Original Article

Experimentation with aerosol bonsetan, pirfenidone, treprostinil and sidenafil

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Introduction: Pulmonary hypertension (PH) has been identified either as a symptom or a primary entity. Several drugs are already on the market and other are being investigated. Idiopathic pulmonary fibrosis (IPF) is also a disease were several drugs are being investigated.

Materials and methods: Three jet nebulizers and three ultrasound nebulizers were used for our experiments with seven different residual cups and four different loadings. Bonsetan, treprostinil, sidenafil and pirfenidone were modified in order to be produced as aerosol in an effort to identify parameters which influence the droplet size production size.

Results: The four-way ANOVA on droplet size using the jet nebulizers revealed two statistically significant factors, drug (F=6.326, P=0.0007) and residual cup (F=4.419, P=0.0007), and their interaction term (F=5.829, P<0.0001). Drugs bonsetan and pirfenidone produce equally the lowest mean droplet size (2.63 and 2.80 respectively) as compared to other two drug mean sizes. The ANOVA results, concerning the ultrasound nebulizers, revealed only the nebulizers as producing significant effect on droplet size (F=4.753, P=0.037).

Discussion: Our study indicates the importance of the initial drug design formulation. Moreover, further investigation of the residual cup design is an additional parameter that can assist in the optimal droplet size production, indifferently of the drug formulation.

Keywords: Pulmonary hypertension (PH); interstitial lung fibrosis; aerosol; bonsetan; pirfenidone

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Introduction

Inhaled therapies have been used for respiratory diseases and symptom relief since many years and vast experience exists for many products (1-4). Several drugs are also being investigated whether they could be administered as aerosol (5-9). The major obstacle for efficient aerosol deposition are the defense mechanisms of the respiratory system (10) and the drug design for efficient tissue distribution (11). Moreover; safety concerns for novel methods of drugs as aerosol administration, still remain to be investigated and elicited (8,12-16). Previous research studies have identified several parameters that influence the droplet size production and can be summarized to the following: (I) residual cup loading; (II) initial loading of

Figure 1 Jet-nebulizer.

the residual cup; (III) refilling of the residual cup when the initial filling has been reduced to half (this can be done only once); (IV) residual cup design; (V) inlet design (if used) and (VI) drug formulation (17-20). Currently there is an increasing interest in the investigation for drugs administered as aerosol for pulmonary hypertension (PH) and idiopathic pulmonary fibrosis (IPF). PH is defined as a mean pulmonary arterial pressure (PAP) ≥25 mmHg at rest when assessed with right heart catheterization (21). It is a fatal disease caused by vascular proliferation, remodeling and small vessel obstruction. Increased pulmonary vascular resistance (PVR) which is often observed, leads to right-sided heart failure (HF) and death (22). Current guidelines have divided PH in five major categories (23). Currently there are new insights presented for the pathophysiology of the disease and progress in the diagnosis. The diagnosis includes (I) right-heart catheterization; (II) optical coherence tomography and (III) computed tomography. Current treatment strategies for pre-capillary PAH include: (I) lifestyle modification; (II) endothelin receptor antagonists; (III) prostacyclins and (IV) immunosuppressive therapy. Novel agents currently considered are: riociquat, imatinib and Rho-kinase inhibitors. Novel agents for post-capillary PAH include: sildenafil, riociquat and Rho-kinase inhibitors. Previous drugs include: angiotensin-receptor blockers, nitrates, diuretics, angiotensin-converting enzyme inhibitors and β-blockers. Pulmonary artery angioplasty is considered the treatment for chronic thromboembolic PH (24). Currently inhaled treprostinil (Tyvaso) has been approved by the FDA [2009] for patients with PAH with NYHA III. Prostcyclin-2 through cycloxygenase-2, leads to vasodilation and platelet aggregation (25). Prostacyclin causes vasodilation in both systemic and pulmonary arteries. Moreover; it has antiproliferative activity as observed with the inhibition of growth of smooth muscle cells. Inhaled prostanoids are usually administered in patients who are not candidates for parenteral treatment, or with declining condition. The major advantage is the delivery of the drug directly to the lungs and therefore less systemic side effects have been observed (4,26,27). Inhaled treprostinil through the TRIUMPH trial provided data where the 6-minute walking test was improved when compared to stable doses of bonsentan or sildenafil (28). IPF is a debilitating disease occurring in adults between 60 to 75 years of age (29). The disease is fatal with a median survival of 2-5 years, and progressive pulmonary function occurs. Pirfenidone is indicated for mild to moderate IPF based on phase III trials using forced vital capacity (FVC) ≥50%, carbon monoxide diffusing capacity (DLCO) ≥35% and 6-minute walking test (6MWT) distance of ≥150 m (30). Additional drugs that have presented positive results as additional treatment for IPF are N-acetylcysteine (NAC) and nintedanib (BIBF 1120), however; further investigation is necessary (31,32). In the current study we have investigated whether modification of the drugs bonsentan, sildenafil, treprostinil and pirfenidone with different nebulizers, residual cups and loadings could be used as a future local administration to the lung parenchyma.

