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Communication

Modification Tadalafil and Macitentan tablets to aerosol

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Abstract

Introduction: Aerosolised drugs have been approved for several diseases such as cystic fibrosis and diabetes. Moreover; there are already drugs for pulmonary hypertension in aerosol form already on the market. **Materials and methods**: Two drugs for pulmonary hypertension (Tadalafil and Macitentan) were milled and transformed from tablets to powder. Three different jet-nebulizers with seven different residual cups were combined. Moreover, we used 3 different ultrasound nebulizers with two different release methods. **Results**: The drug and residual cup designs produce alone or jointly different MMAD diameters. The three large (10 mls) residual cups with the jet-nebulisers produced the smallest aerosol droplets. Both ultrasound nebulisers are capable of producing optimal size aerosol droplets $\leq$5 μm mmad. **Conclusions**: These two drugs can be easily administered as aerosol and an vivo clinical study will prove the safety for the airways.

Keywords: Aerosol; Jet-nebulizers; Ultrasound nebulizers; Tadelafil; Milling; Macitentan; Pulmonary hypertension

1. Introduction

The lung parenchyma consists of airways and finally the alveoli. The alveoli are constructed from a thin tissue layer covered with microvessels. It has been observed that particles $\leq$5 μm go through the oropharynx to the trachea, then the smaller airways and finally the alveoli where they are absorbed to the systemic circulation [1]. The absorption and safety of an aerosol compound depends on the PH, particle size, salt of particles, residual cup loading, residual cup design, patient respiratory function, aerosol production source and drug formulation [1,2]. Antibiotics have been used for several years for cystic fibrosis. Inhaled insulin has been investigated as aerosol administration versus subcutaneous [3,4]. Inhaled antibiotics are also in use for cystic fibrosis [5]. Moreover, we can use inhaled nitric oxide for idiopathic pulmonary fibrosis [6]. Finally, several drugs have been used or investigated as aerosol administration of pulmonary hypertension. Pulmonary hypertension is observed when the mean pulmonary arterial pressure (PAP) is $\geq$25 mmHg at rest [7]. It is considered a fatal disease due to vascular proliferation, small vessel obstruction and remodeling. Moreover; these patients have increased pulmonary vascular resistance (PVR). As a result right-sided heart failure (HF) is observed and death occurs if not treated properly [8]. According to current guidelines the disease has been divided into: (a) Pulmonary arterial hypertension, (b) Pulmonary hypertension due to left heart disease, (c) Pulmonary hypertension due to lung disease and/or hypoxia, (d) Pulmonary hypertension due to pulmonary artery obstruction and (e) Pulmonary hypertension with unclear and/or multifactorial mechanisms [9–11]. The major diagnostic examination is right-heart catheterization,

afterwards an optical coherence tomography follows. The major treatment options for pre-capillary pulmonary arterial hypertension are: General measures, pregnancy avoidance, Influenza and pneumococcal vaccination, Psychosocial support, supervised exercise training and avoid excessive physical activity that leads to distressing symptoms, Inflight oxygen (O_2) therapy for partial pressure of oxygen (PO_2) less than 60 mmHg or class III–IV, avoidance of general anesthesia and use epidural instead, if feasible. Supportive therapy with: (a) Diuretics for RV failure and fluid overload. Long-term O_2 if PO_2 is consistently less than 60 mmHg, (b) Correct anemia and/or iron deficiency, (c) Calcium channel blockers (CCBs). In case of inadequate clinical response to initial combination or monotherapy, sequential double or triple combination therapy is recommended (riociguat and phosphodiesterase type 5 inhibitor (PDE-5i) are contraindicated). In case of inadequate clinical response with sequential double combination therapy, triple combination should be attempted. Refer to lung transplant if there is inadequate response to combination therapy. The following dual combination therapies are recommended on the basis of evidence: (1) Macitentan and sildenafil. (2) Riociguat and bosentan. (3) Selexipag and endothelin receptor antagonist or PDE5i, or both. Other agents: (a) Endothelin receptor antagonists (ERA), (b) Ambrisentan, (c) Bosentan, (d) Macitentan, (e) PDE-5i and guanylate cyclase stimulators, (f) Sildenafil, (g) Tadalafil, (h) Vardenafil, (i) Riociguat, (j) Prostacyclin analogs and (k) prostacyclin receptor agonists, (l) Epoprostenol, (m) Iloprost [12], (o) Treprostinil. Novel agents are currently being investigated such as; Rho-Kinase inhibitors, riociquat and imatinib. Rho-Kinase inhibitors, sildenafil, tadalafil, riociquat and macitentan. Other drugs such as; angiotensin-receptor blockers, diuretics, nitrates, β-blockers and angiotensin-converting enzyme inhibitors. In the case of chronic thromboembolic pulmonary hypertension angioplasty is depended on a case by case evaluation [13]. Almost 12 years ago inhaled treprostinil was been approved by the FDA (2009) for patients with pulmonary arterial hypertension with NYHA III. Prostacyclin is a drug that induces vasodilation in large and small pulmonary vessels. Moreover, it has observed that inhibits the smooth muscle cell growth. Prostcyclin-2 is working through cycloxygenase-2 pathway activation which leads to vasodilation and platelet aggregation [14]. Inhaled prostanoids are administered in patients who can be treated with parenteral treatment. In the TRIUMPH trial treprostinil provided data where the 6-minute walking test was improved when compared to stable doses of bonsentan or sildenafil alone [15]. It has been previously investigated that different types and models of nebulisers in combination with different residual cup design and fillings affect the size of the aerosol production size [4,16,17]. The aim of this experiment was to investigate the possible administration of these drugs as aerosols and which would be the best equipment combination and residual cup filling.

