

Comparison of aerosol delivery efficiency nebulising Colistin by electronic and jet nebulisers

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Summary

Inhaled Colistimethate sodium (CMS) has been demonstrated to be efficacious in the treatment of recurrent bacterial infections of the lower respiratory tract of CF patients. Results of this study show that a wide range of in-vitro Respirable Doses can result if the same formulation is used with different nebulisers and that eFlow[®] rapid can deliver an approximate two fold in-vitro respirable dose compared to other nebuliser systems. This difference needs to be taken into account, particularly with the delivery of antibiotics, where a low dose may be less efficacious and lead to resistance formation against the applied drug. The in-vitro respirable dose can be used as an efficacy indicator and the nebulisation time as a compliance indicator. A shorter nebulisation time of a high respirable dose, improves treatment adherence and the probability that patients will inhale the required dose into the lungs.

Introduction

Recurrent bacterial infections of the lower respiratory tract are one of the major clinical features of cystic fibrosis (CF). Susceptibility to infection is thought to be multifactorial, including impaired mucociliary clearance. A defective protein, cystic fibrosis transmembrane conductance regulator (CFTR) in CF patients causes this impairment [Willumsen et al. 1991].

In younger CF-patients, the predominant infections are caused by *Staphylococcus aureus*, *Haemophilus influenzae* or *Streptococcus pneumoniae* [Pedersen, 1992]. The predominant pathogen in patients age ≥ 18 years is *Pseudomonas aeruginosa* (PA). PA predominates and over time becomes established as a chronic infection [Pedersen 1992; Koch & Høiby 1993]. Once chronic infection is established, the body reacts with a variety of immune mediated responses associated with increased production of viscous mucus, an ideal substrate for bacterial growth for PA. Chronic infection with PA results in airway inflammation, including tissue damage, which causes irreversible pulmonary damage, and eventually death due to complete respiratory failure [Høiby et al. 1987; Høiby & Koch 1990; Berger 1991; Koch & Høiby 1993, Hodson et al. 2002, Ratjen et al. 2006].

A placebo-controlled study of inhaled Colistimethate sodium (CMS) by Jensen *et al.* (1987), confirmed the efficacy of CMS in a small patient population. Subsequent studies have shown improved lung function, a slower decline in respiratory function, and decreased hospital admission rates in patients who received daily nebulised antibiotic treatment. The advantages of nebulised CMS in CF have been shown in a number of randomised controlled trials [Mukhopadhyay et al. 1996]. The widespread use of inhaled CMS for over three decades has proven its safety and efficacy. Contrary to many other antibiotics, resistance formation has been remarkably low [Pitt & Sparrow 2003].

Chemically, CMS belongs to the class of polymyxins and is a cyclic heptapeptide containing the amino acid, L-diamino butyric acid (L-DAB). In CMS each amino group on the L-DAB residue is substituted with a methane sulphonate. CMS is a 'pro-drug' which becomes hydrolysed after administration to release the active substance Colistin base. Colistin will degrade in the dissolved state. Solutions made with diluent (saline) should be nebulized shortly after preparation and should not be stored or reused. If only a portion of the prepared solution is required for use in any given nebuliser, such as the case with I-Neb, the remainder of unused solution should be discarded.

Lung deposition and therapeutic efficacy of nebulised drugs depend on the specific nebuliser system used. The interaction of drug formulation and device can affect aerosol performance in terms of the delivered dose (DD), droplet size distribution, respirable dose (RD) and nebulisation time. Hence, proper bench testing of the drug product in combination with selected nebuliser systems is necessary to estimate potential lung deposition. The aim of this study was to assess the aerosol characteristics of CMS solution in two jet nebulisers (PARI LC PLUS[®] and Ventstream[®]) and three electronic nebulisers (eFlow[®]rapid, Aeroneb[®] Go, and I-neb[®]) by breath simulation and laser diffraction experiments. A dose of 1.0 MIU colistin / 3 ml was loaded into all nebulisers with the exception of the I-neb, where a dose of 0.4 MIU Colistin / 0.4 ml is claimed to be equivalent to 1.0 MIU in other nebulisers.

Materials and Methods

All tests were conducted with COLIFIN[®] containing 1 MIU CMS/vial as lyophilized powder (PARI Pharma, Munich, Germany). The CMS powder was dissolved in 3 ml (1.0 MIU / 3ml) or in 1 ml (1.0 MIU / 1 ml) of isotonic saline (0.9%). The saline was pipetted into the CMS vials, slightly shaken and allowed to stand until the powder was dissolved. After approximately 15 min the foam formation had disappeared and the solutions were pooled in a glass bottle and aliquots used for the experiments. The physicochemical properties were as follows: pH = 7.3, surface tension = 49.6 mN/m, and osmolality = 384 mOsmol/kg: The nebulisers were filled with the respective volume of formulation for each test.