Materials and methods

Drugs

The following drugs were purchased: (I) Tracleer® (bonsetan) 62.5 mg/tab, Actelion; (II) Revatio®, (sildenafil) 20 mg/tab, Pfizer; (III) Esbriet® (pirfenidone) 276 mg/hard capsule, Intermune UK Ltd.; and (IV) Remodulin® (treprostinil) 5 mg/mL solution, United Therapeutics Europe Ltd.

Nebulizers and residual cups

Jet-nebulizers and residual cups

Three nebulizers were chosen from our department for the experiment: Maxineb® (6 liters/minute and 35 psi), Sunmist® (5-7 liters/minute and 35 psi) and Invacare® (4-8 liters-minute and 36 psi). In total seven residual cups were chosen for evaluation, four with a capacity of no more than 6 mLs and two with a capacity no more than 10 mLs. The designs for the large residual cups will be mentioned as A, D and E. The small residual cups will be mentioned as C, F, B and J. The large residual cups were not used with a capacity of more than 8 mLs as explained in the discussion section (*Figure 1*).

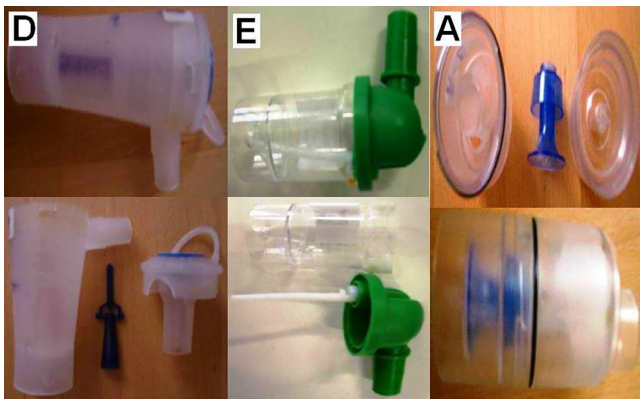

Figure 2 Large residual cups 10 mLs max.

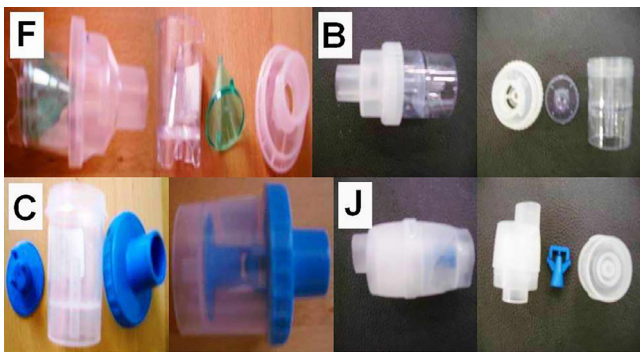

Figure 3 Small residual cups 6 mLs max.

