ORIGINAL RESEARCH

Inhaled tyrosine kinase inhibitors for pulmonary hypertension: a possible future treatment

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Abstract: Pulmonary hypertension is a disease with severe consequences for the human body. There are several diseases and situations that induce pulmonary hypertension and are usually underdiagnosed. Treatments include conventional medical therapies and oral, inhaled, intravenous, and subcutaneous options. Depending on its severity, heart or lung transplant may also be an option. A possible novel treatment could be tyrosine kinase inhibitors. We conducted experiments with three jet nebulizers and three ultrasound nebulizers with erlotinib, gefitinib, and imatinib. Different residual cup designs and residual cup loadings were used in order to identify the best combination to produce droplets of less than 5 μm in mass median aerodynamic diameter. We found that gefitinib could not be transformed into a powder, so conversion to an aerosol form was not possible. Our experiments indicated that imatinib is superior to erlotinib with regard to small droplet size formation using both inhaled technologies (1.37 μm <2.23 μm and 1.92 μm <3.11 μm, jet and ultrasound, respectively) and, at jet devices (1.37 μm <1.92 μm). Cup designs C and G contribute best to small droplet creation uniquely supporting and equally well the activity of both drugs. The disadvantage of the large droplets formed for erlotinib was offset when combined with residual cup C (1.37 μm instead of 2.23 μm). At a 2 mL dose, the facemask and cone mouthpieces performed best and evenly; the facemask and low dose were the best choice (2.08 μm and 2.12 μm, respectively). Erlotinib and imatinib can be administered as an aerosols, and further in vivo experimentation is necessary to investigate the positive effects of these drugs in the treatment of pulmonary hypertension.

Keywords: erlotinib, gefitinib, imatinib, jet nebulizers, ultrasound nebulizers

Introduction

Pulmonary hypertension (PH) refers to increased blood pressure in the large vessels of the pulmonary artery, pulmonary capillaries, and/or pulmonary vein. It affects the entire lung vasculature, leading to orthopnea, fainting, dizziness, fatigue, non-productive cough, peripheral edema, angina pectoris, and leg swelling. PH is considered to be a serious disease, and in the final stages is often associated with decreased exercise tolerance and heart failure. Symptoms usually develop gradually, and patients may delay seeking medical attention for years, only seeking assistance from a physician when symptoms appear, such as coughing up blood and/or syncope. It is known that venous hypertension typically presents with shortness of breath while lying flat, whereas pulmonary arterial hypertension (PAH) usually does not. PH is known to have five variants, so several tests have to be performed to distinguish PAH from venous, thromboembolic, miscellaneous, or hypoxic PH. Moreover, physicians must bear in mind pertinent statutes that induce PH and exclude other possible diagnoses. Common investigations include pulmonary function tests, screening for autoimmune diseases, blood tests to exclude human immunodeficiency virus, electrocardiography, liver function tests, arterial blood gas measurements, ventilation-perfusion studies,

a ventilation-perfusion scan to exclude chronic thromboembolic PH, and chest radiography. Lung biopsy is not indicated unless underlying interstitial lung disease is suspected.

Clinical improvement in such patients is measured by the 6-minute walk test. It has been observed that improved performance on this test correlates with increased survival benefit. The blood brain natriuretic peptide level is nowadays considered a marker for disease stability or progression in patients with PH. PAH pressure can usually be estimated in everyday clinical practice with echocardiography; however, the gold standard is pressure measurement with a Swan-Ganz catheter through the right side of the heart. Pulmonary artery occlusion pressure and pulmonary vascular resistance cannot be measured directly with echocardiography. Right-sided cardiac catheterization is required for diagnosis of PAH. In order to measure cardiac output accurately, we use a Swan-Ganz catheter; this method being by far superior for measuring PAH.[1] Normal pulmonary arterial pressure has been observed to be 8–20 mmHg (1,066–2,666 Pa) at rest. PH is observed when mean pulmonary artery pressure exceeds 25 mmHg at rest.