The aerosol droplet size distribution was measured by laser diffractometry using the MasterSizerX laser diffractometer (Malvern Instruments, Herrenberg, Germany). The nebulisers were connected to the diffractometer via a coupling chamber allowing entrainment of conditioned air through the nebuliser. The nebulisers were charged with the specified volume of test material and operated for two minutes. The measurements were started after one minute of nebulisation. An average droplet size distribution was calculated from 5 single measuring intervals of 5 seconds, every 10 seconds. We have shown in a previous study a good correlation regarding the droplet size of CMS assessed by laser diffraction and cascade impaction with a Next Generation Impactor (NGI) at a flow rate of 15L/min [Bitterle et al. 2007] as apparent from figure 1 below.

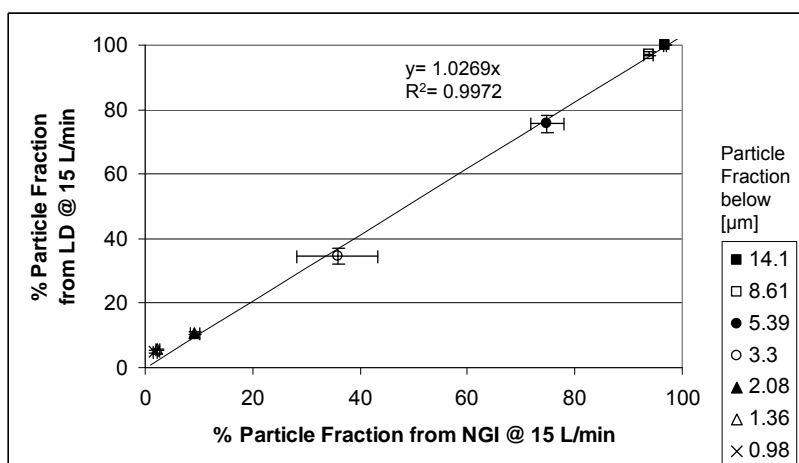


Figure 1: Correlation of cumulative particle size data measured by NGI and LD at a flow rate of 15L/min, each. The particle fractions are shown as % of droplets below a certain particle size [μm] corresponding to the cut-off sizes of the single NGI-stages. From the regression curve a correlation coefficient of 0.9972 can be calculated.

As critical quality attribute of the droplet size distribution the Respirable Fraction (RF) was defined as the fraction of droplets smaller than 5 μm [% < 5μm] and the respirable dose as drug contained [mg] in droplets < 5 μm.

The I-neb[®] nebuliser is a breath triggered nebuliser which requires the application of a dynamic breathing flow to initiate nebulisation. For this nebuliser the droplet size distribution was measured using the Spraytec laser diffractometer (Malvern Instruments, Herrenberg, Germany) coupled to a breath simulator (Compas[™] I, PARI GmbH, Germany). Data collection was conducted continuously with the Spraytec system, but only data collected during the aerosol production phase was used to determine the RF.

To determine the Delivered Dose (DD), breath simulation tests were conducted using a standardised sinusoidal adult breathing pattern (500 ml tidal volume, 15 breaths / min, inhalation/exhalation ratio = 1) as described in [12] and shown in figure 2. Inhalation filters were changed in suitable intervals as tested in pre-experiments. The total active substance delivered (DD = Delivered Dose) is the cumulative drug amount found on the inhalation filters. No filter changes were required for the eFlow[®] rapid and I-neb[®] nebuliser systems.

The Drug Delivery Rate (DDR) is calculated from the drug amount collected on the first filter divided by the time when this filter change occurred. In cases where no filter change was necessary the active substance delivery rate was calculated from the total active substance delivered divided by the nebulisation time. The Respirable Dose < 5μm (RD) is calculated from the drug amount on the inhalation filters DD [mg] x Respirable Fraction [% of delivered amount < 5μm] from laser diffraction experiments. All samples derived from breath simulation experiments were analysed by HPLC using a HSPgelAQ 2.5 column and UV detector.

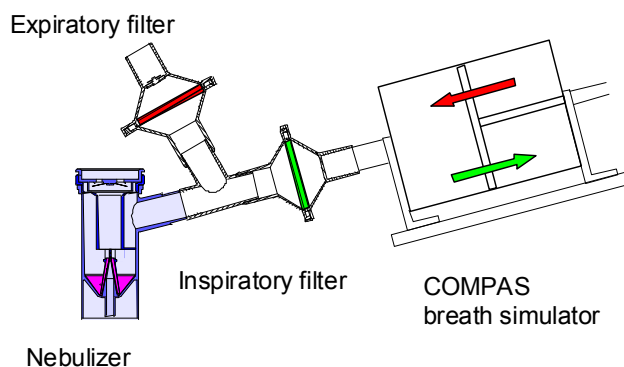


Figure 2: Example of the test set-up for breath simulation measurements

Results

The Respirable Dose, calculated by multiplying the Delivered Dose derived from breath simulation experiments with the Respirable Fraction measured by laser diffraction ranged from about 9.1 to 19.5 mg over all nebuliser systems tested. The highest average respirable doses were achieved by the eFlow rapid (19.5 mg) and the PARI LC PLUS[®] (17.3 mg). In the lower range of the RD values were the Ventstream[®] (10.6 mg), the Aeroneb Go[®] (10.8 mg) and the I-neb[®] (9.1 mg). Figure 3 compares the measured values for DD and the calculated values for RD for the tested nebulisers. Table 1 shows the full data set of measured data (DD and RF) and calculated results (RD).

