In vitro measurement of the deposition of budesonide, ipratropium bromide and salbutamol, administered by jet nebulizing into a neonatal high flow nasal cannula system

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Keywords

Aerosols ; cannula, high flow neonatal ; drugs, in vitro deposition ; infant, newborn ; nebulizers.

Abstract

Objective: to determine what proportion of nebulized drugs is deposited through the prongs of a neonatal high flow nasal cannula circuit. Methods: Total cannula output, aerosol deposition in the tubes of the oxygen nasal cannula and the amount of compounds residue in the sidestream disposable were determined by two different and independent *in vitro* experiments performed in triplicate. Quantification of the compounds was performed using high-pressure liquid chromatography with high resolution mass spectrometric detection. Setting: nebulization of budesonide, ipratropium bromide and salbutamol with a jet nebulizer into a humidified and heated (37˚C) Optiflow™ nasal cannula system for 20 minutes at a flow of 6 L/min. Main outcome measures: Percentage of compounds in cannula output. Results: For all compounds, both *in vitro* experiments resulted in a total cannula output of less than 1%. A small amount of the compounds (i.e., 0.27 to 3.62 % of the nominal dose) could be measured in the rinsing water of the nasal cannula tubes and the highest amount (i.e., 28.43 to 53.63%) remained in the sidestream disposable nebulizer chamber. Conclusion: Routine use of nebulization in neonatal high flow nasal cannula circuits is not recommended. Further research is needed to determine if optimizing nebulizing conditions can result in a therapeutic amount of drugs delivered through an Optiflow™ nasal cannula system in order to simultaneously deliver oxygen and medication without patient manipulation.

Introduction

Nebulization of corticosteroids (e.g., budesonide), anticholinergics (e.g., ipratropium bromide) and beta-agonists (e.g., salbutamol) has been widely accepted in the treatment of neonatal respiratory illnesses, yet its efficiency remains matter of debate (1). Generally, a face mask connected to a jet nebulizer is used for the delivery of pulmonary aerosol medication. However, in neonatal units, newborns frequently receive high flow nasal cannula (HFNC) oxygen therapy for respiratory support. If these patients additionally require aerosolized drugs, the connection of a jet nebulizer to the HFNC circuit may be considered as a simple solution to deliver medication without the interruption or modification of the concomitant oxygen therapy, thereby improving the efficiency and tolerance of the overall procedure (2-5). However, clinical studies on the delivery of drugs via HFNC observed controversial efficacy, questioning the in vitro delivery adequacy of the system (6-8). In vitro studies exhibit controversial results as well. In some studies significant amounts of aerosolized drugs have been measured at the cannula outlet and other studies reported insufficient cannula outlet due to several barriers, like aerosol deposition, impeding efficient nebulization through HFNC circuits (9-11). Several settings like type nebulizer system, nebulizing time, flow rate, temperature, the starting volume, position of the nebulizer, size of nasal cannula and type of humidification system were found to influence aerosol efficiency through an HFNC system (10-13). Nevertheless, an optimized aerosol drug delivery system through an HFNC circuit for infants still does not exist. Consequently, for every specific setup, an in vitro study on the cannula outlet is recommended. According to Rubin and Fink an effective nebulizer should deliver at least 50 % of the total dose as respirable aerosol (14).

The objective of this study is to measure what proportion of frequently used nebulized drugs in neonates (i.e., budesonide, ipratropium bromide and salbutamol) is delivered from the nasal cannula after nebulizing them with a jet nebulizer through a humidified and heated (37˚C) Optiflow™ nasal cannula system for 20 minutes at a flow of 6 L/min, and what proportion remains in the tubing and chamber.