The treatment depends on whether the PH is arterial, hypoxic, venous, thromboembolic, or miscellaneous. In the case of congestive heart failure, diuretics or angiotensin-converting enzyme inhibitors can be given to improve left ventricular function, repair/replace the mitral valve or aortic valve, and beta-blockers. Patients with known left heart failure or hypoxemic lung disease (group II or III PH) should not be treated with vasoactive agents such as phosphodiesterase inhibitors, endothelin antagonists, or prostanoids, because these agents are only approved for PAH.[2] To make the distinction, physicians perform a chest computed tomography scan, cardiac catheterization of the right heart, echocardiography, and a six-minute walk test. In PAH, diuretics, digoxin, oxygen therapy, and oral anticoagulants are usually considered to be appropriate therapy. It has been observed that high-dose calcium channel blockers are useful in only 5% of patients with idiopathic PAH who are vasoreactive on Swan-Ganz catheterization.[3] Only half of these patients respond to calcium channel blockers in the long-term.[4] Several new agents have been introduced for primary and secondary PAH. However, there are few trials supporting the use of these agents, and the only measure consistently used to compare their efficacy is the 6-minute walk test. Most have no data on mortality benefit or time to progression.[5]

Tyrosine kinase inhibitors (TKIs) are presently being evaluated as to whether they could be used as a treatment for PH.[6] Imatinib has been investigated in several studies to determine if it is effective against PH.[7–11] Moreover, other agents, such erlotinib and gefitinib, which have been used effectively in the treatment of lung cancer, could be also considered for PH.[6,12,13] However, orally administered TKIs have serious side effects, which has generated a quest for alternative routes of administration, such as inhalation.[14] In this work, we investigated whether erlotinib, gefitinib, and imatinib could be modified for aerosol administration using jet and ultrasound nebulizers. We also sought to determine the best combination of residual cup design, residual cup loading, and nebulizer, for producing droplets less than 5 µm in size.

Materials and methods
Drugs
Erlotinib (150 mg tablets; Tarceva®; Roche, Basel, Switzerland), gefitinib (250 mg tablets; Iressa®; AstraZeneca, London, UK), and imatinib (400 mg tablets; Glivec®; Novartis, Basel, Switzerland) were sourced from our oncology department.

Aerosol production systems
Jet nebulizers and residual cups
Three jet nebulizers, ie, Maxineb® (Flexicare Medical Ltd., Mountain Ash, UK; 6 L per minute and 35 psi); Sunmist® (Nursing Hygiene, Southwater, UK; 5–7 L per minute and 35 psi); and Invacare® (Invacare Corporation, Elyria, OH, USA; 4–8 L per minute and 36 psi) were chosen for the experiment (Figure 1). We opted to use seven residual cups, of which four had a capacity of no more than 6 mL and two had a capacity of no more than 10 mL. The large cups were designated using the letters A, D, and E, and the small residual cups were designated C, F, B, and G (Figures 2 and 3). Large residual cups with a capacity of 2–8 mL were used. The residual cup loadings were 2, 4, 6, and 8 mL (8 mL only for the large cups).

Ultrasound nebulizers
Three ultrasound nebulizers were selected. The first was the NE-U07 (Omron, Tokyo, Japan), which is compact and weighs less than 350 g, includes a 10 mL medication cup, and generates uniform micrometer-sized vapor particles. The second was a portable Gima device (Gima SpA, Gessate, Italy). The third was a portable EASYneb® II (Flaem Nuova, Martino, Italy). The loadings were 2 mL and 4 mL, since this is the amount held by the residual cup for each of the three ultrasound nebulizers.

Figure 1 Ultrasound nebulizers (upper row) and jet nebulizers (lower row).

Figure 2 Large residual cups.

Figure 3 Small residual cups.

Droplet measurement

The size distribution of the droplets and their mean diameter (d_{32}) were calculated using a Mastersizer 2000 (Malvern Instruments Ltd., Malvern, UK) equipped with a Scirocco module (Malvern). A refractive index of 1.33 was used for the sprayed droplets. Several experiments were performed repeatedly until optimal measurements were obtained, as in our previously reported experiments[15–19] (Figure 4).

Milling

The erlotinib and imatinib tablets were milled in a planetary ball mill (Pulverisette-5; Frisch GmbH, München, Germany) equipped with agate bowls (500 mL) and eight balls (20 mm, 20 g) with a rotational speed of approximately 200 rpm, resulting in an acceleration of about 7.5 g. We initiated our milling at 60 minutes for erlotinib and at 80 minutes for imatinib to obtain a mass median aerodynamic diameter (MMAD) ≤5 μm (measured with the Mastersizer 2000). After milling, we collected powdered drug of the same weight and diluted it with 2 mL of 0.9% NaCl in an effort to simulate a future method/compound of administration as an aerosol. We attempted to mill gefitinib for 320 minutes; however, it was impossible to convert the tablet to a powder (Figure 5).

