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RESEARCH PAPER

Inhalation Performance of Physically Mixed Dry Powders Evaluated with a Simple Simulator for Human Inspiratory Flow Patterns

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ABSTRACT

Purpose To construct a simple simulator reproducing human inspiratory flow patterns and use it to evaluate the inhalation performance of active ingredient particle-carrier particle systems (physically mixed dry powders).

Methods Inspiratory flow patterns were collected and analyzed using a flow recorder. The simulator was constructed using an airtight container, a valve, and a connecting tube. Several of the patterns reproduced by the simulator were compared with those recorded. In addition, the influence of inspiratory flow on the inhalation performance of physically mixed dry powders composed of salbutamol sulfate (SS) and coarse lactose monohydrate was investigated using a twin-stage liquid impinger (TSLI) equipped with the simulator.

Results Human inspiratory flow patterns could be characterized by three parameters: inspiratory flow volume (area under the flow rate-time curve (AUC)), flow increase rate (FIR), and peak flow rate (PFR). The patterns could be reproduced using the simulator. Testing with the simulator *in vitro* revealed that PFR, but not FIR or AUC, greatly affected the inhalation performance of physically mixed dry powders.

Conclusions The simulator is simple to construct and can schematically reproduce human inspiratory flow patterns. Testing with a TSLI and the simulator is useful to evaluate dry powder formulations for clinical application.

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INTRODUCTION

Inhalation therapy plays an important role in the treatment of not only local respiratory diseases, including asthma (1,2), but also systemic diseases (3,4). Therefore, many attempts have been made to enhance its therapeutic effects (5–8). In the local treatment of respiratory diseases, inhalation therapy is most effective at low drug doses, which can attenuate systemic side effects, since inhaled drugs are delivered directly to target sites (9,10). Among the three major delivery systems for inhalation—nebulizers, metered-dose inhalers (pMDIs), and dry powder inhalers (DPIs)—DPIs have several advantages, such as good portability, low cost, and no propellants. Furthermore, the handling of DPIs is easier than that of pMDIs because of breath-actuated passive aerosolization (11).

For effective pulmonary deposition after inhalation, in general, the optimal aerodynamic diameter of drug particles is less than 6 μm (12,13). However, micronized drug particles tend to be highly cohesive and poorly flowable, leading to low performance. To solve these problems, large carrier particles, such as coarse lactose monohydrate, have been mixed with micronized active pharmaceutical ingredient (API) particles to prevent coherence: such preparations are known as physically mixed dry powder formulations (14,15). The micronized API particles and large carrier particles are expected to form an ordered mixture in an inhalation device, which is easily emitted from the device after inhalation, followed by the release of API particles from the mixture. However, it is often difficult for micronized

API particles to detach from carrier particles by inspiratory flow, resulting in poor lung deposition.

To investigate the inhalation performance of DPIs, such as particle emission and pulmonary deposition several inertial impactors, including multistage cascade impactors, such as an Andersen cascade impactor (ACI) and a multi-stage liquid impinger (MSLI), and a twin-stage liquid impinger (TSLI), have been applied. Some of them are described in European and/or US Pharmacopeias (16). In general, these assessments, useful as quality control systems, are performed at a constant flow rate to determine the mass median aerodynamic diameters and/or fine particle fractions of DPIs by the size-based separation of emitted particles on each stage (17–19). However, there would be some discrepancy between conventional *in-vitro* inhaler testing and clinical response because of the variability in human inspiratory flow patterns. In fact, it has been reported that a patient's inspiration pattern greatly affects the performance of DPIs, not only the emission of drug particles from a device but also the pulmonary deposition of the inhaled drug particles (20,21). As far as physically mixed dry powders are concerned, the inspiratory flow pattern might affect the release of API particles from the mixtures. Thus, *in-vitro* inhaler testing for DPIs reflecting human inspiratory flow patterns would give better information on *in-vivo* inhalation performance than conventional testing, the flow pattern of which is far from the inspiratory flow patterns of patients.

Several studies have tried to use realistic inspiratory flow patterns for the assessment of DPIs (22–25). Chavan *et al.* reported that fine particle dose depended on "rate of rise" of inspiratory air flow, although they kept the constant flow rate described in the USP guidelines after the controlled rising of inspiratory flow (22). Furthermore, Martin *et al.* investigated the influence of realistic inspiratory flow patterns on fine particle fraction by using a specific inspiratory flow simulator (Electronic Lung™) consisting of a shuttle valve, a large vertical piston, and a cylinder driven by a computer-controlled servo-motor. Their results showed that the variation of inspiratory flow patterns greatly influenced the lung deposition of the drug (20). Such inspiratory flow simulators would be more effective for inhaler testing of DPIs; however, the large and complicated equipment and high cost limit their application.

Therefore, in this study, a novel inspiratory flow simulator with a simple structure was developed. Using a TSLI connected with the simulator, the influence of inspiratory flow patterns on the dispersion and deposition of physically mixed dry powders composed of salbutamol sulfate (SS) and coarse lactose monohydrate as model drug formulations was investigated. SS is a selective beta-2 adrenoceptor antagonist widely used for the treatment of airway obstruction by asthma, chronic bronchitis, and emphysema (26). We selected SS as a model drug in this study because SS inhalation is widely used clinically. To clarify the influence of the formulations, two physically mixed dry powders with different sized particles of SS were compared.

MATERIALS AND METHODS

Materials

As a model API for DPIs, salbutamol sulfate (SS) (DOLDER LTD., Switzerland) bulk drug (SS-bulk, 14.16 μm) and SS micronized by Spiral Jet Mill (100AS, HOSOKAWA MICRON, Japan) (SS-milled, 2.42 μm) were used. As a carrier particle for dry powder inhalation, lactose monohydrate (Pharmatose® 200 M, DMV, The Netherlands) was used. The other reagents and solvents used were of analytical grade and HPLC grade, respectively.