2. Materials and methods

2.1 Drugs

The following drugs were purchased: (1) Tadalafil® 20 mg/tab, AZ Pharma, ULM, Germany. Typical dosage 5 mg/day and (2) Opsumit® 10 mg/tab (macitentan) Actelion pharmaceuticals Ltd., Neuss, Germany. Usual dosage 10 mg PO qDay.

2.2 Nebulizers and residual cups

2.2.1 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) (Fig. 1). Seven residual cups were included. Four had a capacity of $\leq$6 mL and two with a capacity of $\leq$10 mL. The large residual cups were A, D and E (Fig. 2). The small residual cups were C, F, B and J (Fig. 3). We used 1 gram of powder mixed with 10 mLs of water for injection then we filled the residual cups from 2–8 mL depending the residual cup size from this mixture.

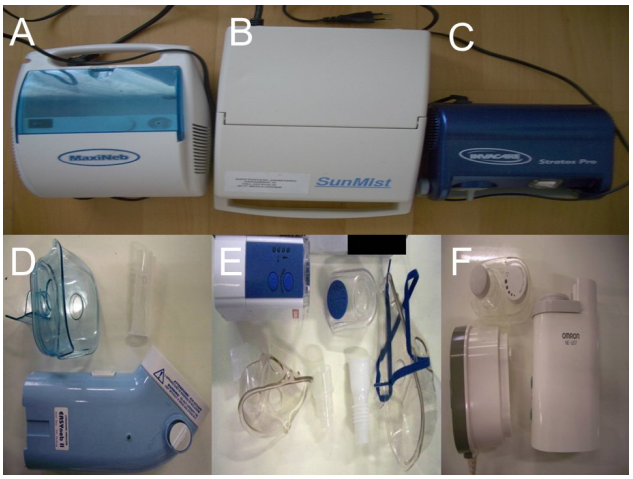

Fig. 1. Upper row with Jet-nebulizers, (A) Maxineb, (B) Sunmist and (C) Invacare. Lower row with ultrasound nebulisers, (D) Easyneb, (E) GIMA and (F) Omron.

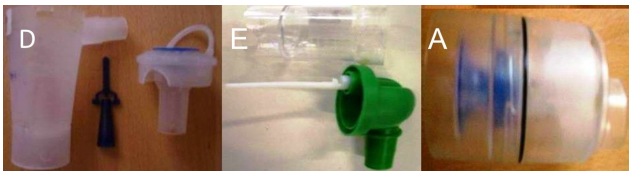

Fig. 2. Large residual cups that can contain up to 8 mls of liquid.

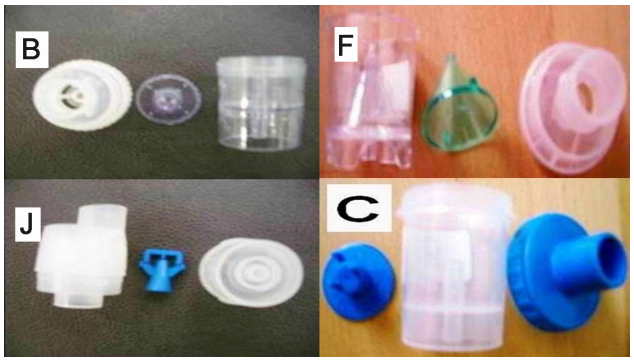

Fig. 3. Small residual cups that can contain up to 6 mls of liquid.