Statistical analysis

Jet technology

Four factors were chosen as having a potential effect on droplet size: two drugs (erlotinib, imatinib), three nebulizers (Invacare, Sunmist, Maxineb), seven residual cups (A–G), and three loading levels (2, 4, and 6 mL). Thus, a four-factor analysis of variance in combination with their interactions was conducted at the 0.05 probability reference level. Pairwise statistically significant differences between means were examined using the 95% confidence intervals of means. Two non-overlapping intervals indicate significant differences between the two means. A similar analysis of variance test was used for cups A, D, and E that could hold an 8 mL dose using the same drugs and nebulizers.

Ultrasound technology

The same drugs as above and three new nebulizers (EASYneb, Gima, Omron) manipulated at two dose levels (2 and 4 mL) were tested for their potential impact on particle size.

Results

Jet technology

The drugs, cup designs, and their interaction effect were the most influential factors affecting MMAD (Table 1, $P<0.001$). Imatinib dramatically decreased the mean droplet size down to 1.37 μm as compared with the effect of erlotinib (2.23 μm). Residual cups C and G lowered the particle size to a similar extent (1.32 μm and 1.37 μm, respectively, Figure 6), whereas the other cups had similar effects but produced droplets of a larger mean size. The strong diminishing effect of cups C and G expands also interactively and uniquely on the two drugs causing both imatinib and erlotinib to perform

Figure 4 Mastersizer 2000.

evenly when these cups are applied (Figure 7), due to the wide overlap between their confidence intervals. The highest loading level (6 mL) appeared to be slightly less effective than the lower doses (Figure 8), but the effect was weakly statistically significant (P=0.048). A loose interactive effect between cup design and the drugs was also established (P=0.039), whereby erlotinib produced a larger mean droplet size (2.57 μm) when combined with the Maxineb device (Figure 9). Another weak effect of a second order interaction (Table 1, P=0.038) was considered negligible and not examined. The experiment focused on the highest loading level (8 mL) had no significant effect on the factors considered and was not investigated further.

Ultrasound technology

Drug activity, loading level, and mouthpiece operation all exerted a rather weak effect on particle size (Table 2, P-values 0.020, 0.036, and 0.043, respectively). Imatinib

Figure 5 Porcelain milling ball.

again performed better than erlotinib, forming smaller droplets (1.92 μm <3.11 μm, Table 3). The facemask adaptation decreased the droplet size more than the cone (2.12 μm <2.91 μm), and so did the 2 mL dose when compared to the 4 mL dose (2.08 μm <2.95 μm). However, the performance of the cone device at the low dose was similar to that of the facemask with regard to droplet size (2.10 μm and 2.05 μm, respectively) as indicated by their interaction effect in Figure 10 (see also Table 2, P=0.038).

Imatinib outclassed the performance of erlotinib in terms of small droplet size formation using both inhaled technologies (1.37 μm <2.3 μm and 1.92 μm <3.11 μm for jet and ultrasound, respectively) and when is considered alone, even better at jet devices (1.37 μm <1.92 μm). Cup designs C and G contributed best to the creation of small droplets, supporting uniquely and equally well the activity of both drugs. The disadvantage of the large droplets formed by erlotinib was canceled out when the drug was combined with the residual cup C (1.37 μm instead of 2.23 μm, Figure 7). At the 2 mL dose, facemask and cone mouthpieces performed best and evenly, otherwise facemask and low dose give the best choice (2.08 μm and 2.12 μm, respectively).

