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Original article

Evaluation of some parameters influencing the drug delivery from a dry powder inhalation device using an in vitro model of the horse airways

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Summary – The aim of this study was to determine the effect of breathing pattern, air humidity and position of the device on the delivery of an aerosol generated by a dry powder inhalation (DPI) device (Inhalator M®). The in vitro inhalation study was performed using the cascade impaction method (Andersen Sampler) adapted to imitate nasal breathing. The amount of ipratropium found in the device, the artificial upper airways and the six stages of the Andersen Sampler was measured using high precision liquid chromatography. Stage 1 of the Andersen Sampler was considered to be the respirable fraction and stages 2 to 6 to be the non-respirable fraction. It was concluded that the theoretical respirable fraction of ipratropium obtained after DPI through Inhalator M® was influenced by relative air humidity, air flow and the position of the device, whereas the number of successive inspirations and the duration of inspiration did not affect this fraction of the drug.

dry powder inhalation / ipratropium bromide / horse / drug delivery

Résumé – Évaluation de paramètres influençant la libération d'un médicament à partir d'un inhalateur de poudre sèche grâce à un modèle in vitro des voies aériennes du cheval. L'objectif de cette étude était de déterminer l'effet de la stratégie respiratoire, de l'humidité relative et de la position de l'inhalateur sur la génération d'un aérosol à partir d'un inhalateur de poudre sèche (Inhalator M®) convenant aux chevaux. La méthode d'impaction en cascade (Andersen Sampler) a été utilisée et adaptée pour imiter une respiration nasale. Les quantités d'ipratropium bromide dans l'inhalateur, dans les voies aériennes supérieures et dans les six niveaux de l'Andersen Sampler, ont été dosées par chromatographie liquide à haute performance. Le niveau 1 de l'Andersen Sampler a été considéré comme représentant la fraction potentiellement respirable du médicament tandis que la somme des

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cinq autres niveaux correspondait à la fraction non respirable. Il a été conclu que la fraction théoriquement respirable d'ipratropium libéré de l'Inhalator M® et dosée après inhalation était influencée par l'humidité relative de l'air ambiant, par le débit aérien et la position de l'inhalateur, tandis que ni le nombre d'inspirations successives ni la durée d'une inspiration ne modifiaient cette fraction du médicament.

inhalation de poudre sèche / ipratropuim bromide / cheval / libération du médicament

INTRODUCTION

Dry powder inhalation (DPI) has recently been developed in veterinary medicine as an alternative to nebulisation and metered-dose inhalation (Duvivier et al, 1997). Inhalation of ipratropium bromide (ipratropium) for the treatment of respiratory diseases is widely used in human (Pakes et al, 1980; Greenough et al, 1993) and equine medicine (Robinson et al, 1993) although it is not licensed for use in animals.

Many factors can affect the delivery of a DPI device, including aerosol characteristics (eg, electric charge, concentration, tonicity, etc) (Melandri et al, 1977; Timsina et al, 1994), air flow (Timsina et al, 1993; Onyechi et al, 1994), relative air humidity (Morrow, 1986; Jashnani et al, 1995), and several factors related to the patient (ie, duration of inhalation and morphology of the respiratory system) (Dickens et al, 1994). During in vivo studies, these parameters are difficult to control or to measure. It is therefore difficult to assess their respective implications on the results of a DPI treatment. In contrast, with in vitro tests, each variable can be examined separately from other variables, at least in theory.

In human medicine empirical in vitro methods have been developed to mimic drug deposition in imitated airways. The most frequent method is based on cascade impaction, which collects drug doses through simulated upper airways onto plates corresponding to the lower respiratory tract (Hallworth and Andrews, 1976), the inhalation procedure reproducing mouth breathing.

Horses are nasal breathers (Barone, 1984) and their nasal cavities include tortuous anatomical structures that create high air flow turbulence. Drug behaviour in the airways of horses may be influenced by their particular upper airway anatomy.

To better understand the factors that influence aerosol delivery from DPI in horses, we developed an in vitro model of nasal breathing. Our specific aim was to determine the effect of breathing pattern, air humidity and position of the device on the delivery of the aerosol generated by a DPI device suitable for horses.

MATERIALS AND METHODS

DPI device and drug

The DPI device used was the Inhalator M® (Boehringer Ingelheim, Germany). It can contain up to six gelatine capsules (ie, the inhalets). The inhalet is punctured by pressing a button linked to needles, rendering its contents available for inhalation. The aerosol is generated by the patient's inspiration through the DPI device and the resulting turbulent airflow in the capsule causes the powder particles to flow out of the device.

Ipratropium dry powder is commercially available as inhalets of Atrovent® (Boehringer Ingelheim, Germany). Each contains 5 mg of micronised powder, including 200 µg of the active ingredient and glucose as carrier.

