In Vitro and In Vivo Aspects of Cascade Impactor Tests and Inhaler Performance: A Review

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ABSTRACT

The purpose of this review is to discuss the roles of cascade impactor (CI) data in inhaler assessment and to examine the relationship between aerodynamic particle size distribution (APSD) and the clinical response to inhaled drugs. A systematic literature search of studies linking APSD to clinical response was undertaken. Two distinct roles for CI-generated data were identified: (1) the control of inhaler/drug product quality; and (2) the provision of data that may be predictive of particle deposition in the respiratory tract. Method robustness is required for the former application, combined with simplicity in operation, resulting in rudimentary attempts to mimic the anatomy of the respiratory tract. The latter necessitates making the apparatus and its operation more closely resemble patient use of the inhaler. A CI cannot perfectly simulate the respiratory tract, since it operates at constant flow rate, while the respiratory cycle has a varying flow-time profile. On the basis of a review of studies linking APSD to clinical response of inhaled drugs, it is concluded that attempts to use CI-generated data from quality control testing to compare products for bioequivalence are likely to have only limited success, as links between laboratory-measured APSD, particle deposition in the respiratory tract, and clinical response are not straightforward.

KEYWORDS: Cascade impactor, inhaler testing, clinical response.

INTRODUCTION

The multistage cascade impactor (CI) is the instrument of choice for the particle size characterization of aerosols from portable inhalers (Table 1).[1] Pharmaceutical aerosols are nonequilibrium dispersions that will be influenced by conditions and timing of sampling, so defined procedures are needed. Thus, various CIs are described in both European[2] and US[3] pharmacopeias. CI measurements require sampling the inhaler-produced aerosol and are, therefore, invasive compared with optical-based particle sizing techniques.[4] Although the CI method is complex,[5] it is favored because the masses of active pharmaceutical ingredients (APIs) in each fraction can be specifically assayed. Furthermore, measurements of API are interpreted in terms of particle size aerodynamic diameter, thereby taking into account both particle density and shape.[1] This scale is indicative of likely API deposition in the respiratory tract, where particle motion in the size range of interest (0.5-10 μm aerodynamic diameter) in the continuously varying flow associated with the respiratory cycle is largely influenced by inertia as well as, to a lesser extent, by gravitational sedimentation and even less by diffusion-related effects.[6] In principle, CI-derived aerodynamic particle size distribution (APSD) data may provide information that is predictive of lung deposition,[7] which might be helpful in estimating the likelihood of clinical response in studies of the efficacy and safety of inhaled medications.[8] The purpose of this review is to discuss the roles of CI data in inhaler assessment and to examine the relationship between APSD and the clinical response to inhaled drugs.

CI METHOD

Inertial impactors size-separate particles subjected to a change in flow direction of their support gas (usually air) moving at constant flow rate under laminar flow conditions.[9] Particles entering a single-stage impactor pass through a plate containing 1 or more jets of well-defined size. A collection surface located immediately beyond the plate at a well-defined separation distance deflects the flow; the inertia of the particles causes them to cross the flow stream, with the result that those with a size greater than a critical value impact on the surface, whereas smaller particles remain airborne. The size at which a given impaction stage collects 50% of the mass entering is termed the effective cutoff diameter (ECD) and defines the calibration for that stage.[1] Several stages are arranged in sequence in a CI, such that particles having progressively finer sizes are collected as the aerosol passes through the instrument. The theory of impactors is well understood,[9,10] and criteria have been established so that stage collection efficiency curves of a given

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Table 1. Suitability of the Cascade Impactor Method for the Characterization of Aerosols From the Various Major Classes of Portable Inhaler

Inhaler Class	Suitability
Pressurized metered dose inhaler with or without spacer/valved holding chamber	High: preseparator may be required for a few high mass/actuation formulations
Dry powder inhaler	High: preseparator often required, especially when sizing carrier–active pharmaceutical ingredient– based formulations
Nebulizer	Moderate: low flow rate operation ≤ 15 L/min with precautions to avoid heat transfer–related droplet evaporation
Nasal metered dose inhaler	Moderate: specialized entry (induction) port required, some droplets may be too large to be size-fractionated
Aqueous nasal spray pump	Low: droplets are almost all too large to be size-fractionated, but adapted cascade impactor method may be useful at quantifying proportion of mass < 10 μm aerodynamic diameter that could penetrate beyond the nasopharynx

design can be made as steep (narrowly size-selective) as possible.[11] The CI is usually preceded by an entry or induction port that is intended to mimic the oro- or nasopharynx, albeit in a rudimentary way.[1] A high-capacity stage (preseparator) with ECD ≥ 10 μm located immediately after the induction port is often needed to evaluate certain types of inhalers, in particular dry powder systems, where API still attached to much larger carrier particles would bias APSD measurements if the particles entered the CI. In terms of offering optimum size resolution without significant overlap of adjacent stage collection efficiency profiles, a minimum of 5 CI stages with ECDs in the range from 0.5 to 5 μm aerodynamic diameter appears to be desirable, preceded by at least 1 stage with ECD between 5 and 10 μm, as well as a collection filter or means of capturing fines < 0.5 μm aerodynamic diameter.[11,12]

Data Interpretation

Raw size distribution data from a CI are obtained as mass of API associated with each component.[1] Expressing mass or mass fraction of API in terms of deposition location has the advantage that contributions from both nonsizing (ie, preseparator and induction port) and size-fractionating components are considered.[13] The resulting profile of the natural order of each component in the CI system (Figure 1A) can be used as the basis to compare inhalers, for instance innovator (reference) and generic (test) products. A refinement is to rank-order the mass collected on each component (Figure 1B).