Discussion

TKIs have been used for a number of years as targeted therapies for non-small cell lung cancer.[20] Until now, neither erlotinib nor gefitinib has presented superiority between them. Both agents have been found to be effective as first-line treatment for epidermal growth factor-positive mutation tumors. Imatinib, on the other hand, is a TKI used in the treatment of multiple cancers, most notably Philadelphia chromosome-positive (Ph+) chronic myelogenous leukemia.[21,22] Current data indicate that TKIs are potent acute pulmonary vasodilators, which prompted this work.[23] The respiratory system has defense mechanisms, including mucus, beating cilia, and macrophages that block efficient aerosol deposition.[24] Moreover, aerosols targeted to the lung have to be designed so that the maximum MMAD is no larger than 5 μm. The reason is that due to the increased humidity of the respiratory system >90%, the aerosol droplets increase at least by 50%.[24] Aerosol drug therapy targeted to the lung has been observed in many diseases to have the advantage of being more effective at lower doses and therefore to have fewer adverse effects. Two examples are inhaled insulin and inhaled chemotherapy, which have produced significant results when compared with intravenous or subcutaneous administration.[25–30] In recent years, inhaled antibiotics have been investigated extensively, and there

Table 1 Analysis of variance for effects of four fixed factors and their interactions on mass median aerodynamic diameter

Effect	SS	df	MS	F	P-value
Drug	**23.50**	**1**	**23.50**	**69.19**	**0.000**
Nebulizer	1.19	2	0.60	1.75	0.194
Residual cup	**13.01**	**6**	**2.17**	**6.39**	**0.000**
Loading	**2.35**	**2**	**1.17**	**3.46**	**0.048**
Drug*nebulizer	**2.52**	**2**	**1.26**	**3.71**	**0.039**
Drug*residual cup	**14.12**	**6**	**2.35**	**6.93**	**0.000**
Nebulizer*residual cup	7.00	12	0.58	1.72	0.126
Drug*loading	0.28	2	0.14	0.41	0.668
Nebulizer*loading	1.58	4	0.39	1.16	0.352
Residual cup*loading	3.61	12	0.30	0.89	0.571
Drug*nebulizer*residual cup	**9.45**	**12**	**0.79**	**2.32**	**0.038**
Drug*nebulizer*loading	1.79	4	0.45	1.32	0.293
Drug*residual cup*loading	3.36	12	0.28	0.83	0.625
Nebulizer*residual cup*loading	6.69	24	0.28	0.82	0.684
Error	8.15	24	0.34		

Note: Statistically significant effects are shown in bold. *denotes interaction between effects.
**Abbreviations:** df, degrees of freedom; MS, mean square; SS, sum of squares.

are already several products on the market.[19,31] However, there are still some safety concerns with these drugs regarding their effect on the lung parenchyma and these need to be investigated further.[32,33] In the case of inhaled insulin, it was observed that local absorption of the drug through the alveoli was different in the presence of a respiratory tract infection, so more frequent glucose level measurements were necessary.[25] Several factors influence aerosol droplet production, the most important being jet nebulizer flow rate,[34]