Methods

In vitro model

The cascade impactor used was an Andersen 1 ACFM six-stage Viable Particle Sampler (Andersen Air Samplers, 2000 Inc, Atlanta, USA) with glass Petri dishes to collect the dry powder samples. It was connected to a previously calibrated rotameter. A simulation of nose breathing was constructed to reproduce in vivo air flow turbulences in the upper airways (UA) (fig 1). The UA was connected airtightly to the opening of the cascade impactor on one side and to the DPI device on the other.

According to the D_p50 equation (Andersen Air Samplers, 2000 Inc, Atlanta, USA), which makes it possible to calculate the size range of the particles for each stage as a function of the air flow, particles of minimum 0.56 µm were deposited on stage 1 of the Andersen Sampler (respirable fraction). Particles ranging from 0.56 to 0.05 µm were deposited on the five next stages and were considered as the non-respirable fraction of the drug.

For each experiment, five DPI devices were used, each of them containing five inhalets of Atrovent®. The DPI device was airtightly connected to the UA, one inhalet was punctured and air was sucked through the entire system by a vacuum pump. The same procedure was then repeated for the next inhalet. The air flow was 72 L.min^{-1}. After inhalation of the five inhalets, the DPI device, the UA and six stages of the Andersen sampler were carefully washed with purified water and the dosage of ipratropium was measured using high precision liquid chromatography (HPLC) (Jacobson and Petersen, 1994).

Effect of breathing pattern

Three aspects of breathing were investigated. Laboratory conditions were the same for each experiment (ie, air at 18 ± 2 °C and 46 ± 4% of relative humidity, used as carrier gas).

First, to assess the effect of air flow on deposition performances of this DPI device, two air flow rates were used, ie, 72 L.min^{-1} and 28.3 L.min^{-1}. An air flow period of 5 s was used.

Second, to assess the variability of the drug deposition resulting from successive inspirations, each inhalet of Atrovent® was inhaled during 1, 2 or 3 successive inspirations. The air flow was 72 L.min^{-1} and the air flow periods were 5 s.

Third, to determine the effect of the duration of one inspiration on the drug delivery from this DPI device at 72 L.min^{-1}, two durations were used (ie, 5 and 15 s).

Effect of relative humidity

In order to determine the influence of relative humidity (RH), the aerosol performances of ipratropium under different environmental conditions were tested. The cascade impactor was housed in a pre-equilibrated environmental chamber. Relative humidity of the room was varied (46 or 95% RH) and air was used as carrier gas. The air flow was 72 L.min^{-1}. An air flow period of 15 s was used.

Effect of the DPI device position

In order to assess the position of the DPI device on ipratropium deposition, two positions in front

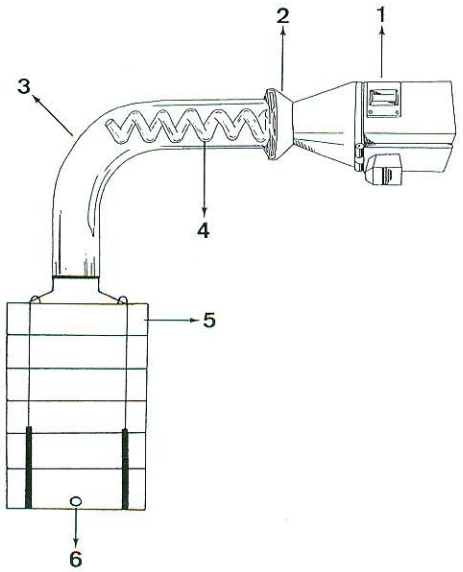

Fig 1. Schematic representation of imitated equine upper airways connected to the DPI device and to the Andersen Sampler. 1: Inhalator M®; 2: airtight connection for the inhaler; 3: glass upper airways; 4: glass helix; 5: Andersen Sampler; 6: connection for the pump.

of the UA were tested. First, the DPI device was connected in front of the UA. Second, the DPI device was placed slantwise to the UA, ie, with an angle of 45° to the longitudinal axis of the UA. The air flow period was 72 L.min^{-1} and an air flow period of 15 s was used. Air at 18 °C and 46% relative humidity was used as the carrier gas.

HPLC analysis

Chromatographic equipment

The HPLC system used to measure the amount of ipratropium consisted of a Model LC-10AD pump and a SPD-10A UV/Vis programmable wavelength detector from Shimadzu Corporation (Kyoto, Japan) as well as a six port Rheodyne valve (Cotati, CA, USA) with a 100 µL external loop. The UV detector was set at 210 nm. A Manu-Cart system, which was made up of LiChroCART analytical column (125 × 4 mm, id) from Merck (Darmstadt, Germany), was thermostated at 25 ± 0.1 °C in a Shimadzu CTO-10AC oven. The control of the HPLC system as well as data collection, storage and treatment were managed by Shimadzu Class-LC10 software version 1.20 loaded in an IBM-AT compatible micro computer (CPU type: 80486 DX 33).