Measurement of Human Inspiratory Flow Patterns

As shown in Fig. 1, the inspiratory flow recorder (Hitachi Automotive Systems, Ltd., Japan), which could visualize human inspiratory flow patterns, consisted of a hot-wire flow meter, a power-supply box, and a personal computer. The hot-wire flow meter was applied for high time resolution (milli-second order) and low flow resistance. In this study, 10 volunteers (21–24 years old, 5 males and 5 females) participated in the measurement of inspiratory flow patterns with a dry powder inhaler (Jethaler® single chamber type, Hitachi Automotive Systems, Ltd., Japan). The pattern in each volunteer was measured in triplicate, and inspiratory flow volume (area under the flow rate-time curve; AUC, L), flow increase rate (FIR, L/min/s), and peak flow rate (PFR, L/min) were calculated.

Reproduction of Inspiratory Flow Patterns Using the Simulator

The inspiratory flow simulator was composed of an airtight container evacuated by a vacuum pump, a valve, and a connecting tube (Fig. 1). The simulator was connected to the outlet port of the TSLI, while the inhalation device and the flow recorder were connected to inlet port in this order. The three critical parameters that characterize inspiratory flow, AUC, FIR, and PFR were regulated by the volume of the container, valve-opening speed, and diameter of the tube, respectively. The container was evacuated with a vacuum pump, and the valve was closed. After the flow recorder was connected to the inspiratory flow simulator, a customized program was started to collect flow rate data every 10 ms for 30 s. Then the valve was opened at a constant rate by a motor in order to regulate the FIR.

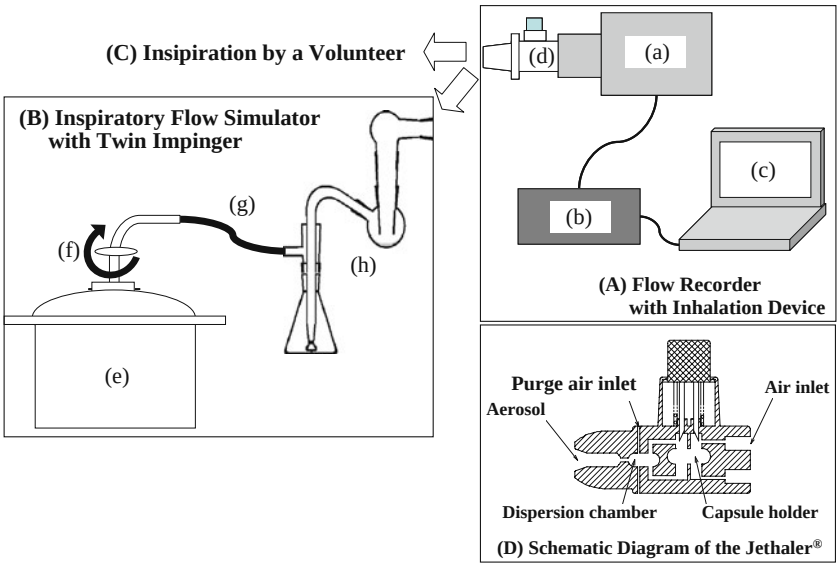

Fig. 1 Schematic diagrams of the system employed in this study to evaluate inhalation performance of physically mixed dry powders. (**A**) The flow recorder for visualization of air flow pattern was composed of a hot-wire flow meter (*a*), a power-supply box (*b*), and a personal computer (*c*) and was equipped with the Jethaler® (*d*). (**B**) The novel inspiratory flow simulator was composed of an evacuated airtight container (e), a valve (*f*), and a connecting tube (*g*) which was connected with a TSLI (*h*). When evaluating inhalation performance of physically mixed powders, the Jethaler® was connected to the inlet port of the TSLI. (**C**) When used for the measurement of the actual human inspiratory patterns, the Jethaler® was directly inhaled by a volunteer. (**D**) Schematic diagram of the Jethaler®.

Thirty seconds was long enough to monitor a flow pattern which usually took only a few seconds.

Preparation of Physically Mixed Dry Powders

Physically mixed dry powders were prepared by mixing 0.5 g of SS and 2.5 g of coarse lactose monohydrate in a glass bottle with a vortex mixer (SCIENTIFIC INDUSTRIES, USA) for 20 min, as reported (27). The powders made with SS-bulk and SS-milled are abbreviated as PM SS-bulk and PM SS-milled, respectively.

The diameters of particles of the two kinds of SS and coarse lactose monohydrate were determined by a laser micron sizer (LMS-30, SEISHIN, Japan) based on laser diffraction. The morphology of these particles was observed under a scanning electron microscope (SEM, JSM-6060, JEOL, Japan).

In Vitro Testing of Physically Mixed Dry Powders with Reproduced Inspiratory Flow Patterns

Aerodynamic particle deposition was determined using a TSLI (European Pharmacopeia Apparatus A, Copley Scientific Ltd., UK) equipped with the proposed inspiratory flow simulator. Stages 1 and 2 in the TSLI contained 7 and 30 mL of purified water, respectively. Aliquots (60 mg) of the physically mixed dry powders (10 mg as SS) were packed in No. 2 HPMC hard capsules (Shionogi Qualicaps, Japan). After the Jethaler® was connected to the mouthpiece of the TSLI, the capsule containing the physically mixed dry powders was placed in the holder with a pin to pierce them. Then, the simulator was operated to disperse the powder in the capsule under several conditions. After dispersion, the dry powders transferred to stages 1 and 2, and the capsule, the device, and the throat were collected by rinsing with purified water. The collected samples were diluted to 100 mL, and the concentration of SS in each sample was measured by high performance liquid chromatography (HPLC). The tests were performed in triplicate.