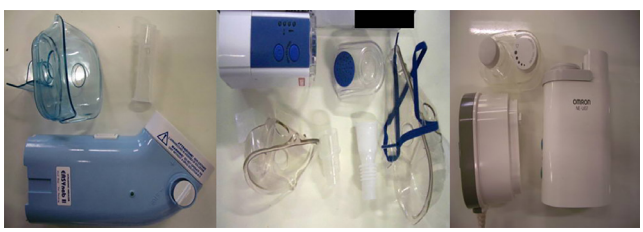

Figure 4 Ultrasound nebulisers.

Figure 5 Matersizer 2000.

Ultrasound nebulizers

Three new ultrasound nebulizers were chosen from the market based on their cost-effectiveness. The first was Omron® NE-U07, Tokyo, Japan. Compact and weight less than 350 gm, includes 10 mL medication cup. Generates uniform micro millimetre-sized vapor particles. The second was a portable GIMA, Gessate, Italy (Choice Smart Health Care Company Limited, Wan Chai, Hong Kong, No. G2061259328002) with the following operating specifications; particle size: 3-5 μm, frequency: 2.5 MHz, medication cup capacity: 1-6 mLs, sound level at 10 cm: <50 db, operating temperature: min. 10 ℃, max. 40 ℃ and air humidity: min. 10%, max. 95% RH. The third was a portable EASYneb® II, FLAEMNUOVA, Martino, Italy. with the following operating specifications; drug max capacity: 8 mLs, frequency: 2.4 MHz, nebulization capacity (adjustable) 0-0.7 mL/min approximately (tests performed with saline 0.9%), particle size: 2.13 μm mass median aerodynamic diameter (MMAD), sound level at 10 cm: 50 db (A), operating temperature: min. 10 ℃, max. 40 ℃ and air humidity: min. 10%, max. 95% RH (*Figures 2-4*).

Measurement of droplet size and droplet size distribution

A laser scattering apparatus (Malvern Mastersizer 2000, Malvern, Worcestershire, UK) equipped with a Scirocco dry accessory module (Malvern, Worcestershire, UK) was used for the determination of the mass median diameter of the produced particles. A specific surface area odd 4.3 was used. Light scattering was used instead of a cascade impactor, as (I) a cascade impactor is, by design, limited to the number of size populations it may discriminate, that is the number of filters. In such a case, a limited number of populations can be distinguished. Our light scattering set-up can measure the light scattering intensity at a very large number of angles (that is the equivalent of a cascade impactor filters), so it can construct a particle size distribution plot of a very large number of points; (II) light scattering is non-invasive to all particles. The above, coupled with the application of the very accurate 'Mie theory' used here for transferring the angle-intensity measurements into size-volume data, and the thorough control of our data fitting, give us confidence in the presented results. Our equipment has been previous used in prior publications (5-8,17) (*Figure 5*).

Milling

The bonsetan, pirfenidone and sidenafil tablets were milled

Figure 6 Milling equipment.

Figure 7 Kern digital scale.

in a planetary ball mill (Frisch, Pulverisette-5) equipped with Agate bowls (500 mL) and 8 balls (20 mm, 20 g) with a rotational speed of approximately 200 rpm which results in an acceleration of about 7.5 g. We initiated our milling at 40 minutes and we acquired a MMAD of 4.4 μm for bonsetan and 3.7 for pirfenidone. The major problem that we encountered was that the sidenafil tablets were influenced by the heat that was developed during the process and a paste was developed around the walls of the agate bowls (*Figure 6*). Therefore we had to seek for an alternative method of milling and we used a grinder FALIING NUMBER S-12611, Stockholm, Sweden, type 120, 3-phase and 280 rpm, made in Finland. Before proceeding to milling we had measured the weight of the tablets and capsules with a digital scale Kern EG 2200-2NM, Kern & Sohn, GmbH, Balingen, Germany (*Figure 7*). After milling we collected powder of the same weight from drug and diluted it with 2 mLs in an effort to simulate a future method/compound of administration.