An exercise addressing innovator/generic APSD comparisons has been recently completed by the Product Quality Research Institute (PQRI),[14,15] driven by the desire to provide regulators with a rigorous methodology. Even though unambiguous in vitro–in vivo correlations (IVIVCs) do not yet exist for inhaled medications used in the treatment of obstructive airways diseases (discussed later), it was assumed that there may be a link between deposition in the CI system and deposition site in the respiratory tract. This initiative resulted in the development of a combined procedure based on calculations of chi-square ratio[14] plus impactor-sized mass-population bioequivalence (ISM-PBE).[16] The

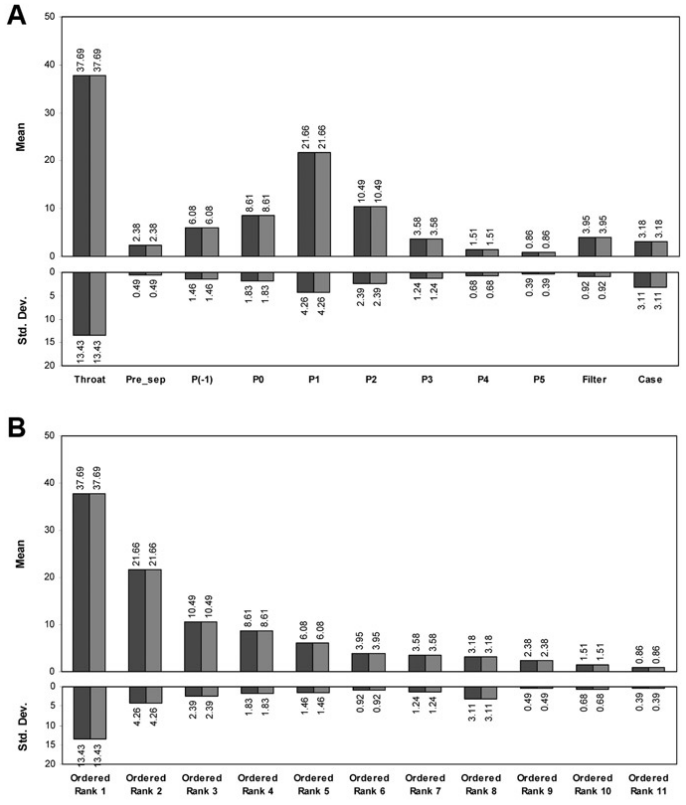

Figure 1. Mass collected on each component of an Andersen 8-stage cascade impactor (A) presented in the natural order of the components in the cascade impactor system; and (B) presented in the rank order of components from highest to lowest associated mass. From Adams et al.[14] In this example, the impactor-measured profiles from test (gray) and reference (black) inhales-generated aerosols were identical.

former is sensitive to differences in sites in the CI system with high deposition, which usually occurs with the nonsizable portion of the profile, whereas the latter was intended to detect differences in the overall mass deposited within the CI. The PQRI group concluded that the outcome of the combined statistical procedure was controlled almost exclusively by the ISM-PBE component. However, it was also found that on a few occasions, the combined statistical procedure may lead to conclusions that are at odds with expert judgments regarding test and reference profile equivalence. As a result, no specific statistical procedure could be recommended.[16] It is important to note that while these forms of data presentation permit such comparisons to be made on a component-by-component basis,[14] no information is provided linking API mass with aerodynamic diameter.

An alternative approach to data interpretation is to link mass deposited on the size-fractionating stages of the CI with the size range, sometimes referred to as the width (ΔECD_i), within which each stage operates. This range is bounded by the ECD for that stage and the ECD of the stage immediately following in the cascade. A closer link with the aerosol deposition profile in the respiratory tract, and therefore the clinical response, may be possible, since mass of API is associated with aerodynamic size. However, although this strategy is useful for comparing data obtained from 1 type of CI, there is still no association between aerodynamic size and the mass collected in the non-size-fractionating components.[13] Such nonsized particles may represent a significant portion of the mass of aerosol emitted from the inhaler.[15] Moreover, comparisons between different CI designs are difficult, as profiles are affected by variations in stage width from 1 stage to another.[13] Such bias is removed if the mass or mass fraction assigned to each size-fractionating stage ($\Delta m_i/M_{EM}$) is normalized by dividing by the appropriate stage width ($[\Delta m_i/M_{EM}]/\Delta ECD_i$), where M_{EM} is the total mass that is emitted at the patient interface of the inhaler. The resulting differential mass-weighted APSD provides a quantitative link between API mass and aerodynamic size that is independent of CI type.[13] It can be further analyzed to provide descriptive statistics, in particular the fine particle fraction (FPF) and coarse particle fraction, typically $<$ and $>$ 5 μm aerodynamic diameter, respectively, for orally inhaled particles.[2] Such metrics reflect both the dependency of particle deposition in the conducting airways and alveolated regions of the lungs on aerodynamic diameter, and the steep rise in oropharyngeal deposition with increasing size.[17,18]