The flow patterns changed in AUC, FIR, and PFR, which ranged from 1.5 to 4.5 L, from 7 to >100 L/min/sec, and from 30 to 80 L/min, respectively. Although the trigger count interval of the inspiratory flow recorder was set to 0.01 s, it was difficult to measure FIR values higher than 100 L/min/sec, so the highest FIR was regarded as >100 L/min/sec.

The inhalation performance of the prepared dry powders was characterized by output efficiency (OE) and stage 2 deposition (St2). OE stands for the amount ratio of drug particles emitted from a capsule and an inhalation device (Eq. 1), while St2 represents the amount ratio of API particles deposited on stage 2 of the TSLI (Eq. 2).

$$\mathrm{OE} = \text{mass recovered from TSLI} / \text{mass balance} \times 100 \quad (1)$$

$$\mathrm{St2} = \text{mass recovered from stage 2} / \text{mass balance} \times 100 \quad (2)$$

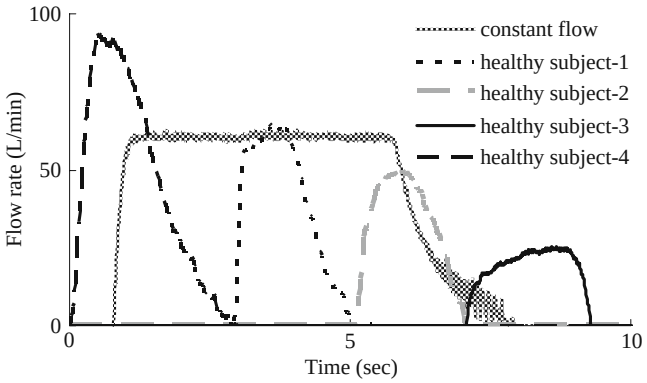

Fig. 2 Inspiratory flow patterns of healthy volunteers. The constant flow was generated by conventional vacuum pump (60 L/min). For clearer presentation of the profiles, the starting points of inspiration patterns were shifted from t = 0 arbitrarily.

Conditions for Measuring Salbutamol Sulfate by HPLC

The SS concentration of the *in vitro* testing samples was determined by a HPLC system (Agilent Technologies, Inc., USA) consisting of a quaternary pump (G1311A), a degasser (G1322A), a UV-vis detector (G1314B), a column oven (G1316A), and an auto sampler (G1329A). The mobile phase was composed of 0.025 M phosphate buffer (pH 2.8), acetonitrile, and methanol (90:9:1, volume ratio). The flow rate was set at 1.0 mL/min. The column (COSMOSIL® 5C18-AR-2, 4.6×150 mm; Nacalai Tesque, Japan) was heated at 35°C. The injection volume was 100 μL. The ultraviolet (UV) absorbance of each sample was measured at 224 nm. It was confirmed that the UV absorbance correlated linearly with the concentration of SS between 1.0 and 150 μg/mL.

Morphological Analysis of Dispersed Physically Mixed Dry Powders following Testing *In Vitro*

To ascertain visually the state of dry powders following inspiration at the highest and lowest PFRs, a specimen mount for SEM with double-sided tape on its top was set on the bottom of stage 1 in the TSLI instead of purified water. The system was operated as described in, "*In Vitro* Testing of Physically Mixed Dry Powders with Reproduced Inspiratory Flow Patterns," at the lowest and highest PFRs (30 L/min and 80 L/min, respectively). The morphology of PM SS-milled and PM SS-bulk on the specimens was observed by SEM.

