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An investigation into the predictive value of cascade impactor results for side effects of inhaled salbutamol

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Abstract

The aim of this study was to compare the Multistage Liquid Impinger (MSLI) and the Andersen Cascade Impactor (ACI) with respect to their power to predict differences in side effects of salbutamol delivered by a dry powder inhaler. Three preparations with the same nominal dose and the same inhaler device but generating aerosols with different aerodynamic particle size distributions were administered to six healthy volunteers in a randomized, placebo-controlled, four-way crossover study. Cumulative doses from 400 up to 1600 μg were given. The serum potassium level (K^+-serum) and the heart rate (HR) were measured at baseline and 15 min after each dose. Both the MSLI and ACI showed large differences between the aerodynamic particle size distributions of the three preparations. The decrease in K^+-serum revealed significant differences between the three active preparations and was significant for doses of 800 μg and higher. The HR results showed differences between the active preparations only at a nominal dose of 1600 μg and only for the preparation with the highest fine particle dose (FPD) compared to the other two preparations. The K^+-serum appears to be a more sensitive measure for side effects than the HR. In vivo-in vitro correlations (IVIVCs) were established between the amounts of salbutamol deposited on the various cumulative impactor stages and the K^+-serum. The best IVIVCs were obtained in the FPD range, resulting in correlation coefficients of at least 0.78. It is concluded that cascade impactor results in the FPD range of the MSLI as well as the ACI correlate well with the K^+-serum. Cascade impactor analysis thus provides a clinically meaningful tool in the development and the quality control of salbutamol inhalation powders.
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1. Introduction

Cascade impactors are widely used in the development and the quality control of medicinal products for

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inhalation and are also proposed for the bridging of small changes in the composition and/or manufacturing process (Weda et al., 2000, 2002). Cascade impactors measure the aerodynamic particle size distribution of the aerosol cloud generated by the product yielding information about the mass fraction that has the potential to enter the deeper part of the lung.

Currently two multistage impactors are described in the European Pharmacopoeia (EP), namely the Multistage Liquid Impinger (MSLI) and the Andersen Cascade Impactor (ACI) (European Pharmacopoeia, 2003). Despite the widespread use of these apparatus, it is yet unknown whether they are capable of detecting clinically relevant differences between two formulations or between batches of the same formulation. Various studies have shown that the aerodynamic particle size distribution of aerosols generated by inhalation products correlates with the amount of drug deposited in the lungs (Laube et al., 1998; Leach, 1998; Seale and Harrison, 1998; Silkstone et al., 2002). Other studies indicate that there is a significant relationship between the amount of drug deposited in the lungs and the pulmonary effects of preparations for inhalation (Pauwels et al., 1997). So, there is evidence that cascade impactor results are correlated with efficacy, albeit indirectly. Moreover, most studies refer only to efficacy and not to safety, i.e., side effects.

In a previous study, we evaluated the capability of impactors to predict differences in the efficacy of inhaled salbutamol (Weda et al., 2002). Three inhalation powders with the same nominal dose and the same inhaler device but generating aerosols with different aerodynamic particle size distributions were administered to asthmatic patients. We showed that despite large differences in the cascade impactor results between the three preparations no clinically relevant differences in FEV_1 were observed in a cumulative dose range of 50–400 μg. This suggests that rather large differences in aerodynamic particle size distribution of polydisperse aerosols do not necessarily result in differences in efficacy.

In contrast to our previous study which focussed on efficacy, the current investigation is dedicated to side effects. The purpose of this study was to compare the Multistage Liquid Impinger (MSLI) and the Andersen Cascade Impactor (ACI) with respect to their power to predict differences in side effects of salbutamol inhalation powders. To the best of our knowledge, our study is the first to directly correlate the aerodynamic particle size distribution of preparations for inhalation with safety data.

2. Materials and methods

2.1. Preparations

All three inhalation powders contained 400 μg salbutamol base per actuation. Two preparations were adhesive mixtures of salbutamol and lactose. They were assigned as coarse (COARSE) and intermediate (INTER), because the aerosol generated by the inhaler with these formulations had large and intermediate aerodynamic particle sizes respectively. One powder preparation consisted of spherical pellets and was assigned as fine (FINE), because with this powder the inhaler generated an aerosol with the smallest particles.