The PQRI assessment did not normalize data to account for CI stage width, because the focus was on making component-by-component comparisons rather than studying APSDs as continuous probability-density models.[14] There is interest in exploring the use of such probability-density functions to describe CI data from inhaler-generated aerosols, but the scope is limited by the fact that in many cases much of the aerosol is collected in non-size-fractionating components.[19] Even when such an approach is possible, data manipulation involving either linear or nonlinear interpolation inevitably leads to loss of accuracy, linked to the goodness-of-fit of the raw data to the regression-model distribution.[19]

Whichever approach is taken to interpret CI-measured APSDs, it is usually assumed that size limits accurately reflect the calibration of the CI, in particular that each stage has a step change in collection efficiency from 0% to 100% at its ECD.[1] Recently, the validity of this assumption has been questioned,[20] since the asymmetric nature of each stage collection efficiency curve on either side of its ECD may introduce bias. Such asymmetry is more evident with older CI designs.[21-23] Although consideration of the complete stage collection efficiency profiles in CI-based measurements is not new,[24] it has not thus far been applied to the assessment of inhaler-produced aerosols. Better-designed CIs, such as the Next Generation Pharmaceutical Impator (NGI), in which the stage collection efficiency curves are steep and symmetrical around the ECD value,[25] should be less susceptible to bias from this source, but at present this hypothesis is unproven.

CI Roles in Inhaler Assessments

Two equally valid roles have developed in recent years for CIs (Table 2). The goal is primarily to characterize emitted APSD for inhaler product quality control.[26] The desired characteristics are a relatively simple procedure that is (1) analytically robust in terms of the APIs being determined, (2) repeatable from 1 measurement to another and from 1 laboratory to another, and (3) as free of operator- and apparatus-caused bias as possible.[5,11,26] The CI is typically operated with minimal attempts to simulate aerosol transport beyond the inhaler.[2,3] A facemask, if present as patient interface, may be removed[27,28]; the induction port is usually a right-angle bend,[2,3,27] and although entry ports of other shapes can be used, they must have simple geometry.[29]

The CI is operated at constant flow rate for testing most types of inhalers. However, for dry powder inhalers (DPIs), the compendial procedures attempt to simulate an inhalation maneuver in order to operate the device so that the powder is aerosolized as a bolus, as would be the case in normal use.[2,3,30] Although stage ECDs are not constant until the flow rate stabilizes, the assumption is made that the bolus passing through the CI is sized as if it were operating at this final flow rate. This approach approximates reality as long as the volume of air sampled is significantly larger than the dead volume enclosed by the CI system.[31] Byron et al recently observed that for such DPI testing in a quality control environment, the setup has to ensure that the flow-time profile is reproducible, irrespective of which impactor, pump, or flow configuration is employed, and that there are no

Table 2. Roles for the Cascade Impactor in Inhaler Assessments: Limitations and Attributes*

Application	Attributes	Limitations	Measured APSD
Product quality control	1. Simple 2. Analytically robust 3. Repeatable (consistent) 4. Accurate in relation to emitted APSD from inhaler/accessory 5. Useful for comparing inhalers	1. APSD data more difficult to relate to respiratory tract deposition behavior	Emitted from the inhaler/accessory device
Predict inhaler aerosol behavior under conditions of use	1. Complex, depending upon degree of realism required 2. Potentially more accurate in terms of APSD entering the lower respiratory tract if combined with anatomically correct upper-airway model	1. Cannot simulate all patient ages, disease states, etc, by 1 model system 2. Unsuited to routine quality control applications	Deposited at the entrance to the lower respiratory tract, if system has anatomically correct oro- or nasopharyngeal geometry

*APSD indicates aerodynamic particle size distribution.

specific controls in the pharmacopeial methods to ensure this goal is achieved.[11] For pressurized metered dose inhaler (pMDI) testing, O'Connor and Tougas have recently highlighted ways in which CI measurement precision can be improved, citing sources of variability such as electrostatic charge on nonconducting components of the CI system, laboratory temperature, and variations in actuation rate (time between individual actuations) of the inhaler.[32]

Even when care is taken to address methodological details, a major limitation of the quality control approach is that the data are obtained with an apparatus that has only rudimentary realization of the anatomy of the respiratory tract. It follows that it is more difficult to relate findings to respiratory tract deposition behavior and ultimately therefore to clinical response.

The alternative strategy is to use CIs in ways that more realistically simulate the behavior of the aerosol after it has left the inhaler.[26] Such systems are less constrained and have, therefore, become quite elaborate. The use of anatomical throat models as inlets is intended to improve correlations between APSD and respiratory tract deposition data.[33-35] Mandhane et al have even extended this approach to simulate delivery to intubated, mechanically ventilated patients, describing a system in which the induction port is replaced with an endotracheal tube.[36] An entry that attempts to recreate realistic facial geometry is important for testing inhalers with accessory devices that have a facemask as patient interface.[37] These systems are also likely to be interfaced with a breathing simulator, introducing further complexity. However, they provide a better realization of aerosol transport conditions in the upper airway. Sampling of polydisperse aerosols typically produced by inhalers should ideally be isokinetic (ie, the air velocity at the inlet should match that of the air flow from which the sample is being taken) to eliminate size-related bias.[1] A more sophisticated approach is to supply sufficient flow of air to operate the CI at its required constant flow rate but at the same time allow the aerosol leaving the inhaler to move under the influence of a breathing simulator.[35,36,38,39] Precautions have to be taken to avoid flow transients arising from pressure pulsations, particularly if solenoid valves are used to supply the CI during part of each breathing cycle. Despite these difficulties, Janssens et al used this approach to acquire APSD data in the development of an in vitro model of a tidally breathing 9-month old infant (Figure 2).[40] The CI-based sampling system was located immediately after the model infant head, in which the nasopharynx was anatomically correct; the model was further enhanced by simulating the mucosa by wetting the internal surfaces of the model nasopharynx with a viscous layer of polyoxyethylene 23 lauryl ether (Brij).