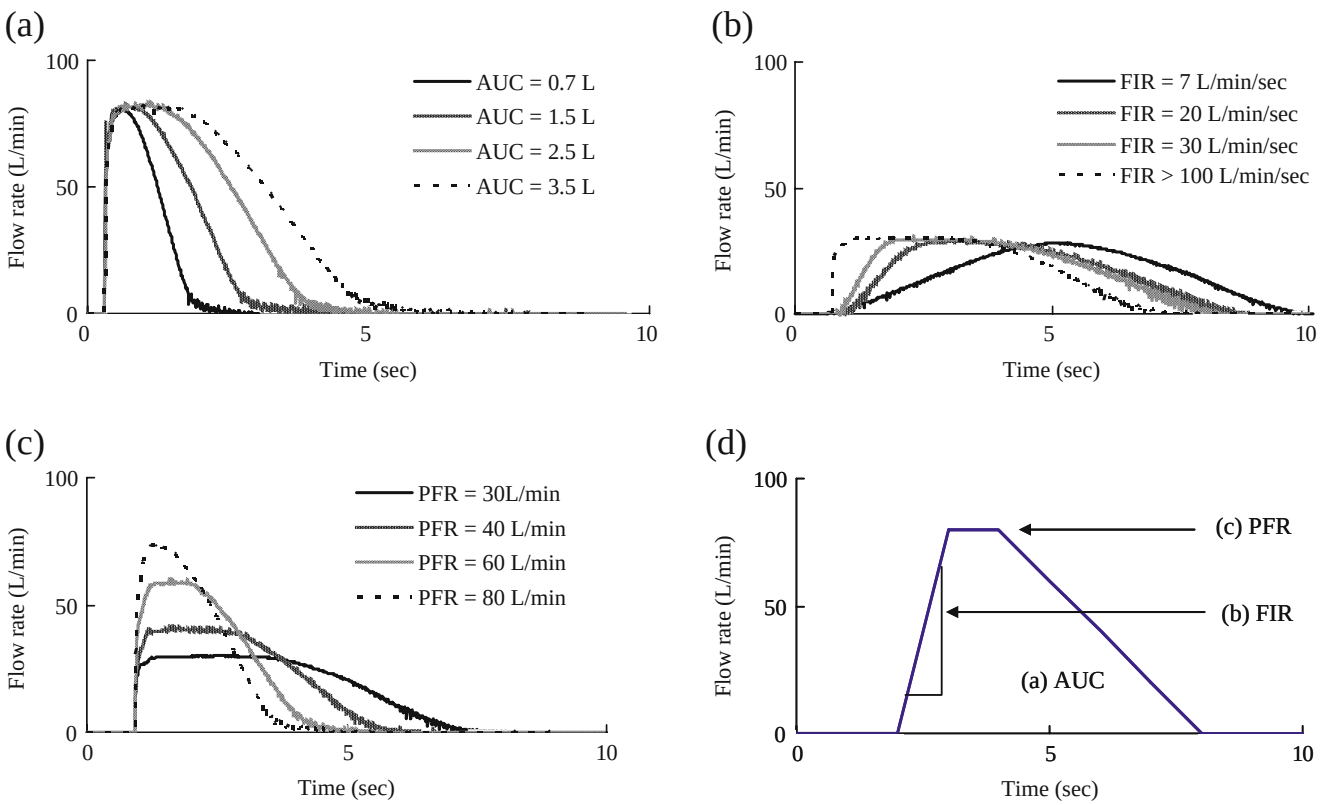

Fig. 3 Inspiratory flow patterns reproduced by the novel inspiratory flow simulator. (**a**) Effect of AUC; FIR and PFR were fixed to >100 L/min/sec and 60 L/min, respectively. (**b**) Effect of FIR; AUC and PFR were fixed to 2.5 L and 60 L/min, respectively. (**c**) Effect of PFR; FIR and AUC were fixed to >100 L/min/sec and 2.5 L. (**d**) The schematic model flow pattern.

Statistical Analysis

Statistical comparisons were made with a one-way analysis of variance (ANOVA). Comparisons of means were performed with the least significant difference test. The significance level was set at $p<0.05$.

RESULTS AND DISCUSSION

Comparison between Human Inspiratory Flow Patterns and Inspiratory Flow Patterns Reproduced by the Simulator

The inspiratory flow patterns of healthy volunteers measured with the inhalation flow recorder are shown in Fig. 2. The patterns changed dramatically in a few seconds and were clearly different from the rectangular flow pattern for the testing *in vitro*. Moreover, the AUC, FIR, and PFR analyzed from human inspiratory flow patterns ranged widely from 0.51 to 2.25 L, from 25.2 to 212.2 L/min/sec, and from 25 to 90 L/min, respectively, although there was no statistical difference between the sexes. Chavan *et al.* reported variability not only among subjects but within the same person, partly supporting our results (28). It is likely that the variability, caused by several factors, including vital capacity and technique of inhalation, can lead to unsatisfactory results in the clinical use of some inhalers.

The novel simulator was developed in order to reproduce human inspiratory flow patterns for more realistic testing (Fig. 1). Several patterns generated by the simulator are shown in Fig. 3. AUC, FIR, and PFR were regulated by the volume of the container, valve-opening speed, and diameter of the tube, respectively. Opening the valve at a constant rate enabled the flow pattern to rise linearly. Each critical parameter, AUC, FIR, or PFR, that determines the inspiratory flow pattern could be changed without affecting the other two parameters. Therefore, the simulator could schematically reproduce various human inspiratory flow patterns and is useful to investigate the influence of inspiratory flow on inhalation performance.

Preparation of Physically Mixed Dry Powders

For testing *in vitro* using the simulator, two physically mixed dry powders composed of SS and coarse lactose monohy-