Statistical assessment

MMAD was measured following three different experimental factorial designs:

(I) Three jet nebulizers in joint with four drugs, seven cup devices with three dosage levels each (2, 4 and 6 mL) were analyzed for potential effects on droplet size by employing a four-factor ANOVA, fixed effects plus their interaction levels, thus totaling 3×4×7×3=252 combined levels;

(II) The same design was repeated including only the large residual cups (A, D, E) at 8 mL dose level using a tree-factor ANOVA and totaling 3×4×3=27 combined levels;

(III) The droplet size was finally checked for potential effects by applying three ultrasound nebulizers, the same drugs as before and "delivery" constructions (face mask and inlet) at two dose levels (2 and 4 mL). A four-factor ANOVA, fixed effects, was performed making up 3×4×3×2=72 combined levels.

Statistically significant effects and interaction terms were tested graphically using pair-wise comparisons of means together with their 95% confidence intervals. Means whose intervals do not overlap are significantly different.

The major aim is focused on the best combination that provides droplets with the least size.

Results

The four-way ANOVA on droplet size using the jet nebulizers revealed two statistically significant factors, drug (F=6.326, P=0.0007) and residual cup (F=4.419, P=0.0007), and their interaction term (F=5.829, P<0.0001). Drugs tracleer and pirfenidone produce equally the lowest mean droplet size (2.63 and 2.80 respectively) as compared

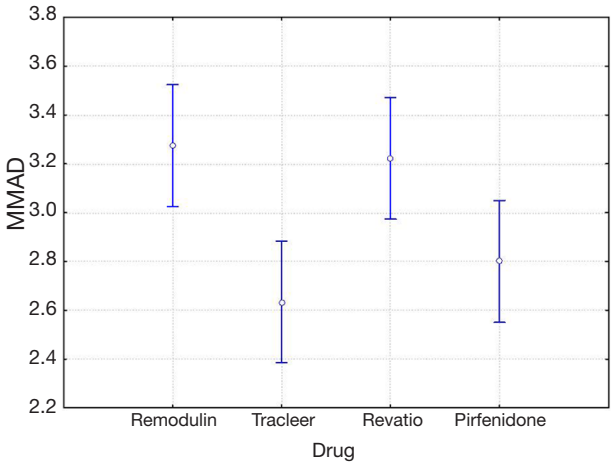

Figure 8 Mean droplet size distribution of four drugs. Vertical bars represent the 95% confidence intervals of the means calculated from the error mean square of ANOVA.

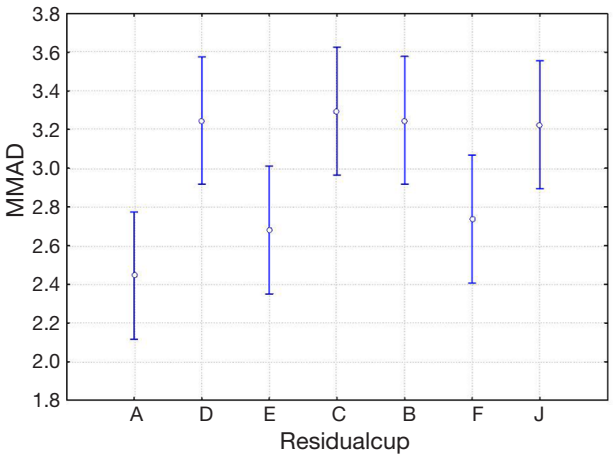

Figure 9 Mean droplet size distribution of seven residual cups. Vertical bars represent the 95% confidence intervals of the means calculated from the error mean square of ANOVA.

to other two drug mean sizes (*Figure 8*). Two groups of different mean droplet size are produced by the residual cups (*Figure 9*). Cups A, E and F are equally the most efficient in small mean droplet size (2.44, 2.68 and 2.74 respectively), while the rest four cups form another group with higher mean sizes, ranging between 3.22 and 3.30). Cup F in joint with drug pirfenidone (*Figure 10*) create the least mean droplet size (1.56), by far lower from all other combined levels and particularly from the combined effect of drug remodulin with cups C, B, and F whose result rises up to 4.1-4.4 μm. The large residual cups (A, D and E), when combined with the four aforesaid