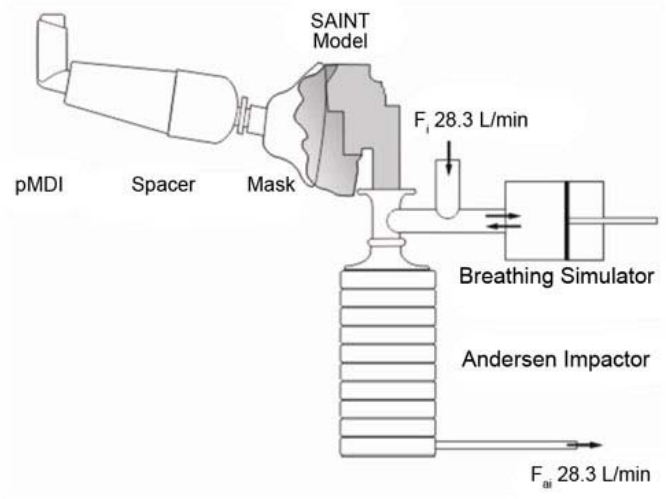

Figure 2. Use of Andersen cascade impactor in conjunction with a breathing simulator set to mimic tidal breathing by infants from a pMDI/holding chamber (spacer). From Janssens et al.[38] pMDI indicates pressurized metered dose inhaler.

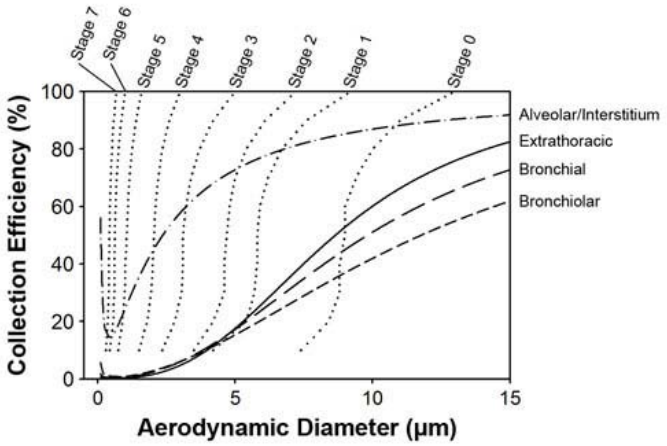

Figure 3. Collection efficiencies of the Andersen cascade impactor (Q = 28.3 L/min) and morphological regions of the lung (peak inspiratory flow rate = 28.3 LPM; V_t = 2 L; healthy male). From Dunbar and Mitchell.[13]

This arrangement represents the state of the art in terms of using the CI as a tool to acquire APSD data that are likely to be representative of the aerosol entering the lower respiratory tract. Models for other patient categories, including oral breathing infants and small children, are the logical next step in this process toward achieving closer clinical realism.

When seeking greater reality in terms of aerosol transport from the inhaler, one is tempted to regard the CI by itself as a form of simulator of the respiratory tract, because particles are fractionated in terms of their aerodynamic size. However, there are 2 major limitations to this approach: (1) CIs are intended to operate with a constant flow rate in order to preserve constant-stage ECD values,[9,10] rather than with the continuously varying flow rate typical of the respiratory cycle; and (2) CIs do not simulate the temperature and relative humidity that exists in the respiratory tract. Under certain circumstances, CIs can cause changes to the size distribution of aerosols containing volatile species (eg, aqueous droplets from nebulizers) by heat transfer–related evaporation.[41] Furthermore, most CI designs operate within only a limited range of flow rates, typically between 30 and 100 L/min,[42] so low-flow versions must be developed to assess inhalers and accessories intended for use by infants and small children.[43] Evidence that CIs are poor respiratory tract simulators was recently presented by superimposing stage collection efficiency curves for 1 particular type of CI (Andersen 8-stage impactor operated at 28.3 L/min) with the corresponding collection efficiency curves for the various regions of the respiratory tract (alveolar/interstitium, extrathoracic, bronchial, and bronchiolar).[13] These regional deposition efficiency curves were established in a model of particle deposition in the human lower respiratory tract for an adult tidally breathing male subject with peak inspiratory flow rate of 28.3 L/min and tidal volume (V_t) of 2 L (Figure 3). The curves of fractional deposition that were based on the model developed by the International Commission on Radiological Protection [44] are relatively shallow compared with the CI stage collection efficiency curves, reflecting the effect of many variables, most notably flow rate change during the breathing cycle. Notwithstanding these limitations, CI-measured FPF correlates with whole lung deposition,[7,33] though the correlation may be relatively poor for a chlorofluorocarbon (CFC)-powered pMDI.[45]

Although respiratory tract deposition models and IVIVCs are being considered for pulmonary drug products, in the absence of such models, inhaler performance is most appropriately ensured by monitoring changes in CI-measured APSDs, without using the CI as a surrogate to mimic respiratory tract deposition.