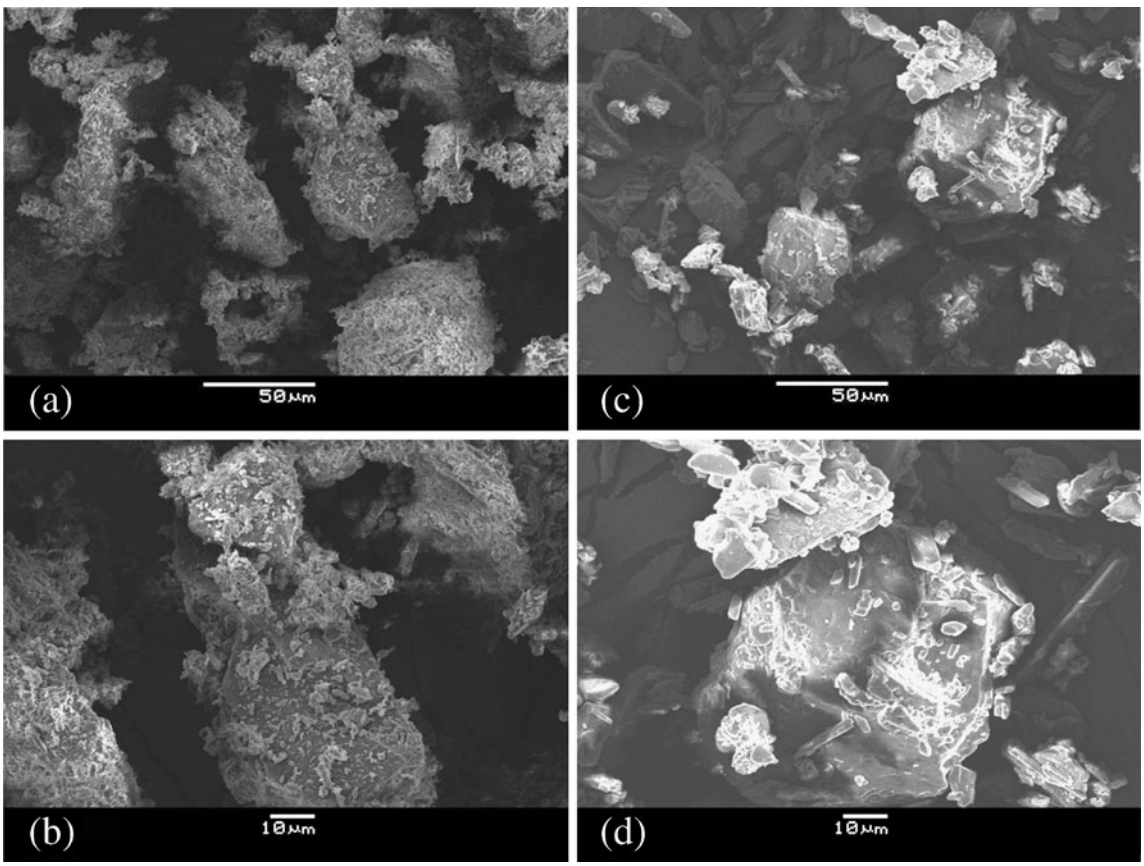

Fig. 4 Scanning electron micrographs of physically mixed dry powders; (**a**) PM SS-milled, (**b**) PM SS-milled with high magnification, (**c**) PM SS-bulk, and (**d**) PM SS-bulk with high magnification.

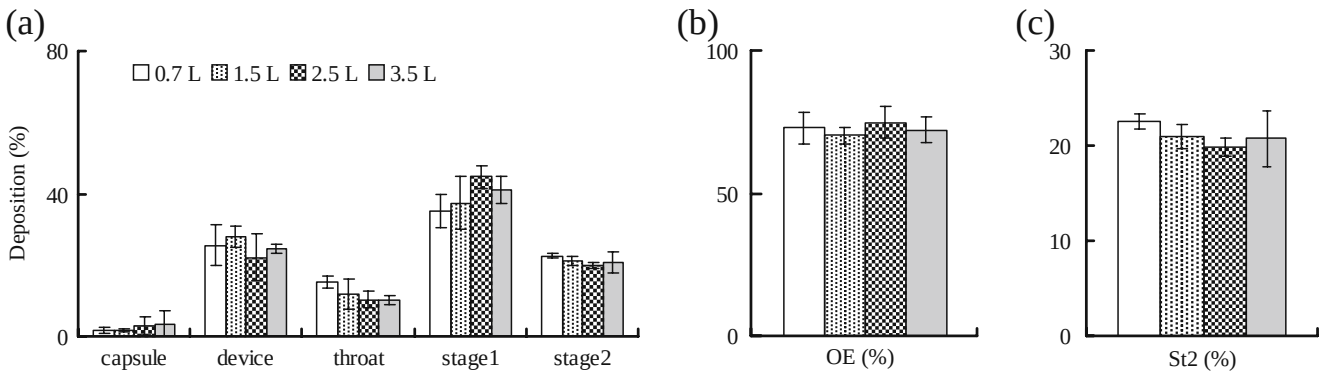

Fig. 5 Influence of AUC on inhalation performance of PM SS-milled; (**a**) the API deposition (%) on the parts of a TSLI, (**b**) OE value, and (**c**) St2 value. The FIR and PFR were fixed to >100 L/min/sec and 60 L/min, respectively. Each value represents the mean ± S.D. ($n=3$).

drate were prepared. The morphology of the two different dry powders is shown in Fig. 4. The volume median diameter (VMD) of SS-milled was approximately 2.4 μm, suggesting it to be the optimal diameter for DPI. On the other hand, the VMDs of SS-bulk and coarse lactose monohydrate were approximately 14 μm and 17 μm, respectively, indicating relatively large sizes. From SEM at high magnification, it was clear that the micronized SS particles were uniformly adhered to coarse lactose monohydrate particles in PM SS-milled, as reported elsewhere (29,30), while some large particles agglomerated in PM SS-bulk. These results indicated that SS-milled could easily adhere to coarse lactose monohydrate particles compared with SS-bulk particles.

In order to verify the homogeneity of these physical mixtures, five 60 mg samples were taken from the mixtures to quantify the content of SS by HPLC. The relative standard deviations of the average drug contents of PM SS-milled and PM SS-bulk were 3.11% and 3.60% ($n=5$), respectively, suggesting satisfactory homogeneity.

Influence of Inspiratory Flow Parameters on Inhalation Performance of Physically Mixed Dry Powders

Using the simulator and TSLI, the influence of AUC, FIR, and PFR on the inhalation performance of PM SS-milled was investigated.

Within the change of AUC from 0.7 to 3.5 L (FIR and PFR were fixed to >100 L/min/sec and 60 L/min, respectively), there was no significant difference of OE and St2 in PM SS-milled (Fig. 5). These results were similar to those reported previously (20,22). Since the open space in the inhalation device was several tens of milliliters, it was considered that the volume needed to emit the packed dry powders would be very small, thereby leading to no influence of AUC on inhalation performance. In addition to the results for AUC, the change of FIR did not affect OE and St2 in PM SS-milled (PFR and AUC were fixed to 30 L/min and 2.5 L, respectively), as shown in Fig. 6. There are reports of a significant influence of FIR on fine particle fractions, in