tapping of the residual cup during nebulization,[35,36] design of the residual cup,[37] the chemical entity used,[38,39] residual cup filling on initiation of nebulization,[35] residual cup loading,[40] viscosity,[34] charge on the drug molecule,[41] surface tension, and concentration of the drug solution. It has been observed that the salts present in the chemical structure of a drug are responsible for absorption of water from the environment and expansion of the molecule. Dry powder absorbs water from the environment, and according to the porosity of the
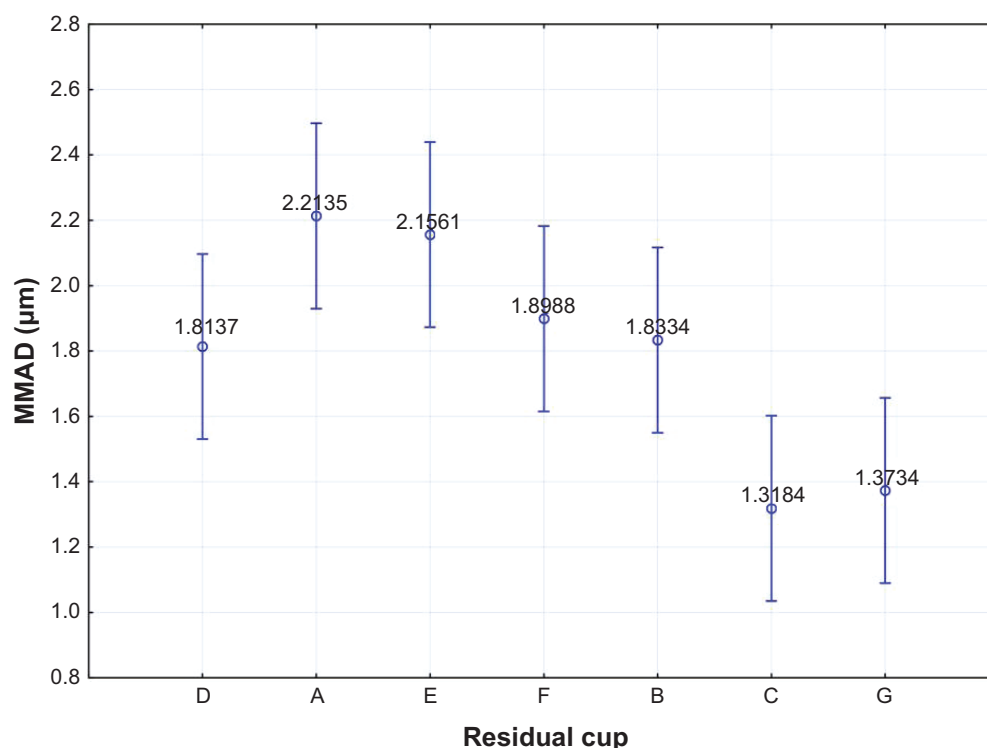
Figure 6 Mean mass median aerodynamic diameter (MMAD) values using various cup designs. Vertical lines denote the 95% confidence intervals extracted from the mean square error (analysis of variance).
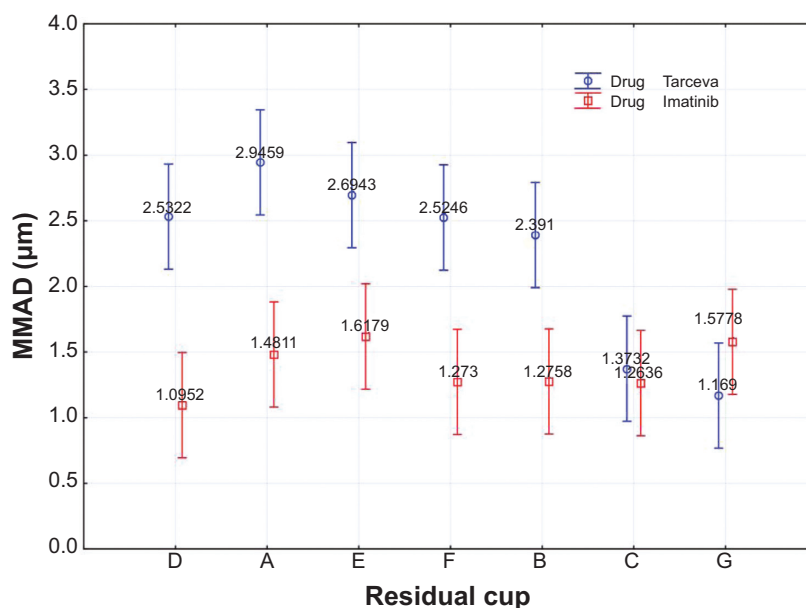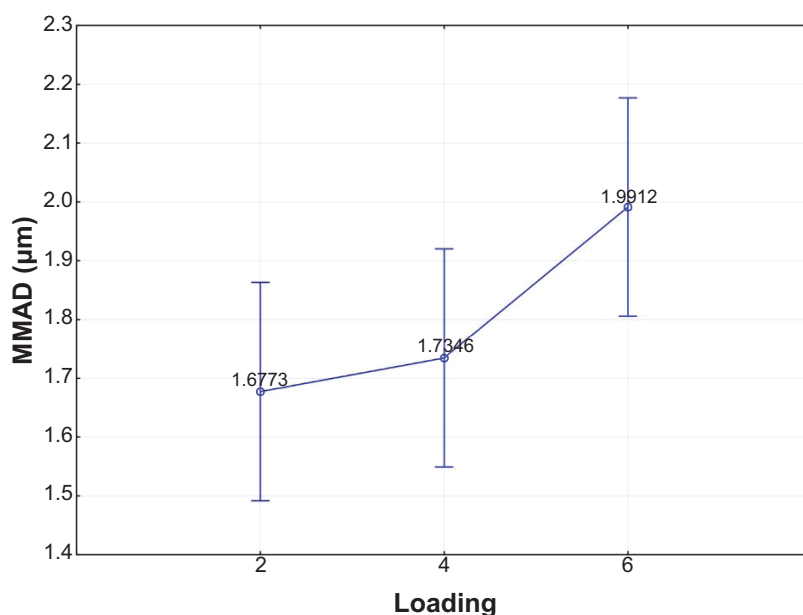
Figure 7 Mean mass median aerodynamic diameter (MMAD) values according to residual cups and drug inhalation. Vertical lines denote the 95% confidence intervals extracted from the mean square error (analysis of variance).