2.2.2 Ultrasound nebulizers

The following ultrasound nebulizers were available in our department. The first was Omron® NE-U07, Tokyo, Japan with a 10 mL medication cup. 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 mL. The third was a portable EASYneb® II, FLAEMNUOVA, Martino, Italy. with the following operating specifications; drug max capacity: 8 mL, Particle size: 2.13 μm mass median aerodynamic diameter (MMAD) We used 1 gram of powder mixed with 10 mls of water for injection then we filled the residual cups from 1–8 mL depending the residual cup size from this mixture (Fig. 1).

2.3 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 od D 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. (ii) Light scattering is non-invasive to all particles [18]. The above, coupled with the application of the very accurate 'Mie theory' used here for transferring the angle-intensity measurements into size-volume data. Our equipment has been previous used in prior publications [4,19–22].

2.4 Milling

The Tadalafil and Macitentan tablets were milled 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 800 rpm which results in an acceleration of about 7.5 g. We initiated our milling at 20 minutes and we acquired a mass median diameter (MMD) of 3.2 μm for tadalafil and 3.7 μm for macitentan.

3. Statistical analysis

3.1 Methods

The main object of the study is to find potential effects of 4 factors on MMAD response keeping in mind that the less mass diameter obtained the better function of the combined treatment.

Concerning the jet-nebulizers, a four-way analysis (fixed effects) on mass median aerodynamic diameter (MMAD) response was employed using three types of nebulizers (INVACARE, MAXINEB, SUNMIST), two types of drugs (Macitentan, Tadalafil), four loading dose (mL) levels (2, 4, 6 ,8) and seven designs of residual cups (alphabet letters).

Regarding the ultrasound nebulizers, a four-way analysis of variance (fixed effects) on MMAD response was also employed using three types of nebulizers (EASY NEB, GIMA. OMRON), same types of drugs, two loading levels (2, 4 mL) and two breathing mouthpiece apparatus (face mask, cylinder).

The statistical procedure first involved a compliance with normal distribution of MMAD variable and secondly an ANOVA performance only on the main effects and hereupon on the interaction terms among the statistically significant factors.

3.2 Results

For jet-nebulizers, the MMAD variable was transformed to a $\log_{10}$ variable since both the size frequency distribution and the boxplot information suggested so (Fig. 4).

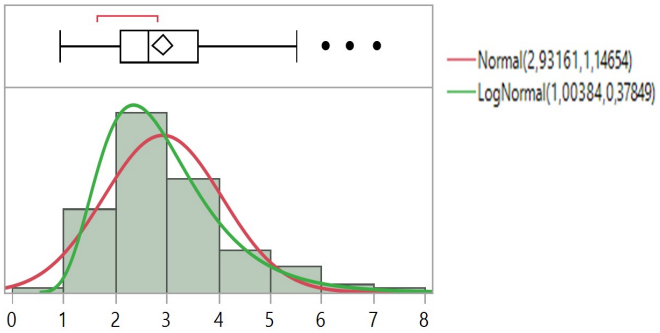

Fig. 4. Size frequency distribution of MMAD and boxplot deployment. Dots indicate outliers and the need for a log transformation of MMAD.

Two factors were found to influence the MMAD when jet-nebulizers were used: drug and residual cup design plus their interaction effect (Fig. 5). The analysis of drug mean values indicated that Tadalafil produced lower MMAD di-