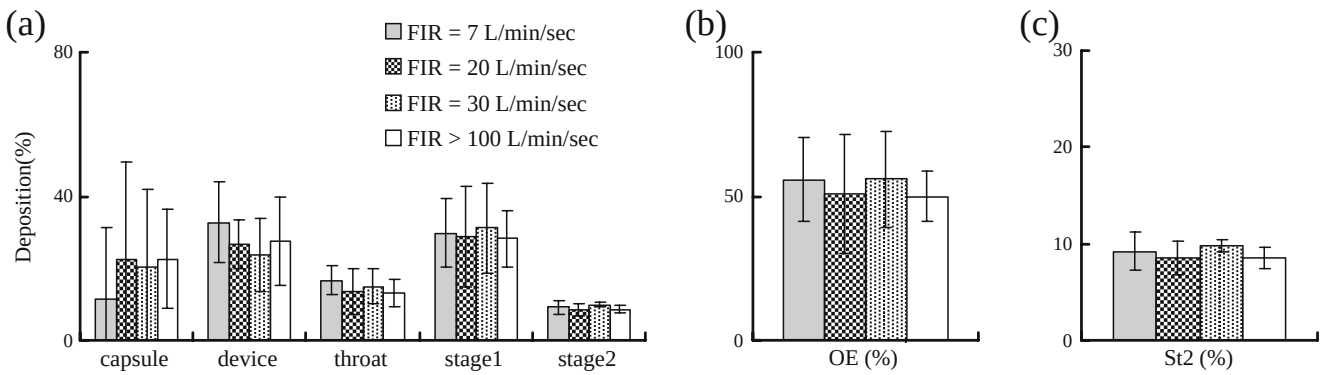

Fig. 6 Influence of FIR on inhalation performance of PM SS-milled; (**a**) the API deposition (%) on the parts of a TSLI, (**b**) OE value, and (**c**) St2 value. The AUC and PFR were fixed to 2.5 L and 30 L/min, respectively. Each value represents the mean ± S.D. ($n=3$).

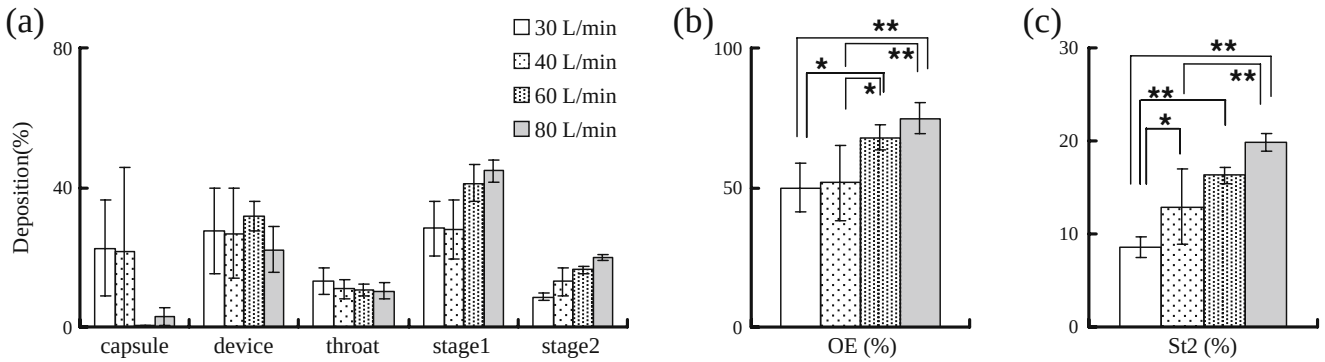

Fig. 7 Influence of PFR on inhalation performance of PM SS-milled; (**a**) the API deposition (%) on the parts of a TSLI, (**b**) OE value, and (**c**) St2 value. The AUC and FIR were fixed to 2.5 L and >100 L/min/sec, respectively. Each value represents the mean ± S.D. ($n=3$). *: $p<0.05$, **: $p<0.01$.

which the degree of influence was greatly affected by the formulation, such as the API/carrier ratio and particle diameter and inhalation device (20,22). Therefore, the combination of the formulations and device used in this study seemed to be insensitive to the change of FIR. As shown in Fig. 7, in contrast, it was obvious that a higher PFR caused a higher OE and St2 of PM SS-milled (AUC and FIR were fixed at 2.5 L/min and >100 L/min/sec, respectively), which was consistent with other reports (20,22).

The cut-off diameter of the TSLI depends on the flow rate being 6.4 μm when operated at a constant flow rate of 60 L/min. This means the cut-off diameter varied when the PFR was changed. Keeping a constant flow rate would be significant for quality control purposes; however, it does not reflect the human inspiration pattern. Even on inhaling particles of the same size, the pulmonary deposition should depend on the inspiration pattern, especially on the PFR (31). A particle size around 6 μm or less is generally recognized to be suitable for deep lung deposition; however, actual lung deposition in patient usage is influenced by their inspiratory flow patterns (31). Although the cut-off size for stage 1 varied with the PFR, the St2 determined in the present system may be a better index of the deposition of API in the lungs than that determined at a constant flow rate.

To closely assess the influence of PFR on the inhalation performance of physically mixed dry powders, PM SS-bulk was also examined using the TSLI and inspiratory flow simulator. As shown in Fig. 8, a higher PFR tended to cause a higher OE but a lower St2 for PM SS-bulk (AUC and FIR were fixed at 2.5 L and >100 L/min/sec, respectively), which was the opposite of the result for PM SS-milled. SS-bulk particles were larger in diameter than SS-milled particles (Fig. 4), indicating that the inertial force working on them is stronger. A slow PFR might be enough for the SS-bulk particles to detach from the coarse lactose monohydrate particles. A fast PFR might result in SS-bulk particles settling on stage 1 because of the larger inertial force. This insight strongly suggests that the balance of inertial force and adhesion force between API and the carrier was a critical factor influencing PFR in the inhalation performance of physically mixed dry powders.