particles, expansion may be 50%–75% of the initial particle size. Further hydration may cause either expansion or contraction of the molecule (not applicable in the case of powder for concentration). The shape of the dry powder particle plays an important role in induction of cough. If one axis of a dry powder particle is larger, the mucosa of the respiratory system becomes irritated and cough is induced.

The major limitation of our study was that we did not evaluate the timing of aerosol production accurately in each experiment. This parameter is very important in designing

any future aerosol product since the timing of administration is very important for the patient.

TKIs have been observed to block or reverse pulmonary fibrosis.[42] Activation of the epidermal growth factor receptor (EGFR) pathway has been reported to activate proliferation of the microvasculature, so inhibition of EGFR has been proposed as an alternative treatment approach for PH.[43] A novel inhaled EGFR inhibitor has also been investigated for chronic obstructive pulmonary disease and could be used for PH.[44] Imatinib has been identified as a possible treatment

Figure 8 Mean mass median aerodynamic diameter (MMAD) changes with increasing loading levels. Vertical lines denote the 95% confidence intervals extracted from the mean square error (analysis of variance).
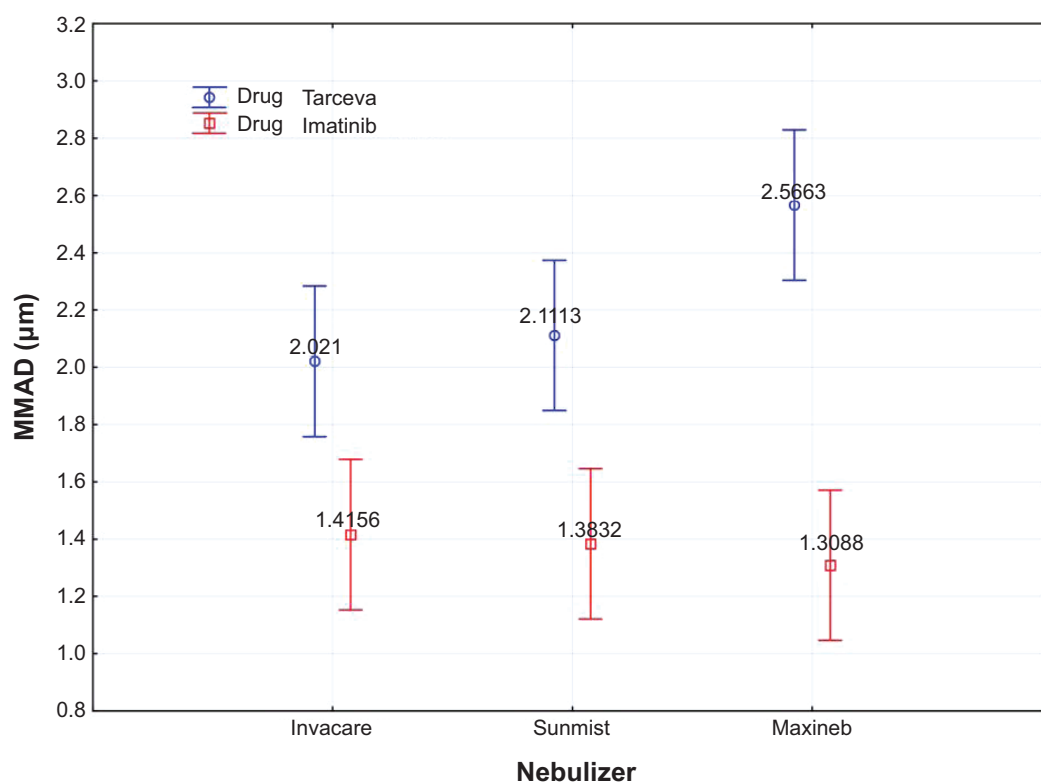
Figure 9 Mean mass median aerodynamic diameter (MMAD) values according to the combined performance of drugs and nebulizers. Vertical lines denote the 95% confidence intervals extracted from the mean square error (analysis of variance).