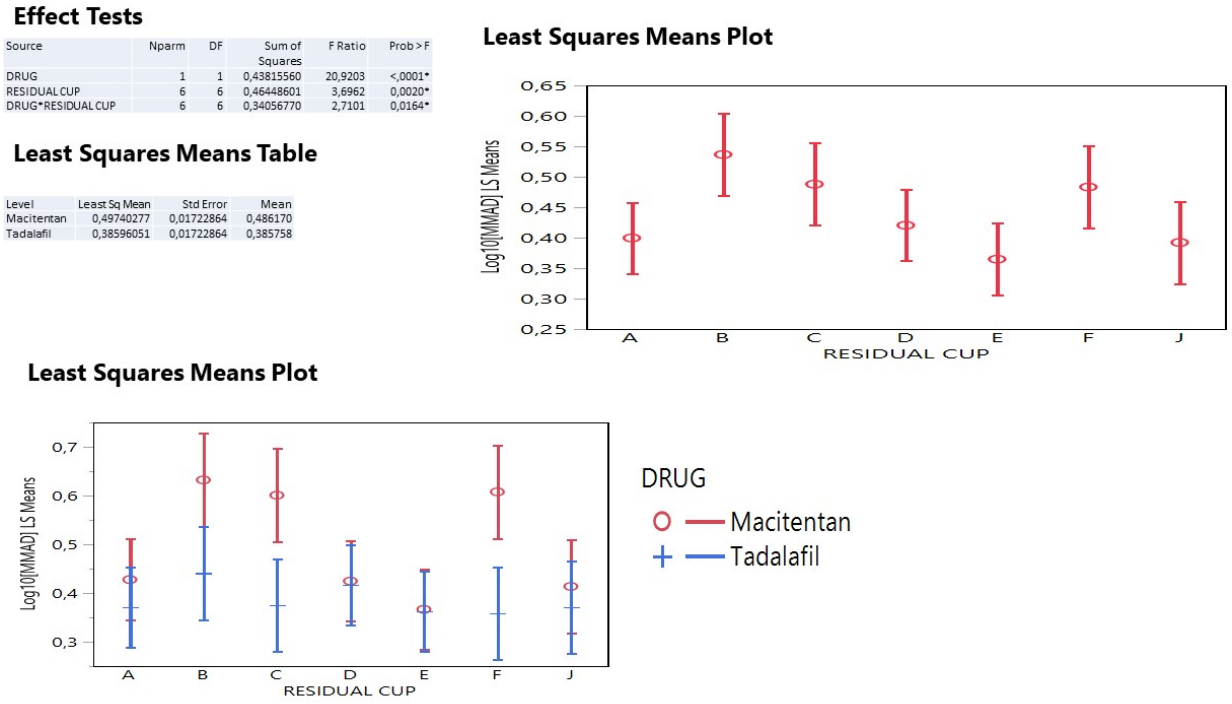

Fig. 5. The analysis of drug mean values indicated that Tadalafil produced lower MMAD diameters (2.43 μm) than Macitentan (3.14). The residual cup B contributed to greater MMAD diameters as the 95% interval of mean values based on the ANOVA mean square clearly indicated, followed by cups C and F.

Table 1. AVOVA output showing the F and *p* values of the four main effects under study.

Effect Tests					
Source	Nparm	DF	Sum of squares	F ratio	Prob > F
Drug	1	1	0,01397550	1,1912	0,2895
Nebulizer	2	2	0,04570143	1,9477	0,1715
Loading	1	1	0,01473125	1,2556	0,2772
Mouthpiece	1	1	0,02564812	2,1862	0,1565

ameters (2.43 μm) than Macitentan (3.14 μm). The residual cup B contributed to greater MMAD diameters as the 95% interval of mean values based on the ANOVA mean square clearly indicated, followed by cups C and F. The previous interval plot is much better clarified when the interaction means between drug and residual cap are plotted. The residual cups B, C and F, when Macitentan is administered, produce significantly higher MMAD diameters (3.5–5.0 μm) than those of the same cups when Tadalafil is administered (2.0–3.2 μm).

For ultra sound nebulizers the MMAD variable was also transformed to $\log_{10}$ base variable because values were slightly better normalized.

None main effect was observed to influence the MMAD response as Table 1 clearly indicates.

Overall, none of the two groups of nebulizers seems to affect the MMAD response, indeed however, the drug and some residual cup designs produce alone or jointly different MMAD diameters.

4. Conclusions

Underlying disease is a serious issue that has to be evaluated in every patient. There has been an extensive research initially for inhaled insulin for patients with asthma and chronic obstructive pulmonary disease (COPD) [3]. The mucus, airway clearance mechanisms and local genes/transporters play a crucial role in the local absorption of an inhaled drug [23]. The viscosity of the mucus does not let the drug to be released to the airway surface [1]. Patients with COPD and cystic fibrosis have thick mucus and the clearance mechanisms do not work properly [24,25]. There are different transporters in different sites of the airways. In the airways there is also 90% humidity, therefore molecules with high salt concentration will expand by almost 50% as they reach the alveoli from the upper respiratory system [1]. Exacerbations of COPD and asthma or another underlying disease is a contraindication for inhalational drugs [26]. Moreover, a careful respira-