The same batch of micronised salbutamol sulphate (X_{90} = 3.7 μm, X_{50} = 1.7 μm, and X_{10} = 0.7 μm) was used as drug substance for all three preparations and was a gift of Genfarma B.V., The Netherlands. Sieve fractions from lactose monohydrate 80 Mesh (250–315 μm) and 100 Mesh (90–106 μm) were applied as carrier excipient for COARSE and INTER respectively. Micronised lactose (X_{50} = 3.4 μm) was used as excipient in the preparation of FINE. All lactose types were a gift of DMV International, The Netherlands. The particle size distributions of micronised salbutamol sulphate and micronised lactose were measured by a Sympatec HELOS laser diffraction apparatus, using a RODOS dry powder dispenser (Sympatec GmbH, Germany).

COARSE and INTER were prepared by mixing lactose and salbutamol sulphate in a stainless steel container of 160 cm^3 during 10 min in a tumbling mixer at 90 rpm (Turbula T2C, W.A. Bachofen AG, Switzerland). Batches of 25 g adhesive mixture containing 3.7% salbutamol sulphate were prepared. FINE was manufactured by homogenising, densification and pelletising of the salbutamol and micronised lactose blend in the same stainless steel mixing container (10 min at 90 rpm), using stainless steel beads with a well defined size distribution as pelletising aid. Next, the fines were removed and the remaining pellets were spheronised on a 200 μm vibratory sieve for 20 min (Fritsch

Analysette 3, Fritsch GmbH, Germany). Finally, the pellets were classified into different size fractions by mild hand sieving; the fraction 315–630 micron was used for the experiments. A batch of 20 g pellets containing 7.4% salbutamol sulphate was prepared.

All three preparations were filled into a bulk container of a Novolizer® multi-dose dry powder inhaler having an intermediate air flow resistance of 0.028 $kPa^{0.5}\,min\,l^{-1}$ (Viatris GmbH, Germany) (Berner et al., 1998; Fenton et al., 2003). A placebo was prepared from lactose monohydrate 80 Mesh. Before release by the responsible pharmacist, the quality of the preparations was checked by the Centre for Quality of Chemical-Pharmaceutical Products of the National Institute for Public Health and the Environment, The Netherlands. The dose uniformity of all preparations was amply within USP and EP standards, with relative standard deviations of 0.4–3.0%. The preparations were stored at 18° C and 32% relative humidity.

2.2. Impactors

The aerodynamic particle size distribution of the active preparations was measured with the MSLI and the ACI, both described in the EP. The USP throat was used for both impactors. For the ACI a preseparator was added atop of stage 0.

The MSLI consists of four impaction stages and a final collection filter (Gelmann A/E glass microfibre filter). The four impaction stages were kept moist with 20 ml methanol containing 20 μl phosphoric acid 85% (m/m) and 10.7 μg fenoterol hydrobromide per millilitre as internal standard. For the MSLI a pressure drop across the inhaler of 4.0 kPa was applied, resulting in a flow rate of 80 l/min for the MSLI.

The ACI consists of eight stages and a final collection filter (Gelmann A/E glass microfibre filter). Unlike the MLSI the stages were kept dry, but were coated with 2% viscous oil in hexane. The preseparator contained 10 ml methanol with 20 μl phosphoric acid 85% (m/m) and 10.7 μg fenoterol hydrobromide per millilitre as internal standard. For the ACI the maximum achievable flow rate of 60 l/min was used, corresponding to 2.5 kPa across the inhaler.

The operating conditions and theoretical cut-off diameters at the flow rates used are shown in Table 1 (European Pharmacopoeia, 2003; Nichols, 2000).

Table 1
Operating conditions and characteristics of the impactors

	MSLI	ACI
Flow rate (l/min)	80	60
Time per actuation (s)	3	4
Volume per actuation (l)	4	4
Cut-off diameter (μm)		
Stage 0		5.9
Stage 1	11.3	4.1
Stage 2	5.9	3.2
Stage 3	2.7	2.1
Stage 4	1.5	1.4
Stage 5	–[a]	0.62
Stage 6		0.35
Stage 7		0.15
Stage 8		–[a]

[a] Stage 5 of the MSLI and stage 8 of the ACI are the final collection filters.

2.3. In vitro measurements

For each preparation, two actuations were delivered to the impactors. The amount of salbutamol deposited in the mouthpiece of the Novolizer® inhaler (M), mouthpiece adaptor (MA), impactor throat (T), and the various impactor stages were determined with HPLC using a 125 mm × 4 mm Hypersil BDS 5 μm C18 column with guard column, a detection wavelength of 278 nm and methanol:water 15:85 (v/v) as eluent. This method was validated with respect to accuracy, precision, linearity, range, quantification limit and detection limit (0.15 and 0.05% of the nominal dose, respectively), and proved to be suitable according to internal GLP standards. The in vitro measurements were performed in triplicate.