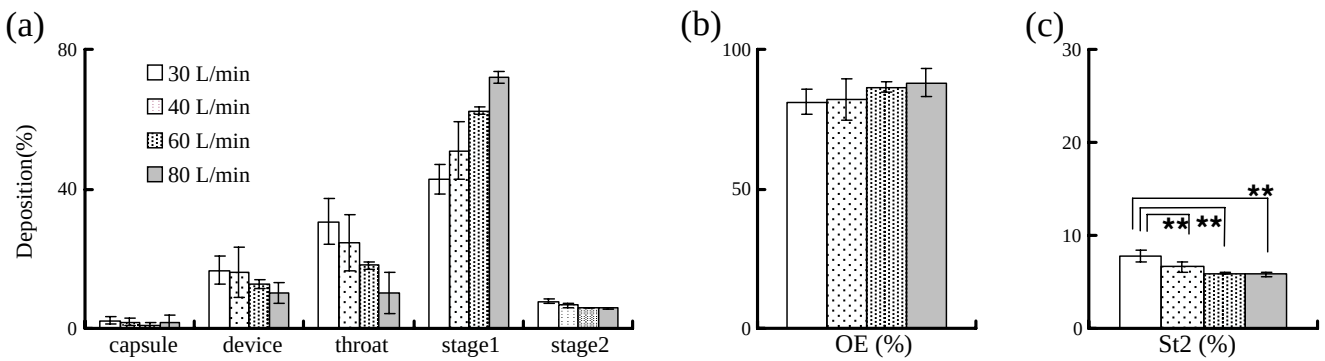

Fig. 8 Influence of PFR on inhalation performance of PM SS-bulk; (**a**) the API deposition (%) on the parts of a TSLI, (**b**) OE value, and (**c**) St2 value. The AUC and FIR were fixed at 2.5 L and >100 L/min/sec, respectively. Each value represents the means ± S.D. ($n=3$). **: $p<0.01$.

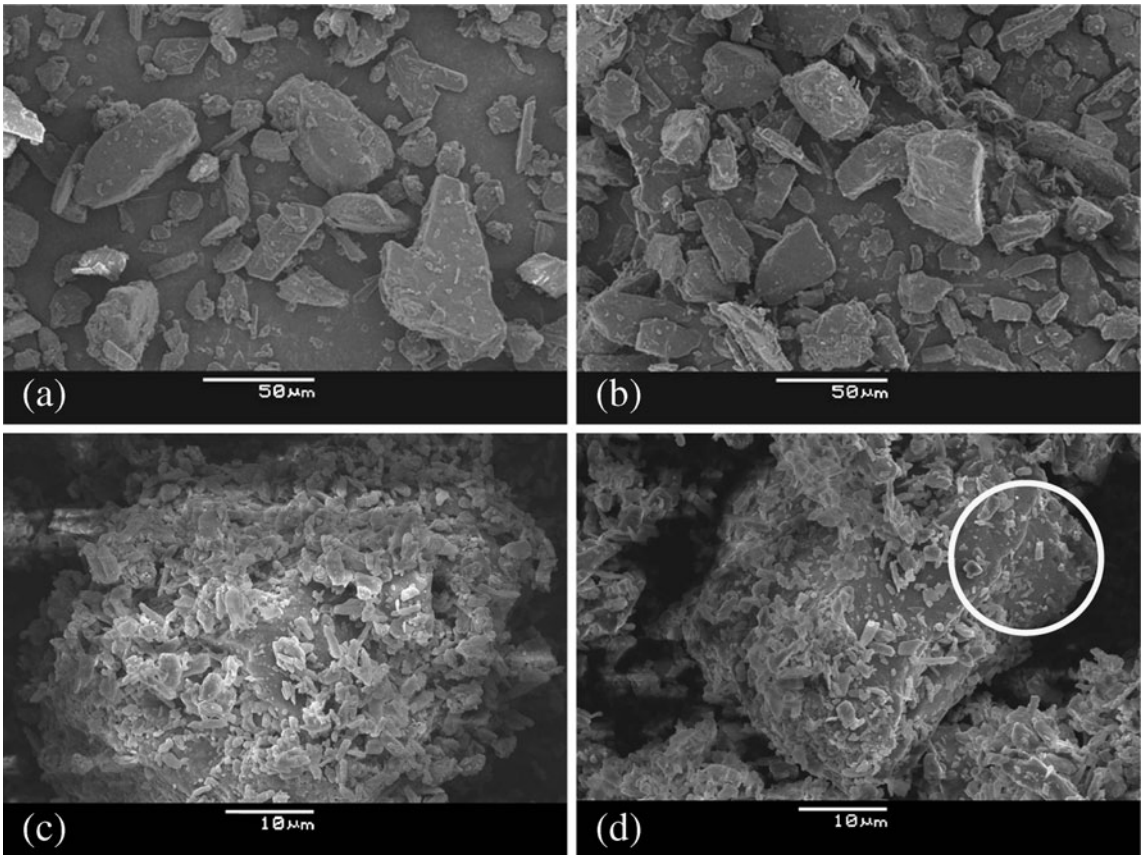

Fig. 9 Scanning electron micrographs of dispersed physically mixed dry powders on stage 1 following *in-vitro* inhaler testing; (**a**) PM SS-bulk at 30 L/min of PFR, (**b**) PM SS-bulk at 80 L/min of PFR, (**c**) PM SS-milled at 30 L/min of PFR, (**d**) PM SS-milled at 80 L/min of PFR. The lactose nonohydrate particle of PM SS-milled dispersed at 30 L/min is extensively covered with SS (**c**), while that dispersed at 80 L/min has more surface free from SS ((**d**): *circle*).