APSD, Deposition, and Clinical Effects

Deposition of drugs in the respiratory tract determines their clinical effects,[46] although this relationship is often concealed by the use of high drug doses that achieve maximal clinical response.[8] The systemic side effects of inhaled asthma drugs are also thought to be caused chiefly by the fraction deposited in the lungs.[47] The relative importance of total lung deposition vs regional lung deposition, and how this influences both beneficial effects and side effects of inhaled drugs, is incompletely understood.[48]

Since APSD is the most significant characteristic determining deposition in the respiratory tract and deposition influences clinical response, APSD must predict clinical response to inhaled drugs, at least to some degree.[49] For an inhaled corticosteroid, a 4-fold change in dose may be needed in order to detect a difference in clinical response.[50] While unlikely to be true for all types of inhaled drug, this result may have led to a more general belief that for drugs with relatively wide therapeutic windows, such as those used in the treatment of asthma, APSD can vary considerably without the clinical response being affected. However, it is unclear whether this belief is supported by evidence.

APSD is only one of many factors that could affect the clinical response to an inhaled drug.[51] However, to increase the understanding of the nature of the relationship between APSD and clinical response, primarily as it applies to pMDIs and DPIs, published studies linking clinical response and particle size have been reviewed. These studies involved a primary data set, comprising comparisons between 2 or more aerosols of different sizes from the same or very similar pMDIs or DPIs, and a secondary data set involving a range of data from pMDIs, DPIs, and nebulizers, and using monodisperse pharmaceutical aerosols. Studies linking APSD to either lung deposition or pharmacokinetic parameters were not considered.

Table 3. Primary Data Set Studies, Where Aerosols With Different APSDs From the Same or Similar pMDIs or DPIs Are Compared*

Inhaler	Drug and Dose	Particle Sizes	Study Population	Results	Reference
pMDI	Terbutaline sulfate, 250 μg	<5, 5-10, and 10-15 μm fractions placed in pMDI	10 asthmatics and 10 healthy subjects	Responses to < 5 μm particles higher ($P < .05$) than to larger particles	52
Turbuhaler DPI	Terbutaline sulfate, cumulative to 2 mg	FPMs 90, 40, and 5 μg	12 asthmatics	Response to 90 μg FPM significantly ($P < .05$) higher than to 5 μg FPM; no difference between 90 μg/40 μg, or 40 μg/5 μg	53
pMDI	Unspecified anticholinergic agent, cumulative to 1600 μg	FPFs 35% and 10%	10 "volunteers"	Response significantly greater for aerosol with higher FPF; ratio of increase in specific airways conductance in response ~2:1 at likely therapeutic dose of 200 μg	54
pMDI	Albuterol, 200 μg	Not stated, but sprays delivered via 4 nozzles ranging from 0.23 to 0.59 mm in diameter	19 asthmatics	Not quantified, but smallest nozzle provided significantly higher response than largest nozzle	55
DPI	Albuterol, 400 μg	Experimental powder formulations with FPFs 25% to 40%	12 asthmatics and 12 healthy subjects	No correlation between response and FPF	56
Novolizer DPI	Albuterol, cumulative dose to 400 μg (lowest dose 50 μg)	Experimental powder formulations with FPFs 18% to 47%	12 asthmatics	Similar response for each formulation, even at lowest dose	57
Novolizer DPI	Albuterol, cumulative dose to 1600 μg	Experimental powder formulations with FPFs 10% to 51%	6 asthmatics	Changes in serum potassium and heart rate increased with dose and with FPF	58

*APSD indicates aerodynamic particle size distribution; pMDI, pressurized metered dose inhaler; DPI, dry powder inhaler; FPM, fine particle mass; FPFs, fine particle fractions.

Data Review: APSD vs Clinical Response—Primary Data Set

Clinical studies were identified that compared the clinical effects of aerosols from the same or similar pMDIs or DPIs (Table 3). All the studies were undertaken in relatively small groups of subjects (n = 6-19) and were published between 1982 and 2004. Two of the studies are available only as abstracts, and 1 of these 2 studies was entirely qualitative. Six of the seven studies used CI methods to measure APSD, but in none of them was the CI used in a manner intended to simulate patient use.

Using terbutaline sulfate pMDIs, Rees et al confirmed that the size band < 5 μm aerodynamic diameter is clinically effective, while larger size bands may be ineffective.[52] Particle size spectra by mass were measured by scanning photosedimentometry, but these were the size distributions of the particles placed in the pMDIs, not the APSDs of the emitted aerosols. Therefore, this study did not allow the relationship between APSD and clinical response to be examined.

Persson and Wiren found a statistically significant difference in the forced expiratory volume in 1 second (FEV_1) response between aerosols of terbutaline sulfate delivered by Turbuhaler DPI with fine particle masses (FPM, < 5 μm) 90 μg and 5 μg.[53] Owing to the high variability in clinical response between individuals, neither amount gave a response significantly different from an aerosol with an FPM of 40 μg. FPM was the only detail provided about the APSDs.