The amounts deposited at the various stages were expressed as μg salbutamol base per actuation. The fine particle dose (FPD) was calculated, defined as the amount of drug deposited on the stages below the stage with the cut-off diameter with the closest value to 5.0 μm, i.e., stage 3–5 of the MSLI and stage 1–8 of the ACI.

For each preparation the mass median aerodynamic diameter (MMAD) and the geometric standard deviation (GSD) were calculated.

2.4. In vivo measurements

Six healthy volunteers (four female, two male) participated in the study. The average (± S.D.) age was

37 ± 14 years. All subjects were non-smokers and did not have any history of asthma and/or COPD. The study was approved by the Ethic Committee of the St. Antonius Hospital Nieuwegein, The Netherlands. All subjects gave their written consent to participation.

The in vivo study followed a randomised, placebo-controlled, four-way crossover design. On four separate sessions with intervals of one week, the three active preparations and the placebo were administered with the Novolizer®. At each session, subsequent doses of 400, 400 and $2\mu g \times 400\,\mu g$ were administered with a dosing interval of 20 min. Before starting each session, the subjects were given detailed inhalation instructions. The inhalation manoeuver consisted of a deep and forceful inhalation, followed by a breath-holding period of 10 s. The inhalation flow through the Novolizer® was recorded by measuring the pressure drop over the inhaler, from which the flow was calculated. A peak inspiratory flow of >60 l/min and an inhalation time of >2 s through the Novolizer® was necessary in order to start a session.

The heart rate (HR) and the serum potassium level (K^+-serum) were measured at baseline and 15 min after each dose of salbutamol. The HR was determined by manual counting over a period of 60 s. The K^+-serum was measured by taking blood samples from a cannula in an antecubital fossa vein, and subsequent analysis by means of ion selective chromatography.

2.5. Statistical analysis

For the three active preparations, the significance of differences between the amounts of salbutamol deposited at the various stages of the MSLI as well as the ACI was determined with analysis of variance, using NCSS for Windows (Number Cruncher Statistical Systems, Kaysville, Utah, USA). The same was done for the FPD. The absence of differences was chosen as H_0-hypothesis.

Dose-effect curves of the three active preparations and the placebo were constructed. These curves were analyzed by repeated measurements analysis of variance for preparation and dose differences, and for a dose by preparation interaction using SPSS for Windows (Statistical Products and Service Solutions Inc., Chicago, Illinois, USA). The absence of differences and the absence of a dose by preparation interaction was chosen as H_0-hypothesis.

For each 400 μg preparation, cumulative amounts of salbutamol deposited in the impactor stages were calculated, starting at M up to the final collection filter, subsequently MA up to the final collection filter, then T up to final collection filter, etc. The differences in K^+-serum versus baseline value (delta K^+-serum) as well the HR versus baseline value (delta HR) were calculated for the 400, 800, and 1600 μg dose. In vivo–in vitro correlations (IVIVCs) were developed by plotting the mean amounts of salbutamol of all three preparations at all three dose levels against the individual delta K^+-serum data. This was done for all cumulative stages, for both impactors. Least squares regression analysis was used to determine the slope, intercept, and correlation coefficient of these relationships, using NCSS. The H_0-hypotheses of the correlation being zero and the intercept being zero were investigated.

In all statistical tests, an α value of 0.05 was considered to be significant.

3. Results

3.1. In vitro results

For the MSLI and ACI, the complete deposition pattern of the preparations is presented in Figs. 1 and 2, respectively. For the MSLI, none of the three preparations were significantly different from one another regarding the amounts of salbutamol deposited in the mouthpiece adaptor and stage 5. For the ACI, no significant difference was seen in the mouthpiece of the inhaler and between INTER and FINE at stage 7 and 8 of the ACI. At all other impactor stages, significant differences were observed between the three preparations with the MSLI as well as with the ACI ($P < 0.05$).

The FPD (mean ± S.D.) obtained with the MSLI was $39.8 \pm 1.8\,\mu g$, $83.5 \pm 10\,\mu g$, and $204 \pm 7.3\,\mu g$ for COARSE, INTER, and FINE, respectively. The FPD measured with the ACI, was $40.7 \pm 0.2\,\mu g$, $144 \pm 1.9\,\mu g$, and $321 \pm 16\,\mu g$, respectively. For both impactors, the differences in FPD between the three preparations are significant ($P < 0.001$).

The MMAD (GSD) of the three preparations were 6.8 μm (2.6), 3.4 μm (3.1), and 1.7 μm (2.2) for COARSE, INTER, and FINE, respectively.

