior probability distribution for the parameter is obtained, which provides the basis for statistical inference concerning the parameter. To use this methodology in estimating clinical efficacy of aerosolized drugs, several variables of interest have to be defined and coded first. After a substantial amount of information per variable per patient has been collected, a population model can be obtained, which can in turn be optimised by the data of each patient that is collected in future. If proven successful, this approach can support clinical decision making by predicting with a certain probability that, for example, a male patient, 25 years old, FEV\textsubscript{1} of 54% with a chronic \textit{Pseudomonas aeruginosa} infection for 2 years, might have the best clinical result using an inhaler X with drug Y and dose Z twice daily. This hypothesis certainly deserves further exploration.
To conclude

**Inhaler development**

Rapid administration of an antibiotic aerosol with a small device, which is easy to use and has an effective, reproducible and reliable performance, reflects the ideal situation for CF patients. Present developments, such as the Twincer® inhaler, are promising. In addition to its simple design and therefore low production costs, the concept of a single use disposable inhaler is an advantage in preventing cross contamination of (resistant) bacteria and possible deterioration of the performance of the nebuliser and stability of the drug because of accidental exhalation into the inhaler. Additionally, the risk of environmental contamination during inhalation of a drug dose, associated with conventional (jet) nebulisation, is negligible with the use of a dry powder inhaler.

However, the position of dry powder inhalation of antibiotics in cystic fibrosis treatment is still confined to pilot studies (this thesis, Pilcer et al. 2008, Geller et al., 2007). Future perspectives are promising, as many potential improvements have not yet been explored. Long-term studies are needed to assess the true value of the potential benefits of dry powder antibiotic inhalers in cystic fibrosis.

**Innovations and pharmaco-economics**

The cystic fibrosis population is estimated at ~ 100,000 patients worldwide, with a high likelihood of underreporting and underdiagnosing, especially in developing countries (Cystic Fibrosis Worldwide, 2005). In terms of cost-effective drug development for manufacturers, this population is small, making this field unprofitable for larger innovative pharmaceutical companies. Therefore, an opportunity has been created to develop drugs in the framework of an orphan drug programme both in Europe and the USA.

Well-designed, long-term studies with a large number of CF patients are required to assess the clinical value of all newly developed drug-device combinations. This is a challenge by itself, as the number of eligible CF patients per (specialised) CF centre is generally small. This thesis includes three pilot-studies with a small number of subjects. These studies may form the basis for the larger clinical trials. The limited number of patients in these three pilot-studies is partly a result of the difficulty to find eligible patients.

To be able to include large numbers of patients, well-designed randomised multicentre studies are required under professional supervision and coordination. Great expenses are generally involved in this type of research, which is beyond the scope of many clinicians and (academic) institutes. To gain new results from scientific work in an efficient way, multidisciplinary cooperation between scientists, clinicians, pharmacists and other professionals, under which condition the research described in this thesis has been performed and as advocated by the EuroCareCF (www.eurocarecf.eu) initiative, is essential for research in cystic fibrosis. Consensus on e.g. outcomes, end-points and definitions of parameters in research and performing
studies accordingly will facilitate the comparison of results from various bench- and clinical studies. Furthermore, this will hopefully enable and facilitate the participation of CF patients in an efficient way.

Next to technical innovations, the search for effective antibiotics to combat *Pseudomonas aeruginosa*, *Burkholderia cepacia* and other Gram-negative bacteria will remain an ongoing quest. Besides colistimethate sodium and tobramycin, other anti-pseudomonal antibiotics for inhalation, such as amikacin, ciprofloxacin and aztreonam are also under investigation, either for dry powder inhalation as for wet nebulisation. It is likely that these approaches will result in further improvement in the daily treatment and life expectancy of cystic fibrosis patients.

In terms of the diversity in technical developments and drug formulation requirements, it is mandatory to keep the total costs of inhalation treatment within a realistic range and to perform pharmaco-economic research, as state-of-the-art treatment should be within reach for every patient with cystic fibrosis. This is a shared responsibility of scientists, the drug and device industry, national and international regulatory authorities and prescribers.

For cystic fibrosis patients, the last two decades have been characterised by an improved life expectancy, better treatment options and rapidly developing new therapies. CF has become a chronic disease with multiple treatment targets, resulting in increasingly complex treatment strategies. More than ever it is important that multidisciplinary teams of physicians, nursing staff, physiotherapists, dieticians, social workers and specialised pharmacists collaborate to optimise treatment now and in the future. In addition, a joint effort with scientists and pharmaceutical companies to continuously search for new developments in drug aerosol therapy will hopefully further improve life expectancy and quality of life.
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Concluding remarks and future perspectives


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