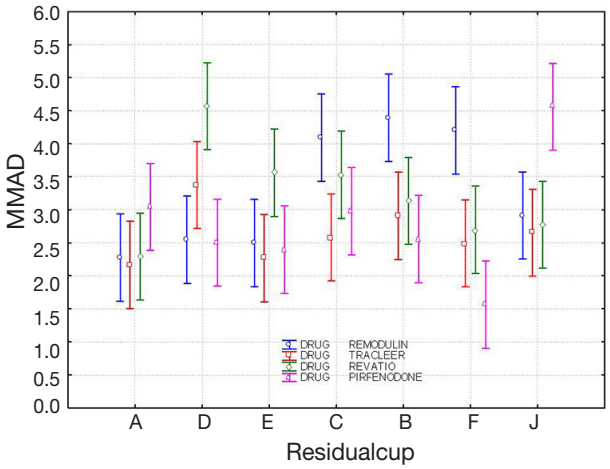

Figure 10 Mean droplet size distribution of the combined levels between drugs and residual cups. Vertical bars represent the 95% confidence intervals of the means calculated from the error mean square of ANOVA.

drugs and the same nebulizers, do not produce statistically significant results at 8 mL dose level (P>0.05 in all factors and interaction terms). The ANOVA results, concerning the ultrasound nebulizers, revealed only the nebulizers as producing significant effect on droplet size (F=4.753, P=0.037). This effect is easily clarified in the *Figure 11* in which the OMRON nebulizer outweighs the others' effect providing a mean droplet size of 2.69 μm. To capitalize, jet nebulizers, although not significant on droplet size, best perform with drugs tracleer and pirfenidone, using cup designs A, E and F, and particularly when combined with cup F and pirfenidone. Loadings greater than 6 mL together with any other factor combined do not exert any significant effect on droplet size. Ultrasound nebulizers produce a unique effect on droplet size, no matter considering any other factor in the study, from which OMRON generates the smallest droplets.

Discussion

Inhalation therapies in order to be efficient they have to bypass the defense mechanisms of the respiratory system. These can be summarized to: (I) beating cilia; (II) macrophages; (III) mucus; (IV) local enzymes; and (V) local transporters (10). Furthermore; an underlying respiratory disease modifies the deposition and absorption of aerosol therapies. Bronchoconstriction which might occur during an exacerbation of asthma or chronic obstructive pulmonary disease (COPD) reduces the distribution and deposition of an aerosol therapy (33,34). The production of mucus

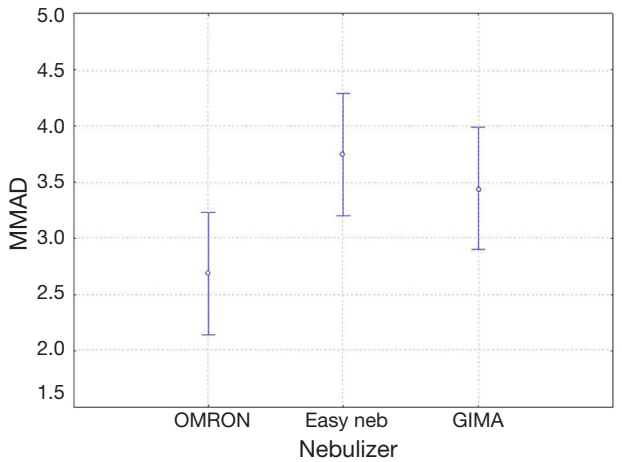

Figure 11 Mean droplet size distribution of three ultrasound nebulizers. Vertical bars represent the 95% confidence intervals of the means calculated from the error mean square of ANOVA.