Materials and methods

1. Materials

The setup for this experiment was the same as we use in clinical practice in the neonatal intensive care unit and consisted of an Optiflow™ tubing kit, the Optiflow™ nasal cannula, a heating element (Fisher & Paykel Healthcare, Auckland, New Zealand) and a Sidestream Disposable Nebulizer Chamber (Philips Respironics, Chichester, UK). Commercially available budesonide (0.25 mg/mL), ipratropium bromide (0.25 mg/2mL) and salbutamol (0.5 mg/mL) were used for both the experimental design and as reference standards for the mass spectrometric analysis of budesonide, ipratropium and salbutamol respectively. NaCl 0.9% solution was obtained from Braun (Meisungen, Germany), the internal standard salbutamol-D3 from the National Measurement Institute (North Ryde, Australia) and triamcinolone acetonide-D6 from Clearsynth (Mumbai, India). The collection device for drug detection, ExaBreath®, was placed at our disposal by SensABues (Stockholm, Sweden). Acetonitrile and methanol were purchased from Biosolve (Valkenswaard, The Netherlands). For all experiments, ultrapure water produced by a Milli-Q® Reference A plus system from Merck KGaA (Darmstadt, Germany) was used.

2. In vitro Optiflow™ system setup

The position of the nebulizer is close to the nasal prongs, downstream of the humidifier (Fig. 1). To the sidestream disposable nebuliser chamber, 0.250 mL of commercially available budesonide, ipratropium bromide and salbutamol were added and diluted with 1.250 mL of NaCl 0.9% solution. This 2 mL suspension was nebulized using a jet nebulizer through a humidified and heated (37˚C) OptiflowTM nasal cannula system for 20 min at a flow rate of 6 L/min and FiO2 = 21%.

Total cannula output was determined by collecting the nebulized aerosol on the basis of two different and independent in vitro experiments performed in threefold. In the first experiment the nasal prongs of the oxygen nasal cannula were directly plugged into the ExaBreath®. The ExaBreath® was tightly sealed with Parafilm. This device contains an electrostatic based filter which is used to capture and retain the aerosol particles originating from the nebulizer. The mouthpiece and control bag from the ExaBreath® were not used. After completion of the nebulization experiment, compounds were extracted from the filter of the ExaBreath® by removing the filter from the device, spiking it with 50 µL internal standard solution (50 mg/L salbutamol-D3 and triamcinolone acetonide-D6 in methanol) and placing it in a 10 mL test tube containing a 1 mL sterile pipet tip (Fig. 2). The sterile pipet is used as a tool to hold the filter at the top of the tube so that after centrifugation the centrifuged solution can be separated from the filter. The pipet is sterile to minimize any contamination or effect of the tip on the analysis. The inner part of the ExaBreath® was rinsed with 2 mL of methanol to collect the aerosol drops that stayed behind on the inner surface of the device. The 2 mL rinsing solution was then added to the filter. The test tubes were vortexed (10 seconds), placed on a roller mixer (5 min) and centrifuged (10 min, 3200 rpm). The filter and pipet tip were removed from the test tube and 100 µL of the centrifuged solution was transferred to an autosampler vial containing 900 µL of ultrapure Milli-Q water. Vials were manually vortexed for 15 seconds and budesonide, ipratropium and salbutamol were quantified (see below). To determine the percentage of the compounds that reached the ExaBreath® after nebulizing, the ExaBreath® filter was, in a separate experiment also performed in threefold, spiked with the same total amount of compound that was added to the sidestream disposable (i.e., 0.250 mL of the respective solutions) and with the same amount of internal standards. These filters were not subjected to any nebulization and subsequently extracted with the same extraction protocol. The mean instrumental response obtained from these experiments was regarded as a 100% recovery and compared with the mean response from the in vitro experiments.

A second independent in vitro setup to determine total cannula output was additionally performed. In that experiment, the nasal prongs of the oxygen nasal cannula were directly submerged into a test tube containing 1 mL ultrapure water, that was tightly sealed with Parafilm. After completion of the nebulization, the solution was directly analyzed as described below. The mean instrumental response from these experiments (n=3) was compared with the mean response obtained from dissolving the total amount of compound that was added to the sidestream disposable into 1 mL ultrapure water (same volume as in the test tube of the experiment).

Besides determining the total cannula output, in all experiments, the substantial deposition (droplets that remained in the tubes of the oxygen nasal cannula) and amount of compound that remained in the sidestream disposable were also determined by rinsing both devices with 1 mL ultrapure water after completion of the nebulizing experiment. Compounds in this rinsing solutions were quantified as described below.