Chromatographic technique

The stationary phase of the LiChro CART column was LiChrospher 60 RP-Select B (particle size: 5 µm) (Merck, Darmtadt, Germany). The mobile phase (isocratic mode) was prepared by mixing 240 mL of acetonitrile (Acetonitril far UV, Acros Chimica, Geel, Belgium) and 1 000 mL of 0.05 M phosphoric acid containing 1-butanesulfonic acid sodium salt at a concentration of 0.1% (w/v). The water used in all experiments was purified on a Milli-Q system (Millipore, Bedford, MA, USA). The flow rate of the mobile phase was 1.5 mL.min^{-1}.

Linearity and reproducibility

Two calibration lines ranging from 0.1 µg/mL to 10 µg/mL (n = 5) and from 1 to 100 µg/mL (n = 5) were constructed before each analysis using a linear least square regression method. Coefficients of determination were superior to 0.999.

Calculations and statistical analysis

Assessment of the drug delivery from Inhalator M® was based on the dosage of ipratropium in four parts of the in vitro system: the device, the UA, stage 1 of the Andersen and stages 2 to 6 of the Andersen (non-respirable fraction).

The total dose (TD) was calculated as the sum of the powder dosed at each stage of the deposition apparatus (DPI device + UA + 6 stages of the Andersen Sampler). The percentage of TD (%TD) was calculated as the ratio between the amount of drug dosed in a specific part of the model after inhalation and the TD.

Mean values and standard deviation were calculated and analysed using a non-parametric Mann–Whitney test. The limit of statistical significance was defined as $p < 0.05$.

RESULTS

Effect of breathing pattern

High flow rate significantly decreased the amount of drug remaining in the device after inhalation from 91.43 ± 8.21%TD at 28.3 L.min^{-1} to 37.92 ± 10.40%TD at 72 L.min^{-1}.

Table I presents the results of successive inspirations on ipratropium delivery. The drug delivery from the device and its deposition pattern were not modified after successive inspirations through the in vitro system.

An inspiration lasting 15 s did not change the deposition pattern of ipratropium in our model (table I).

Effect of relative humidity

The drug remaining in the device after inhalation was higher at 95%RH (54.49 ± 17.09%TD) than at 46%RH (37.93 ± 10.39%TD) but the difference was not statistically significant. Low relative humidity significantly increased the amount of

Table I. Effect of successive inhalations and duration of one inspiration on ipratropium delivery from the device. Values are expressed as mean (standard deviation), $n = 5$.

Inspiration	*Device (%TD)*	*Upper airways (%TD)*	*Stage 1 Andersen (%TD)*	*Stages 2–6 Andersen (%TD)*
1 inspiration (5 s)	44.98 (14.55)	23.07 (7.36)	18.66 (5.12)	18.51 (8.29)
2 inspirations (2 × 5 s)	36.96 (14.29)	26.41 (6.18)	18.36 (4.12)	19.40 (3.43)
3 inspirations (3 × 5 s)	40.68 (12.66)	22.52 (5.10)	20.55 (4.20)	20.77 (6.98)
1 inspiration (15 s)	37.92 (10.40)	23.04 (2.21)	18.38 (1.57)	21.67 (7.01)

%TD: percentage of total dose.

drug collected on stage 1 of the Andersen from 11.88 ± 3.93 to 18.38 ± 1.57%TD.

Effect of the DPI device position

Results are summarised in table II. When the DPI device was placed slantwise compared to the opening of the upper airways, the quantity of ipratropium remaining in the device after inhalation was the same as when the DPI device was in front of this opening, but it significantly decreased the amount of powder deposited on stage 1 of the Andersen Sampler by increasing the dose impacted in the UA.

DISCUSSION

As reported in human studies, all in vitro models simplify respiratory anatomy but are essential to characterise inhalation products (Ganderton, 1995) as well as to determine factors affecting DPI devices (Dickens et al, 1994). The present study simplified the anatomical and physiological properties of the equine airways and standardised the measurements. The in vitro model therefore allowed us to induce variation of one specific parameter and to determine whether or not it affected the drug delivery from the Inhalator M®.

The fractions of ipratropium collected on the different stages of the model were expressed as a function of the total amount of drug deposited and dosed after inhalation through the system. However this may overestimate the amount of dosed drug. Indeed two calibration equations were needed to cover the potential ranges of drug concentrations. The slopes determined by these equations might be slightly different, whereas their coefficients of determination were always greater than 0.999. This therefore ensures that if there was overestima-

Table II. Effect of the device position on ipratropium delivery. Values are expressed as mean (standard deviation), $n = 5$.