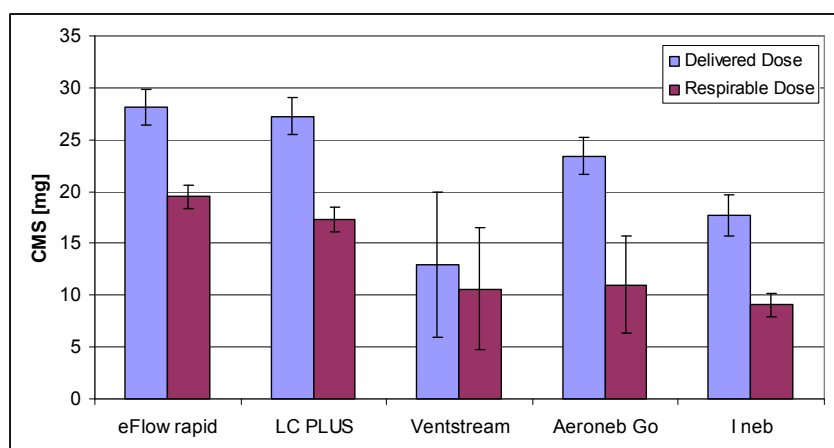


Figure 3: Comparison of Delivered and Respirable Dose for the investigated nebulisers systems. A dose of 1.0 MIU of CMS was loaded for all nebulisers except for the I-neb[®], where according to instructions for use for colistin only 0.4 MIU were loaded.

Table 1.: Aerosol characteristics of colistin solutions nebulized by different nebulisers.

	PARI LC PLUS	eFlow rapid	I-neb	Aeroneb Go	Ventstream
Loaded Dose (CMS)	1.0 MIU / 3ml = 79 mg	1.0 MIU / 3ml = 79 mg	0.4 MIU / 0.4ml = 31.6 mg	1.0 MIU / 3ml = 79 mg	1.0 MIU / 3ml = 79 mg
Delivered Dose [mg]	27.3	28.1	17.7	23.4	13.0
RF [%<5µm]	63%	69%	51%	46%	82%
Respirable Dose [mg]	17.3	19.5	9.1	10.8	10.6
Nebulisation time [min]	7.8	4.0	3.5	9.9	6.7
Respirable drug delivery rate [mg/min]	2.2	4.9	2.6	1.1	1.6

Discussion and Conclusion

Results of this in-vitro study show that a wide range of in-vitro Respirable Doses (an approximate two fold difference) can result if the same formulation is used with different nebulisers. This needs to be taken into account when choosing a suitable nebuliser for inhalation treatment, especially in the case of antibiotics, where a low dose may not only be clinically less effective but may also lead to resistance formation against the applied drug. The data of this study show that the in-vitro performance of the eFlow[®] rapid is comparable to the PARI LC PLUS[®]. Although the Ventstream[®] achieves the highest Respirable Fraction, the Delivered Dose is lowest and the Respirable Dose 1.8-fold lower compared with the best performing nebuliser, the eFlow[®] rapid delivering the highest Respirable Dose and exhibiting also the highest Respirable Drug Delivery Rate. The I-neb[®] was able to deliver a Respirable Dose similar to the Ventstream[®] and the Aeroneb Go[®], in spite of only loading 0.4 MIU instead of 1.0 MIU of colistin.

It must be noted in this context, that drug solutions not needed due to a lower required fill volume (I-Neb[®]) or being left in the medication reservoir (jet-nebs and eFlow[®] rapid) should not be collected, stored or re-used, since Colistin will be degraded upon storage and can be harmful for patients (FDA homepage alert). Close attention must be paid to the dissolution step to dissolve the powdered Colistin. For complete dissolution of approximately 79 mg (1 mio units), a sufficient amount of diluent (3 ml saline) is required and vigorous shaking should be avoided to prevent foaming. Smaller volumes are less appropriate since this can affect solubility as well as physicochemical properties (e.g. viscosity and surface tension) including the transferability of the drug into the medication cup of the nebuliser.

Data show, that the Respirable Dose and the combined parameter Respirable Drug Delivery Rate are key parameters for a comparison of device performance, since they reflect the amount and rate of drug potentially being deposited in the intrapulmonary region. While the in-vitro respirable dose can be used as an efficacy indicator, the nebulisation time may serve as a compliance indicator, provided that an efficacious dose is delivered by the respective system. The shorter the nebulisation time for a target respirable dose, the higher the probability patients will adhere to treatment and inhale a therapeutically required and effective dose into the lungs. Our data show that COLIFIN[®] designed for use in the eFlow[®] rapid can also being administered via other nebuliser systems, such as the Ventstream[®], AeroNeb Go[®] and I-neb[®]. However, due to their inferior delivery efficiency an impaired therapeutic efficacy and the occurrence of resistance formation may be possible [14].

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