3. Quantification of the compounds

For every experiment, 1 µL of extract, solution or rinsing water was injected into an Ultimate 3000 ultra-high pressure chromatographic system (Thermo Fisher Scientific Waltham, MA, USA) consisting of an Accucore phenylhexyl-column (2.6 µm, 100 mm x 2.1 mm, Thermo Fisher Scientific). The column and autosampler temperature were set at 40°C and 4°C respectively. The mobile phase consisted of a mixture of A (2 mM ammonium formate, 0.1% formic acid in ultrapure water) and B (2 mM ammonium formate, 0.1% formic acid, 1% water in 50/50 (v/v) methanol/acetonitrile). A linear gradient with the following proportions of solvent B was applied: 0.0 – 2.0 min at 2%, 2.0 – 5.0 min from 2% to 80%, 5.0 – 6.0 min at 80%, 6.0 – 6.5 min from 80% to 90%, 6.5 – 7.0 min at 90%, 7.0 – 7.5 min from 90% to 95%. The flow rate was 0.4 mL/min. After separation, the compounds were detected using a Q-Exactive Hybrid Quadrupole orbitrap mass spectrometer (Thermo Fisher Scientific) equipped with a heated electrospray ionization source, operating in positive ionization mode. The following ionization source parameters were used: sheath, auxiliary and sweep gas flow rate at 45, 15, and 0 arbitrary units (au), respectively; heater and capillary temperature at 350 and 300 °C respectively, spray voltage at 350 kV and S-lens RF level at 70.0. A full scan mode was used, scanning from 150 to 500 mass to charge ratio (m/z) with a resolution of 70000. The automatic gain control target was set at balanced (1 × 1e6 ions) with a maximum injection time of 200 ms. Diisooctyl phthalate (391.28429 m/z) was used as lock mass. The data were processed using Tracefinder 3.3 (Thermo Fisher Scientific). Detection of each compound was based on their retention time and exact mass to charge ratio (Table 1). Analysis of components was performed by ultra-high-performance

Figure 1: Experimental setup for the collection of nebulized aerosol by the ExaBreath®. A: Sidestream disposable nebuliser chamber with aerosol solution, B: ExaBreath® with electrostatic based filter, C: Tube connected to compressed air, D: Optiflow™ tubing kit, E: Optiflow™ nasal cannula.

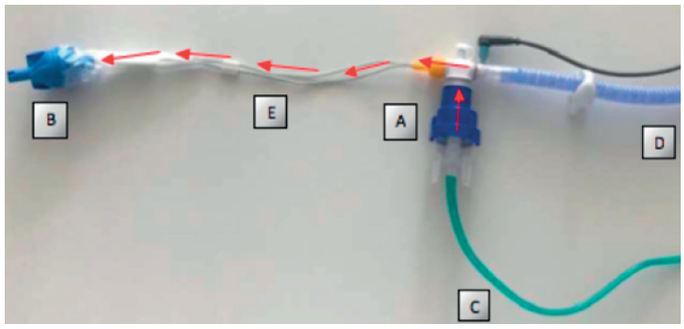

Figure 2: Extraction of budesonide, ipratropium and salbutamol from the filter.

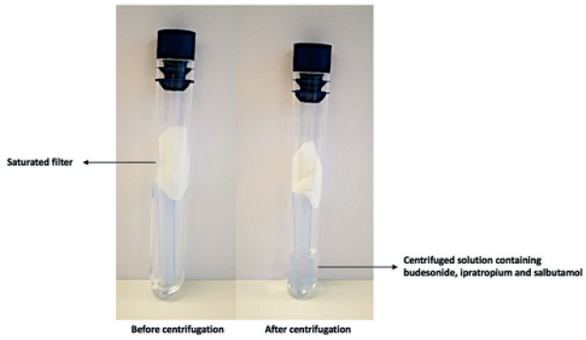

Table 1: Ions used for quantification, exact masses and retention times of the compounds. m/z = mass to charge ratio; (M+H)+ = protonated parent ion; (M)+ = parent ion.