Position	*Device (%TD)*	*Upper airways (%TD)*	*Stage 1 Andersen (%TD)*	*Stages 2–6 Andersen (%TD)*
Normal	37.92 (10.40)	23.04 (2.21)	18.38 (1.57)	21.67 (7.01)
Slantwise	31.79 (12.26)	37.84* (10.94)	12.85* (1.03)	17.51 (1.31)

% TD: percentage of total dose; * significantly different from normal position, $p < 0.05$.

tion of some ipratropium dosages, it was not of statistical significance.

Whatever the method of inhalation, size distribution of inhaled particles is one of the determinants of the amount that reaches the lungs. It is assumed that particles from 5 to 10 µm are trapped in the upper airways, whereas 90% of particles less than 0.5 µm are exhaled (Mitchell, 1960; Clarke and Pavia, 1988). It is therefore important to produce particles from 0.5 to 5 µm to maximise the chance of their deposition in the lower respiratory tract (Clarke, 1991). In our in vitro model, stage 1 of the Andersen Sampler collected particles from 0.56 µm and above allowing us to focus on this theoretical respirable fraction. Particles deposited on stages 2 to 6 of the Andersen Sampler ranged from 0.56 to 0.05 µm. They were therefore considered as the non-respirable fraction of the drug that would be exhaled during in vivo DPI treatment. This fraction of ipratropium dry powder appears to be constant whatever parameter was varied. This suggests that whatever the conditions of DPI treatment with ipratropium and Inhalator M®, the user has no means to decrease this non-respirable fraction of the drug.

The loss of powder in the Inhalator M® was large but it seemed to be consistent with measurements made with other DPI devices (Ganderton and Kassem, 1992). Two reasons could explain the quantity of drug remaining in the device after inhalation. First, relative humidity and electrostatic forces created by the air flow into the plastic device fix the powder on the walls of the gelatine capsules. Second, the design of the devices influences the emptying of the capsules, acting on both air flow turbulences and deaggregation of the powder (Bell et al, 1971; Vidgren et al, 1988a). The DPI device used in this study had a complicated internal structure. It was supposed to create high air flow turbulences, resulting in an effective deaggregation of the powder. On the other hand, it also acted partly like a powder trap that may retain agglomerates of particles too large to be efficiently inhaled. For this reason, it could be compared to the spacers used in addition to metered-dose inhalers (Corr et al, 1982; Dolovich et al, 1983), resulting in selective delivery of smaller respirable particles to the lung.

The flow rate at which a patient inhales through a powder inhaler affects the amount of drug reaching the lung (Vidgren et al, 1988b; Newman et al, 1989; 1994). In our model, the flow rate was constant and did not exactly reflect an in vivo breathing pattern. However the difference in drug delivery between the two tested air flows was significant, probably because the low flow rate was not able to create sufficient turbulence to deaggregate the micronised dry powder that remained in the device after inhalation. Moreover horses can attain much higher rates of air flow (Lekeux and Art, 1994), which suggests that during in vivo DPI procedure, they would empty the inhalets at least to the extent that they were at 72 $L.min^{-1}$ in the in vitro model.

At equal air flow, neither two or three successive inspirations through the device nor the duration of the inspiration modified the deposition pattern of the drug obtained after a single inspiration. This suggests that the duration of an in vivo DPI procedure should be shorter. This represents an advantage over nebulisation, which lasts about 10 min (Robinson et al, 1993).

Relative humidity is known to influence the aerosol delivery from DPI devices (Dickens et al, 1994; Jashnani et al, 1995). Using Inhalator M®, it decreased its delivery performance. At 95% relative humidity, water probably reaches the powder within the device, resulting in agglomeration of powder in the inhalets that were not efficiently emptied. Relating these last results with in vivo situations suggests that DPI should be performed in an environment with a low rel-

ative humidity to minimise loss of powder within the device.

The position of the DPI device in front of the airway opening is critical for its use in horses. Indeed, because horses breathe only by the nose, inhaled drugs must go through the nasal cavities before being deposited in the lower airways. It seems therefore essential to find a position for the device that permits the best drug delivery. When the device is placed slantwise compared to the opening of the airway, the indirect trajectory of air flow between the output of the device and the upper airways results in increased impaction of the powder at this site. This suggests that the more beneficial position of the device for inhalet emptying is in the longitudinal axis of the nasal cavities of the horse.

In conclusion, this in vitro study showed that under standardised conditions, the theoretical respirable fraction of ipratropium obtained after DPI through Inhalator M® is influenced by relative air humidity, air flow and the position of the device, whereas the number of successive inspirations and the duration of the inspiration did not affect this fraction of the drug.

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