Padfield et al assessed the efficacy of a formulation of an unspecified anticholinergic agent delivered by pMDI but prepared by 2 different mixing processes.[54] The 2 methods were shown to result in 35% (A) and 10% (B) of the dose penetrating to the lower stage of an impinger (particles < 6.4 μm diameter). In a study of 10 subjects (possibly healthy volunteers), a cumulative dose-response study showed that at a likely therapeutic dose of 200 μg, the increase in specific airways conductance for method A was approximately double that of method B.

Evans et al compared bronchodilator responses to 200-μg albuterol aerosols delivered from a pMDI to 19 asthmatic

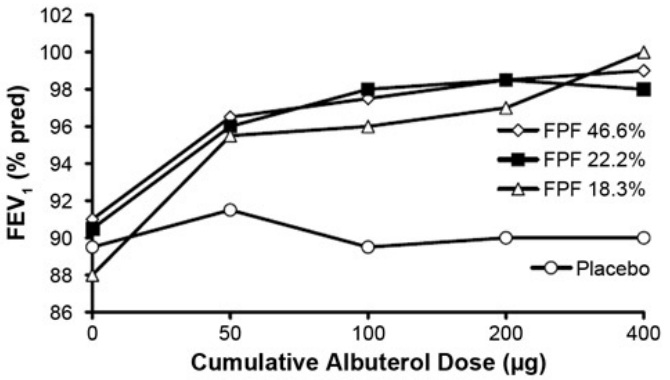

Figure 4. Mean cumulative response of FEV_1 to doses of albuterol in 3 formulations with different FPFs from Novolizer DPI in 12 asthmatic patients. From Weda et al.[57] FEV_1 indicates forced expiratory volume in 1 second; pred, predicted; FPF, fine particle fraction.

patients via 4 actuators with different orifice diameters (range 0.23-0.59 mm).[55] Significantly smaller particle sizes were found for narrower nozzles. There were significant differences in peak expiratory flow rate between the largest and smallest orifices, but no differences in FEV_1 or forced vital capacity.

In a study involving experimental powder formulations delivered from an in-house DPI, Srichana et al found that lung function responses did not correlate with the FPF, which ranged from 25% to 40%.[56] However, this negative finding may have resulted from the large albuterol dose (400 µg), which would probably have been at the top of the dose-response curve.

Weda et al undertook interesting studies involving experimental albuterol powder formulations delivered by Novolizer DPI to 12 asthmatics.[57] Three formulations with FPFs ranging from ~18% to 47% were given in cumulative doses beginning at 50 µg and finishing at 400 µg. All active treatments were more effective than placebo, but there were no significant differences in bronchodilator responses between active treatments, even at the lowest albuterol dose (Figure 4).

In a follow-up study, Weda et al used 3 further powder formulations (FPFs ~10%-51%) to deliver cumulative doses of albuterol to 6 healthy volunteers (lowest dose 400 µg, highest dose 1600 µg).[58] The highest dose was markedly larger than that likely to be used in routine clinical practice by patients with mild to moderate asthma. The objective of this study was to assess changes in side effects (fall in serum potassium and rise in heart rate). There were significant differences between active preparations, with the decrease in serum potassium correlating with both dose and FPF (Figure 5). The increase in heart rate was highest for the formulation with the highest FPF.

The 2 studies by Weda et al[57,58] appear to be the most useful of those in the primary data set, since they provide full APSD data that can be related to both efficacy and safety of inhaled albuterol in a quantitative manner. It was argued that for the albuterol formulations tested, therapeutic equivalence between formulations could be assumed providing that the 90% confidence interval for the ratio of fine particle doses fell within the range 0.5 to 1.2.[57,58] The lower end of this range was defined by the observation of equivalent efficacy when the fine particle mass of albuterol was doubled,[57] and the upper end of the range was based on analysis of the comparative side effect data.[58]

Other Inhaler Data

Many studies have compared the clinical effects of aerosols delivered from 2 dissimilar inhalers that happen to have different particle size distributions, for instance CFC pMDIs vs hydrofluoroalkane (HFA) pMDIs. While an HFA aerosol with reported mass median aerodynamic diameter (MMAD) 1.1 µm was shown to be more potent than a CFC aerosol with MMAD 3.5 µm,[59] the 2 products differed not only in APSD but also in other spray characteristics, including impaction force and spray temperature.[60] These differences between products make it problematic to establish the relationship between APSD per se and clinical response.

Two studies compared innovator and generic pMDI products with slightly different APSDs, finding no differences in clinical response.[61,62] However, both investigations used doses that were probably close to or at the top of the dose-response curve.