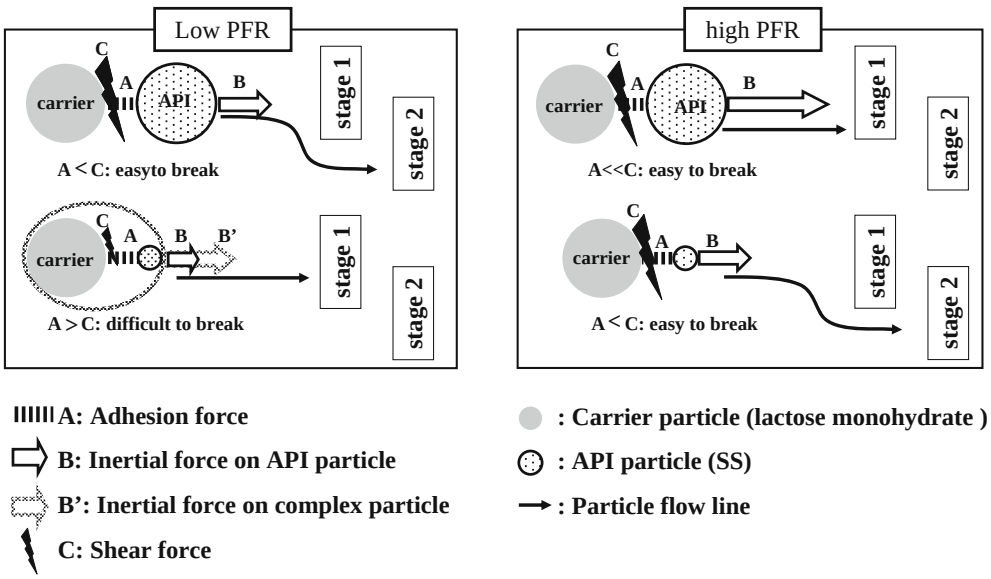

Fig. 10 Schematic diagram of hypothetical mechanisms behind influences of API particle size and PFR on inhalation performance of physically mixed dry powders. At a low PFR, the shear force acting on the large API particles (*left-upper*) is stronger than the adhesion force between API and carrier, so the API particles easily separate from the carrier, resulting in higher deposition on the stage 2 of the TSLI. Inversely, the shear force acting on small API particles (*left-lower*) is weaker than the adhesion force between API and carrier, resulting in difficulty of separation from carrier particle, so the large complexes of small APIs and carrier particle are likely to deposit on the stage 1 by the strong inertial force (B'). At a high PFR, the large API particles (*right-upper*) are easy to separate from carrier as at a low PFR. However, the inertial force is too strong for the API particles to avoid deposition on the stage 1. On the other hand, the small API particles (*right-lower*) separate from carriers by the higher shear force derived from a high PFR; however, the inertial force is still small enough to avoid deposition on the stage 1.

Morphological Comparison of Dispersed Physically Mixed Dry Powders Following Testing *In-Vitro*

As further confirmation of the influence of PFR on the inhalation performance of physically mixed dry powders, the morphology of PM SS-milled and PM SS-bulk on stage 1 following testing *in vitro* at the lowest and highest PFRs (30 L/min and 80 L/min, respectively) was compared using SEM. SS-bulk particles were detached from the coarse lactose monohydrate particles and uniformly dispersed at both the lowest and highest PFRs (Fig. 9a, b), which was consistent with the high OE in *in-vitro* testing (Fig. 8b). These results indicated that a high dispersibility of PM SS-bulk could be achieved even at the lowest PFR. On the other hand, there was a great difference between the behavior of PM SS-milled at the lowest and highest PFRs; most SS-milled microparticles adhered to coarse lactose monohydrate particles at the lowest PFR, while the microparticles were detached from the lactose monohydrate particles and dispersed at the highest PFR (Fig. 9c, d). This finding was in accordance with the result of *in-vitro* testing that higher OE and St2 values could be achieved with a higher PFR (Fig. 7). Taking these observations into consideration, it was obvious that PM SS-milled required a higher PFR for dispersion.

Hypothetical Mechanisms behind Influences of API Particle Size and PFR on Inhalation Performance of Physically Mixed Dry Powders

The opposite effect of PFR on PM SS-bulk and PM SS-milled can be explained by the balance of shear force and adhesion force between API and carrier particles and the inertial force acting on the API particles, as shown in Fig. 10. As far as the inhalation performance of physically mixed dry powders is concerned, it is widely recognized that API particles must easily detach from carrier particles following inhalation. The detachment is achieved by an inspiratory flow above a critical level, which is dependent on the balance of shear force and the adhesion force between API and carrier particles. The shear force working on physically mixed dry powders depends on the PFR, that is, the higher the PFR is, the stronger the shear force is in the same inhalation device (32). The SS-bulk particles have a larger mass than the micronized particles, resulting in larger shear force in a flow, which contributes to the easy detachment of SS-bulk particles from the coarse lactose monohydrate carrier at a low PFR. However, at a high PFR, the behavior of SS-bulk particles is greatly affected by a strong inertial force, consequently leading to the larger deposition on stage 1 (i.e., lower St2). On the other hand, the PM SS-milled particles with a smaller mass experience weak shear force. Therefore, the detachment of SS-milled microparticles from coarse lactose monohydrate requires a PFR high enough to increase the shear force to overcome the adhesion force. Even at a high PFR, however, the inertial force acting on SS-milled microparticles may still be small enough to avoid impaction to stage 1, and thereby transferred to stage 2.

CONCLUSIONS

In this study, a simple simulator was developed for reproducing human inspiratory flow patterns. The simulator closely regulated three inspiratory flow parameters—PFR, FIR, and AUC—which enabled us to reproduce various human inspiratory flow patterns schematically. From the *in-vitro* testing of physically mixed dry powders using the simulator, it was demonstrated that PFR was the critical factor determining the inhalation performance of physically mixed dry powders, which was greatly affected by the size of API particles. The effect of PFR on inhalation performance depended on the size of the API particles that could be explained by a balance of the shear force and the adhesion force between API and carrier particles and the inertial force on API particles. These results indicate that the simulator is an attractive apparatus for testing *in vitro*, reflecting human inspiratory flow patterns, which will not only aid the development of effective DPIs but also offer information for optimum inhalation.

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