Compound	Ion used for quantification	*m/z*	Retention time (min)
Budesonide	$(M+H)^+$	431.24282	5.48
Ipratropium	$(M)^+$	332.22202	4.04
Salbutamol	$(M+H)^+$	240.15942	3.26
Salbutamol-D3	$(M+H)^+$	243.17825	3.25
Triamcinolone acetonide-D6	$(M+H)^+$	441.25540	5.15

Table 2: Mean percentage (± standard deviation) of budesonide, ipratropium and salbutamol initially added to the sidestream disposable measured in the ExaBreath®, the sidestream disposable nebulizer chamber and the rinsing water of the nasal cannula tubes after completion of the experiment (n=3).

	ExaBreath® (%)	Residue in sidestream disposable (%)	Nasal cannula tubes (%)
Budesonide	0.22 ± 0.10	33.15 ± 3.91	0.45 ± 0.22
Ipratropium	0.35 ± 0.20	48.89 ± 3.86	2.07 ± 1.10
Salbutamol	0.42 ± 0.21	44.99 ± 4.71	1.93 ± 1.34

liquid chromatography-high resolution mass spectrometry, resulting in a chromatogram for each component and for an internal standard. Quantification of the compounds was performed by calculating the corresponding responses (ratio of area of the compound and area of the corresponding internal standard). For budesonide, the deuterated internal standard triamcinolone-acetonide-D6 was used. For salbutamol and ipratropium, salbutamol-D3 was chosen as internal standard.

As no human or animal subjects were involved, ethical committee approval was not obtained for this study.

Results

Based on the mean instrumental response obtained from the spiked ExaBreath® filter experiment without nebulizing (100% recovery) and the mean instrumental response obtained from the in vitro experiment, the amount of budesonide, ipratropium and salbutamol that reached the ExaBreath® after nebulizing was found to be only 0.22 ± 0.10 %, 0.35 ± 0.20 % and 0.42 ± 0.21 % respectively of the initial amount of compound added to the sidestream disposable nebulizer chamber (Table 2). In a second experiment where nasal prongs of the oxygen nasal cannula were directly submerged into a test tube containing 1mL ultrapure water, no budesonide and salbutamol could be detected and the total cannula output from ipratropium was less than 1%. The solution that remained in the sidestream disposable nebulizer chamber and in the tubes of the oxygen nasal cannula after the experiment were also collected and analyzed. The collected data showed that 33.15 ± 3.91 %, 48.89 ± 3.86 % and 44.99 ± 4.71 % of the initial amount of budesonide, ipratropium and salbutamol, remained in the sidestream disposable nebulizer chamber after completion of the experiment. For all the compounds, only a small fraction could be detected in the nasal cannula tube (Table 2).

Discussion

When budesonide, ipratropium bromide and salbutamol were nebulized using a jet nebulizer through a humidified and heated (37˚C) Optiflow™ nasal cannula system for 20 minutes at a flow of 6 L/min, for all compounds, less than 1% actually leaves the nasal prongs of the oxygen nasal cannula. A small amount of the compounds could be measured in the rinsing water of the nasal cannula tubes and the highest amount remained in the sidestream disposable nebulizer chamber. These results are in line with Dugernier et al. who found that pulmonary drug delivery of diethylenetriaminepentaacetic acid using a jet nebulizer through the HFNC was between 0.7 and 2.0 % of the nominal dose and substantial deposition was observed in the single limb circuit, the humidification chamber and the nasal cannula (13). Zhou et al found that 65 to 67% of solution left behind after nebulization with the SideStream, irrespective of relative humidity conditions (15). O'Callaghan and Barry found that more than 90 percent of the primary droplets become trapped on internal structures or remained in the sidestream disposable nebulizing chamber when using jet nebulizers (16).

The nebulizer performance is much less for budesonide than for salbutamol. Budesonide is a suspension medicine, with a particle size distribution peak during nebulization that is larger than the one of a solution medicine, such as salbutamol (15). That is one of the reasons why the output of a suspension is less than that of an aqueous solution when nebulization is used (17).