also reduces the absorption of an aerosol therapy since a concentration of the drug is enclosed within the mucus. Local enzymes and transporters are parameters that their interaction with the aerosol drugs has not been fully investigated. It has been presented that there are different enzymes and transporters at different locations of the respiratory system which modify the absorption of a drug locally (35). Moreover, the aerosol mist has to be composed of droplets of MMAD of <5 μm. The third important factor for an efficient aerosol therapy is the drug design. There are two major delivery systems for local absorption; the passive and active transportation. The passive transportation is based on the principals of the drug formulation molecules and interaction with the local enzymes and transporters. The active transportation is based on an antigen-antibody connection of the drug molecules and local tissue (11). Active transportation tends to be usually less expensive in production, however, in several situations less effective. Cost-effective design is another parameter that has to be included in drug design development. Previous investigation of aerosol production systems have indicated that jet-nebulizers and ultrasound nebulizers are easy to use in the everyday practice, however, jet-nebulizers are currently cheaper than ultrasound nebulizers. Moreover, it has been observed that several drugs are more efficiently delivered with one system versus the other, meaning that we should choose the aerosol production system based also on the drug that we want to deliver (20). The production of aerosol regarding the jet-nebulizers is also influenced by the residual cup design, initial filling, time of nebulisation and drug. Moreover; it has been observed that reduction of the produced droplet size can be achieved with the usage of inlets design (17). Temperature increase from the piezoelectric crystal results in a higher concentration effect of the drug solution, than in the jet-nebulizers (36). However, the combination of temperature and drug concentration cause a shift in the tension and viscosity and consequently change the droplet size distribution (37). The nebulization time also plays a role in the viscosity, surface tension, saturated vapour pressure and finally to the droplet size distribution. Increase of the concentration in the produced aerosol is mostly observed in the ultrasound nebulizers than the jet-nebulizers (38). The variations of the viscosity are also time dependent (low the first 2 minutes, higher after the 4 minutes) and temperature dependent in ultrasound nebulizers. An increase in the mean droplet size range has been also observed when adding buffer (39). The increasing drug concentration is also a factor inducing bronchoconstriction (40). In conclusion the major factors affecting the produced mist are: (I) temperature; (II) viscocity; (III) drug formulations (salts, buffer) and (IV) time of nebulization.

PAH is a complex disease characterized by increased PVR which eventually leads to right-sided HF and death (41). Current recommendations for patients with NYHA II, III, IV symptoms indicate first-line monotherapy with agents targeting the following pathways: endothelin, prostacyclin and nitric oxide. Sequential combination of agents is indicated when there is no evidence of clinical improvement with one specific-PAH agent (42). However, again there are patients who do not present improvement even after a combination treatment (43). Combination treatment has been proposed in advanced stage disease for initial treatment (42). The concept for combination treatment has the advantage of targeting simultaneously different pathological pathways (43). Most studies have investigated the sequential addition of agents and very few initiation of a combination treatment (44). Currently pharmacogenomics are investigated for targeted treatment of PAH as a future treatment (45). Possibly in the future we could target the disease with a combination of aerosol treatment. Treatment for IPF is based on the updated recommendations of 2013 (29). Several agents have been presented and discussed such as, corticosteroids, NAC, interferon-gamma-1b, azathriopine, anticoagulant therapy, oxygen therapy, lung transplantation and pirfenidone (46). Combination of treatments has been also been proposed (46-58). Additionally, "weak no" has been indicated for treatment of PAH due to IPF according to the last updated

recommendations (29). IPF despite current treatment efforts still remains a fatal disease with a median survival between 2-5 years. Currently we investigated whether inhaled pirfenidone could be redesigned to be delivered as aerosol. Major obstacle for IPF is firstly the safety of aerosol administration, because several drugs tend to induce exacerbation of an underlying disease and secondly the efficiency. Production and deposition of a drug does not necessarily mean that the formulation will be distributed from the site of administration to the blood circulation, in specific in patients with IPF since the parenchyma is destroyed. Further investigation has to be investigated probably in a 3D model of lung parenchyma simulating IPF disease.

Conclusions

In our study we investigated drug solutions close to a possible future treatment and we observed that the dilution as it was done it could be a possible method of administration for all drugs. Large concentrations >8 mLs were not necessary as in our previous studies and it is only matter of how much concentration of the drug we want to deliver. Again it was observed that residual cup design plays an important role in the production of the aerosol and the drug formulation. Certainly we need additional local therapies for PH and IPF, local administration has possible advantages, however, future experiments have to present data for the safety of this administration method to the lung.

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