tory evaluation has to be performed for every patient not only with spirometry, but also with diffusing capacity of the lung (D_{LCO}) which evaluates the capacity of the smaller airways. Six-minute walking test is another test that is also necessary for pulmonary hypertension where the respiratory evaluation is combined with the cardiologic evaluation [27]. Inhaled drugs for pulmonary hypertension are available however; certainly we need more, since different drugs are effective for each patient and disease stage. The necessary technology is available to transform the drugs and also several aerosol and production systems are already on the market [4,16,28–31]. The major advantage of inhaled delivery of the drug directly to the lungs are less systemic side effects and large drug concentration in a small period of time [32–34]. There is indication for patients with pulmonary hypertension that need a treatment combination and therefore rapid absorption is feasible with aerosol administration. The aerosol characteristics does not depend only on the nebulizer design but from the combination of nebulizer, nebulizer cup design, drug characteristics and quantity of the drug within the residual cup. All these factors interact between them. In our study we observed that Tadalafil produced the smallest aerosol droplets with residual cup B 2.43 μm with a filling of 6 mL followed by residual cup C and F. The smallest aerosol droplets were produced with the jet-nebulisers. Regarding Macitentan the smallest droplet size was 3.14 μm again with residual cup B with a 6ml filling followed by C and F. Again the smallest droplet sizes were produced with jet-nebulisers. The residual cup design and filling probably affects the impact of the droplets within the walls of the residual cup along with the air pressure inserted from the jet nebulizer through the connection cable. The multiple impacts assist in the production of smallest droplets. In a previous study the inlet in front of the nebulizer cup further reduced the mass median aerodynamic diameter of the droplets and in a recent study novel nozzle designs were able to further reduce the aerosol droplets [4,35]. In another study other factors such as the electrostatic precipitation was investigated as a factor affecting the aerosol distribution within the peritoneal environment and a new methodology was proposed to overcome this issue [36]. The underlying pulmonary disease plays a crucial role in the absorption of aerosols, as with other drugs, we should avoid administering aerosol drugs if we have pulmonary infection or COPD and asthma exacerbation. Based on our results the drugs tadalafil and macitentan can be administered as aerosol with current nebulisers, both jet- and ultrasound. We more experiments to evaluate several aspects of the produced aerosol such as the drug concentration and the absorption. If possible if the future we could have a sustain release aerosol formulation. The aerosol administration is easier to use than subcutaneous pumps and maybe a portable system such as a metered dose pressurized inhaler could be manufactured (mdpi) in the future. Aerosol administration has certainly the advantage of fast absorption as previously observed with other drugs. In any case every patient should have a variety of choices for his medication. Tablets have the disadvantage of non-linear sustain release, and in some patients with gastric issues the absorption is not complete. The aerosol administration can be performed in any patients even in those with a Levin or gastrostomy and they the aerosol administration is fast acting. In our manuscript we investigated whether the drugs macitetan and tadalafil were possible to be converted firstly to powder and secondly aerosol mist. We used a simple technique which has been previously used in other publications [28]. Major limitations were that we were not able to measure the concentration of the drugs within the aerosol mist and several other aspects of the aerosol such as the z potential which are important for a future drug design. We concluded that the residual cup design and residual cup filling play a crucial role in the formation of mmad $\leq$5 μm which is the threshold for optimal distribution and absorption within the airways. The larger residual cups produce the smallest aerosol droplets. The three different residual cups with the jet-nebulisers produce the smallest aerosol droplets. Both ultrasound nebulisers are capable of producing optimal size aerosol droplets $\leq$5 μm mmad. Additionally, targeting multiple pathways in this disease is necessary [37–40]. We need long term studies to evaluate the safety of these drugs to the lung parenchyma, in in vitro study would elucidate this aspect. Our next steps will be to make the appropriate measurements with HPLC and then agree on the appropriate dosage that has to be delivered according to each disease.

Author contributions

PZ, DP, CS, CK, KT, DM, HH, CB, WHS, GP, IK, GG, SP and CK wrote the manuscript and performed the experiments. DP and PZ performed the statistics. IK, GP and GG provided useful insights.

Ethics approval and consent to participate

Not applicable.

Acknowledgment

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Conflict of interest

The authors declare no conflict of interest.

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