As nebulizing budesonide, ipratropium and salbutamol using a jet nebulizer through a humidified and heated (37˚C) Optiflow™ nasal cannula system for 20 minutes at a flow of 6 L/min resulted in a delivery percentage far less than 50%, routine use of this specific set-up is not recommended. Numerous in vitro studies reported a range of factors strongly affecting delivery efficacy (i.e., nebulizer system, delivery gas type, nebulizing time, flow rate, temperature, droplet size, the starting volume, positioning of the nebulizer, size of nasal cannula), of which the administered gas flow rate is believed to play a critical role (8,9,11-14,18,19). Both Perry in 2013, and Daily in 2017 demonstrated that increasing gas flow rates significantly decreases the inspired dose of aerosol (11,12). High flow gas rates might induce particle impaction in the HFNC circuit. In our set-up, we suspect that temperature played an important role in the low delivery efficacy. The concentration of budesonide, ipratropium and salbutamol in the sidestream disposable was higher after the experiment than before the experiment, indicating that water had evaporated. A likely cause is the use of heated air (37˚C), necessary for the HFNC. The conventional procedure for nebulizing compounds involves the formation of an aerosol, without the use of heated air. In a conventional setup, the concentration of the compounds in the liquid droplets will be equal to the concentration of the compounds in the sidestream disposable nebulizer chamber. However, when the nebulizer is coupled to an Optiflow™ nasal cannula system for the administration of oxygen, heated air is required. Furthermore, budesonide, ipratropium and salbutamol are known to be thermolabile compounds (20-22). This explains the low efficiency when using heated air during nebulizing and why the combined amount of compound measured in the ExaBreath®, nasal cannula tubes and the sidestream disposable after nebulizing does not add up to 100%. On the other hand, it might be that not the full amount of component in the tubes was collected after rinsing.

In our neonatal unit we used to position the nebulizer downstream of the humidifier. In adult intensive care units the nebulizer is mainly placed at the inlet of the humidifier (23). Réminiac demonstrated that, in adult HFNC circuits, placing nebulizers immediately upstream from the humidification chamber is the most efficient position (8). Placement of aerosol devices between the humidifier and the patient results in a greater aerosol deposition in the tube that can occlude the nasal prongs (24).

This study has some limitations. The experimental setting was restricted to a fixed flow and one size of prongs. Perry et al showed that the inspired dose of salbutamol decreased with smaller sized cannulas (11). The use of different flows and of prongs with other sizes will influence the deposition of medication, as might the type of circuit used. As such, results of this in vitro study can not be generalized to all types of circuits and all flow settings. Therefore, each neonatal unit practicing nebulization in high flow circuits should measure the efficacy of deposition of medication with their own set-up. The in vitro setting excludes the influence of the patient, whose breathing efforts might influence the quantity of medication that effectively reaches the respiratory system. In adults with "quiet" breathing patterns, the inhaled dose seems to increase with lower flow rates while in a "distressed" breathing pattern, the aerosol delivery is higher when gas flow reaches approximately 50% of the inspiratory flow (19). If the same would be applicable in (preterm) neonates, in whom the inspiratory flow is low (ranging from 0.8 L/min to 3.5 L/min), lowering the flow rate to less than 50% of the inspiratory flow might compromise some physiologic benefits of HFNC (19,25). Finally, in the second experiment the nasal prongs were submerged into a sealed test tube. Additional pressure built up in the test tube during the experiment may have affected the final deposition of medication. To verify this, the experiment should be repeated with components for which total cannula output is known. Unfortunately, knowledge about this is currently lacking.

Conclusion

This in vitro study showed that nebulizing budesonide, ipratropium bromide and salbutamol using a jet nebulizer through a humified and heated (37°C) Optiflow™ nasal cannula system with a flow of 6L/min, does not result in clinical relevant deposition of these drugs at the nasal interface. Therefore, routine use of that specific set-up in neonatal units should not be recommended. However, in vitro studies miss several important patient factors, underscoring the need for more anatomically accurate models. Validated deposition models using the airway geometry of children of different ages do not exist to date. Therefore, well-designed studies in neonatal patients are necessary to determine how nebulizing conditions can be optimized in order to result in a therapeutic amount of drugs delivered through an Optiflow™ nasal cannula system, enabling delivery of oxygen and medication simultaneously without patient manipulation.

Conflicts of interest

The authors have no conflicts of interest to declare.

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