for hypereosinophilic syndrome presenting with cough.[45] Another multikinase inhibitor that could be investigated as an aerosol is sorafenib, which has been found to be effective for vascular remodeling in arterial hypertension.[46] In a study by Moreno-Vinasco et al[47] it was noted again that sorafenib can be used to treat severe PH since it uses the mitogen-activated

protein kinase pathway. Imatinib has been used successfully to treat PH in a patient with chronic eosinophilic leukemia[48] and also for patients with arterial hypertension.[10,49,50] In the studies by Tang et al[51] and Le Cras et al[52] it was observed that vascular endothelial growth factor inhibitors can be used successfully to treat PH. Platelet-derived growth factor (PDGF)

Table 2 Analysis of variance of effect of four fixed factors and their interactions on mass median aerodynamic diameter

| Effect	SS	df	MS	F	P-value
Drug	**8.58**	**1**	**8.58**	**49.01**	**0.020**
Nebulizer	3.12	2	1.56	8.93	0.101
Loading	**4.60**	**1**	**4.60**	**26.29**	**0.036**
Mouthpiece	**3.79**	**1**	**3.79**	**21.65**	**0.043**
Drug*nebulizer	3.20	2	1.60	9.13	0.099
Drug*loading	1.17	1	1.17	6.71	0.122
Nebulizer*loading	1.79	2	0.90	5.12	0.163
Drug*mouthpiece	0.50	1	0.50	2.85	0.233
Nebulizer*mouthpiece	0.85	2	0.43	2.44	0.291
Loading*mouthpiece	**4.27**	**1**	**4.27**	**24.43**	**0.039**
Drug*nebulizer*loading	0.25	2	0.13	0.73	0.579
Drug*nebulizer*mouthpiece	3.22	2	1.61	9.21	0.098
Drug*loading*mouthpiece	0.30	1	0.30	1.70	0.322
Nebulizer*loading*mouthpiece	0.49	2	0.25	1.41	0.416
Error	0.35	2	0.18		

Note: Statistically significant effects are shown in bold. *denotes interaction between effects.
Abbreviations: df, degrees of freedom; MS, mean square; SS, sum of squares.

Table 3 Mean values of mass median aerodynamic diameter and 95% confidence intervals for drugs, mouthpiece devices, loading levels, and nebulizers calculated from the mean square error (analysis of variance)