Many studies have compared the clinical effects of inhaled drugs delivered as nebulized aerosols of different sizes (Table 4).[63-73] These studies generally provided limited APSD data (eg, MMAD and geometric standard deviation, or FPM). Many of these studies involved bronchodilator aerosols in crossover studies in small (<20) groups of

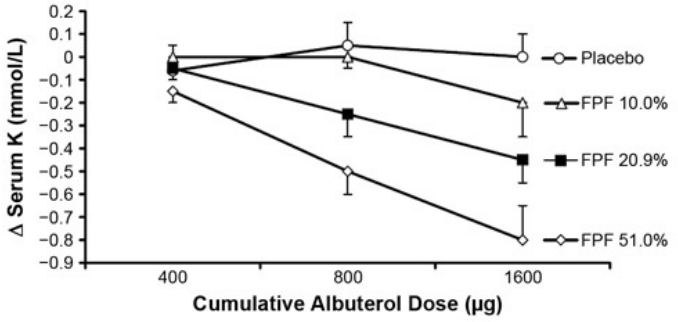

Figure 5. Mean + SEM changes in serum potassium (Δ serum K) following cumulative doses of albuterol up to 1600 µg, given as 3 formulations with differing FPFs, in 6 healthy subjects. From Weda et al.[58] FPF indicates fine particle fraction.

asthmatic patients, while 3 studies compared the effects of inhaled recombinant human DNase (rhDNase, Pulmozyme, Genentech) in much larger parallel groups of cystic fibrosis patients. In these studies, changes in APSD have been engineered by using different nebulizers and/or by changing the compressed gas flow rate used to drive the nebulizer. In further studies[63,64,74-77] there were changes between regimens not only in APSD but also in inhalation technique. APSD data for the nebulizers were generally obtained by laser light scattering[78] rather than by CI.

Most of the nebulizer studies listed in Table 4 were conducted to answer a practical and clinically important question: what is the clinical response when different nebulizers are operated in different ways? The studies were not designed to examine the relationship between APSD and clinical response. It is difficult to assess the nature of this relationship, owing to the presence of confounding variables described above, plus differences in volume fill, nebulization time, and drug concentration. In addition, most nebulizer studies probably used drug doses at or near the top of the dose-response curve, and most were probably underpowered. Even without these difficulties, it could be challenging to extrapolate data linking APSD and clinical response from nebulizers to other inhalers, especially pMDIs.

Monodisperse Pharmaceutical Aerosol Studies

Clinical effects of monodisperse bronchodilator aerosols of differing particle sizes, produced by a spinning disk generator, have been investigated. In 3 studies, monodisperse bronchodilator aerosols of diameters 1.5 μm, 2.8 μm, and 5.0 μm were compared.[79-81] These articles suggest that the optimal bronchodilator particle size in mild asthmatics is ≤ 3 μm and in severe asthmatics is around 3 μm.

Studies with a similar protocol were undertaken in mild to moderate asthmatics,[82,83] using monodisperse albuterol aerosols of 1.5 μm, 3 μm, and 6 μm. The results of the first of the investigations, by Usmani et al,[83] differed from those of Zanen et al,[79-81] because bronchodilator response was found to be higher for 3 μm and 6 μm aerosols than for the 1.5 μm aerosol. However, in a follow-up study, bronchodilator response increased with increasing particle size at an inhaled flow rate of 30 L/min but decreased with increasing particle size at an inhaled flow rate of 67 L/min (Figure 6).[83]

Monodisperse aerosols provide the cleanest data linking particle size to response, yet paradoxically they may tell us only a little about the relationship between APSD from inhaler devices and clinical effects. Monodisperse aerosols of a fixed and uniform size differ markedly from those released from inhaler devices, which are polydisperse and may change in size through processes such as liquid evaporation and powder deaggregation. Therefore, it is necessary to use caution when extrapolating the findings of studies using monodisperse aerosols to inhaler devices.

Discussion of Data Review

It is striking how few studies have been designed with the primary objective of investigating the relationship between APSD of pharmaceutical aerosols and clinical response. While many of the studies described in this review are excellent, they are of limited help in the quest to understand this relationship, owing to the presence of confounding variables, the use of doses near the top of the dose-response curve, and the lack of full APSD data. The clinical responses are often highly variable, and yet studies generally involve only small groups of patients. The 3 rhDNase nebulizer studies are a notable exception.[71-73] It has been observed elsewhere[84] that underpowering of clinical studies comparing inhaler devices is common. It is interesting to speculate about whether the relationships between APSD and clinical response in the primary data set studies would have been any clearer if the APSD data had been obtained in a manner more closely simulating patient use—for instance, using an impactor inlet based on a human oropharyngeal model.[33-35]

There can be no doubt that APSD influences the clinical response to inhaled drugs, and the reviewed studies confirm this. However, even for well-established drugs such as inhaled bronchodilators, it is still poorly understood how large a change in APSD has to be, before it becomes clinically relevant. It is to be expected that the relationship between APSD and clinical response will depend on the type of drug being delivered and perhaps also on the type of inhaler, the nature and severity of the lung disorder (eg, asthma vs chronic obstructive pulmonary disease, and mild vs severe obstructive airways disease), and the inhaled flow rate. There are few data comparing the side effects of pharmaceutical aerosols administered in different sizes. The regulatory authorities generally require APSD data to fit within certain specifications, and with the advent of the regulatory paradigm of quality by design, there is an added requirement that these specifications, or the design space for APSD, be clinically relevant.[85] However, the literature provides limited help in setting such specifications.

In the future, some companies may consider it helpful to undertake a study allowing estimation of the change in APSD from their product that is clinically significant. Some of the issues highlighted in this review could be used to guide the design of such a study. These issues include ensuring that a study has adequate power to detect a clinically relevant difference in response between 2 aerosols, and avoids confounding variables. Studies should compare doses both on the slope and at the top of the dose-response curve, as such studies provide complementary data pertinent to a clear

Table 4. Nebulizer Studies Where Aerosols of Different Sizes Have Been Compared Without a Change in Inhalation Technique*

Nebulizers	Drug and Dose	Particle Sizes	Study Population	Results	Reference
Not stated	Cumulative doses of fenoterol; maximum dose 40 μg to right lung	MMADs 0.55 and 2.4 μm	6 asthmatics	Trend toward better response for smaller aerosol	[63]
Wright nebulizer (7 L/min) vs DeVilbiss 40 (6 L/min) vs Bennett Twin (7 L/min)	Methacholine in varying concentrations	MMADs 1.3-3.6 μm	8 asthmatics	2.7-fold difference in MMAD did not significantly alter response	[64]
Sandoz nebulizer at 4, 6, and 8 L/min	Albuterol, 1 mg	MMDs 11-17 μm	10 asthmatics	No differences in responses between regimens	[65]
Inspiron nebulizer at 4 and 8 L/min	Rimiterol, cumulative to 8 mg	MMDs 4- 11 μm	8 asthmatics	No differences in responses between regimens	[66]
Inspiron nebulizer at 4 and 8 L/min	Albuterol, 1 mg and 5 mg	MMDs 4-11 μm	40 asthmatics	No differences in responses between 4 regimens	[67]
Two nebulizer/ compressor combinations	Albuterol, aiming to achieve lung doses 20-250 μg	MMADs 1.4 vs 5.5 μm; FPFs 94% vs 50%	8 asthmatics	Identical responses to both regimens	[68]
Turret (8 L/min) vs Upmist (6 L/min) vs Inspiron (4 L/min)	Terbutaline sulfate, 2.5 mg	MMDs 1.8-10.3 μm	7 asthmatics	Smallest aerosol gave statistically significantly better improvement in tests of small airways function than 2 larger aerosols	[69]
Turret (12 L/min) vs Inspiron (6 L/min)	Albuterol, cumulative to 2 mg; ipratropium bromide, cumulative to 400 μg	MMDs 3.3 and 7.7 μm	8 asthmatics	Albuterol: 3.3 μm aerosol gave statistically significantly higher response Ipratropium bromide: no differences in response between regimens	[70]
Three nebulizer/ compressor combinations	RhDNase, 2.5 mg twice daily for 15 days	FPFs 48%-57%	397 cystic fibrosis patients randomized to 3 groups	Similar improvements in lung function for each regimen	[71]
Two nebulizer/ compressor combinations	RhDNase, 2.5 mg daily for 7 days	MMDs 6.9 and 3.4 μm; FPFs 35% and 71%	173 cystic fibrosis patients randomized to 2 groups	Similar improvements in lung function for each regimen	[72]
Two nebulizer/ compressor combinations	RhDNase, 2.5 mg daily for 14 days	MMDs 4.9 and 2.1 μm; FPFs 50% and 83%	749 cystic fibrosis patients randomized to 2 groups	Study powered to detect 50% difference in response between regimens; difference in response between regimes was statistically significant for forced vital capacity ($P = 0.03$) but not for forced expiratory flow in 1 second ($P = 0.06$)	[73]

*Studies were crossover in design except where stated. MMADs indicates mass median aerodynamic diameters; MMDs, mass median diameters; FPFs, fine particle fractions.

understanding of the relationship between APSD and clinical outcome. While assessment of response to bronchodilators in studies of short duration is relatively straightforward, assessing response to other drugs, including inhaled corticosteroids or systemically acting peptides, is more difficult and may require studies of significantly greater complexity. This partly explains why there are few published data linking APSD to the clinical effects of these drugs. It is important to

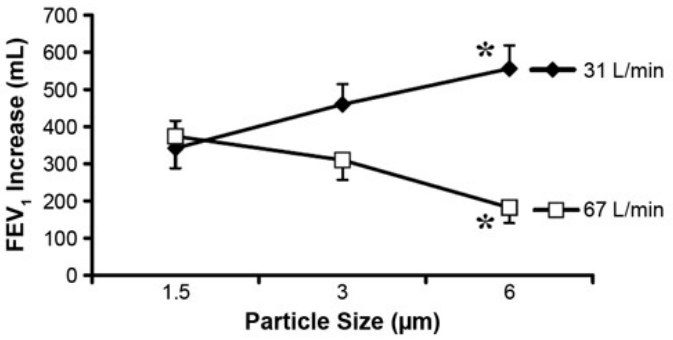

Figure 6. Mean + SEM changes in FEV_1 following inhalation of 1.5 μm, 3 μm, and 6 μm monodisperse albuterol aerosols at slow (31 L/min) and fast (67 L/min) inhalation rates. From Usmani et al.[83] FEV_1 indicates forced expiratory volume in 1 second. Asterisk (*) indicates a statistically significant difference at $P = 0.001$ exists between the measurements with 6 μm particles.

remember that in clinical practice, response will be dependent not only on APSD but also on inhaler technique, patient education, and adherence to therapy.[86] As 1 clinical study concluded, the CI method "offers a guide to clinical response and does not predict it accurately."[55]

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