	Mean	−95%	+95%	n
Drug				
Erlotinib	3.11	2.59	3.63	12
Imatinib	1.92	1.40	2.44	12
Mouthpiece				
Facemask	2.12	1.60	2.64	12
Cone	2.91	2.39	3.43	12
Loading				
2	2.08	1.56	2.60	12
4	2.95	2.43	3.47	12
Nebulizer				
EASYneb	2.88	2.25	3.52	8
Gima	2.64	2.00	3.27	8
Omron	2.02	1.39	2.66	8
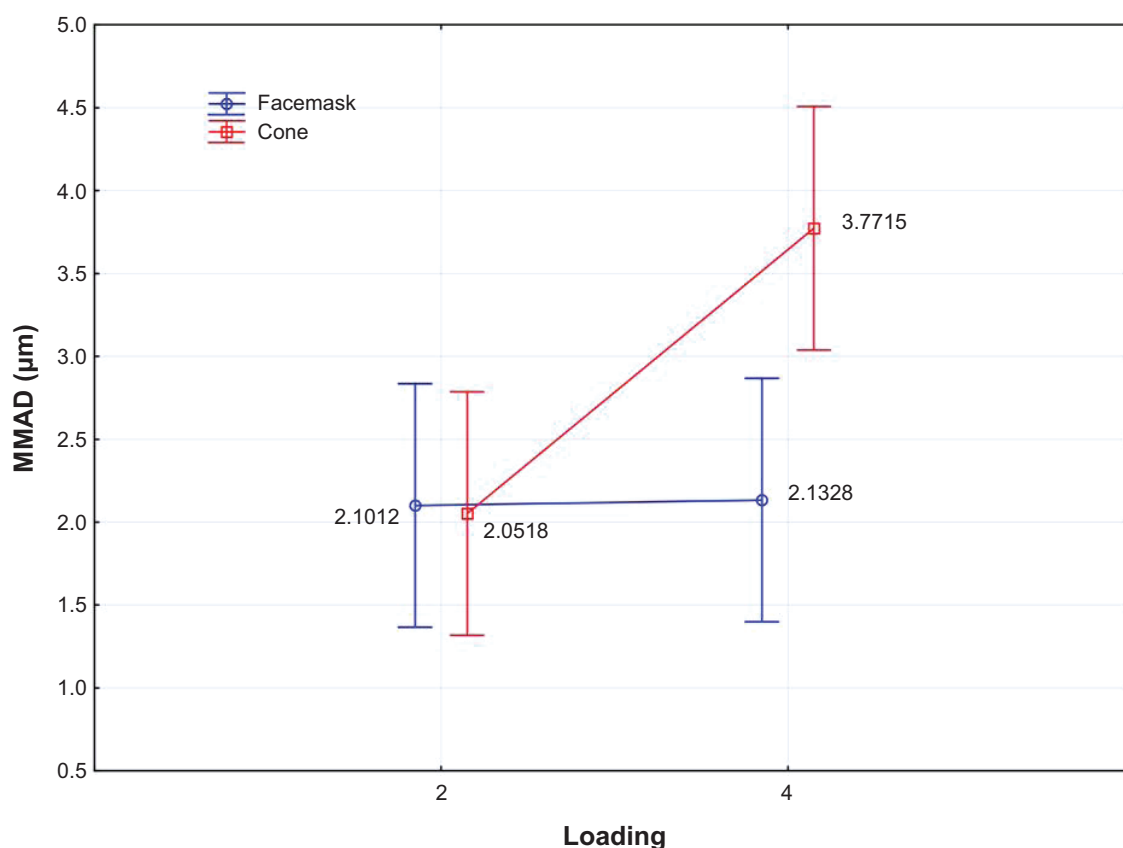
Figure 10 Mean mass median aerodynamic diameter (MMAD) values according to loading levels and mouthpiece devices. Vertical lines denote the 95% confidence intervals extracted from the mean square error (analysis of variance).

inhibitors have been also proposed as a treatment for PH.[53] In a study by Nakamura et al[54] it was observed that imatinib inhibits the PDGF pathway. Kosanovic and Schermuly[55] further proposed that inhibiting the PDGF pathway is more effective in the treatment of PH because it also blocks fibrinogenesis. The rho-kinase inhibitors have also been suggested as a treatment for PH.[56,57] Dasatinib was reported to induce PH when used in a patient with chronic myeloid leukemia.[58] In a study by Hennigs et al[59] dasatinib was again identified as a novel cause of severe precapillary PH. However, safety concerns have been raised when using TKIs to treat PH, with a special focus on cardiac repolarization.[60,61] Currently, another Syk kinase inhibitor is under development for inhalation by Pfizer and is being investigated in a Phase I study.[62] Rapamycin was found to reverse proliferation of pulmonary artery smooth muscle cells, indicating that inhaled rapamycin should be investigated for this disease.[63] Finally, Src TKIs could be another novel treatment for PH.[64]

Our current research indicates that TKIs already on the market can be modified to be produced as aerosols that could be used as an aerosol treatments for PH. Specifically, imatinib,

which we already know causes severe dose-dependent side effects when administered systemically, could be administered as an aerosol. A future clinical trial is needed to determine the effectiveness of aerosolized TKIs for PH. In our current study, the major findings were that the performance of imatinib was superior to that of erlotinib with regard to small droplet size formation using both inhaled technologies (1.37 µm <2.23 µm and 1.92 µm <3.11 µm, jet and ultrasound, respectively) and when the drug is considered alone with jet devices it produces even smaller droplets. (1.37 µm <1.92 µm). Cup designs C and G contributed best to small droplet size creation supporting uniquely and equally well the activity of both drugs. The disadvantage of the large droplets formed by erlotinib was canceled out when combined with residual cup C (1.37 µm instead of 2.23 µm). At the 2 mL dose, the facemask and cone mouthpieces perform best and evenly (2.08 µm and 2.12 µm, respectively). Gefitinib was impossible to manipulate in its current tablet formulation.

Disclosure

The authors report no conflicts of interest in this work.

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