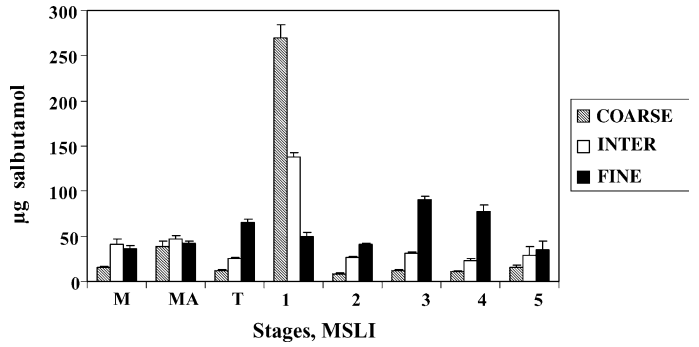

Fig. 1. Deposition pattern of the three preparations, obtained in triplicate with the MSLI, including standard deviations. M, mouthpiece of the inhaler device; MA, mouthpiece adaptor; T, impactor throat.

3.2. In vivo results

All volunteers completed the four sessions. The dose-effect curves for the delta K^+-serum and the delta HR are shown in Figs. 3 and 4, respectively. The dose by preparation interaction was significant for both parameters ($p < 0.001$), indicating that the dose-effect curves for the placebo and the active preparations did not run parallel.

Statistical evaluation of the K^+-serum data showed significant differences between placebo and the three active preparations ($p < 0.02$). The difference between the three active preparations was also significant ($p < 0.03$). The dose-effect evaluation revealed that the K^+-serum decrease from baseline was significant starting from the 800 μg dose ($p < 0.05$).

Statistical evaluation of the HR results showed significant differences between the placebo and the three active preparations ($p < 0.001$), but not between COARSE and INTER. The dose-effect evaluation showed that the increase in HR was only significant at the 1600 μg dose level ($p < 0.01$).

3.3. In vivo–in vitro correlations

The IVIVCs between the cascade impactor, results and the changes in K^+-serum are given in Tables 2 and 3, respectively, including the correlation coefficients and the parameters describing the regression lines. The intercept did not differ significantly from zero for the K^+-serum regression lines, with the exception of the IVIVC obtained for stage 5 of the

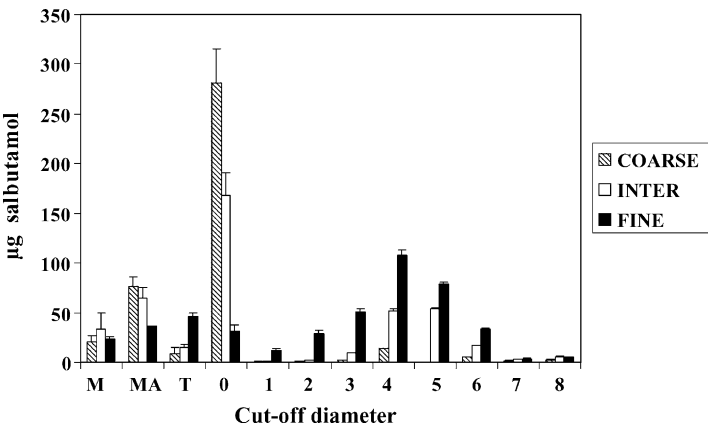

Fig. 2. Deposition pattern of the three preparations, obtained in triplicate with the ACI, including standard deviations. M, mouthpiece of the inhaler device; MA, mouthpiece adaptor; T, impactor throat.

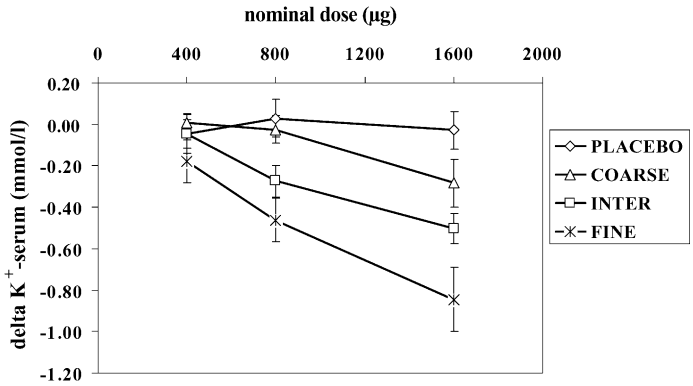

Fig. 3. K^+-serum 15 min after administration of nominal doses of 400, 800, and 1600 μg placebo and three salbutamol inhalation powders with different aerodynamic particle size distribution, including standard errors of the mean. The K^+-serum is expressed as difference vs. baseline values.

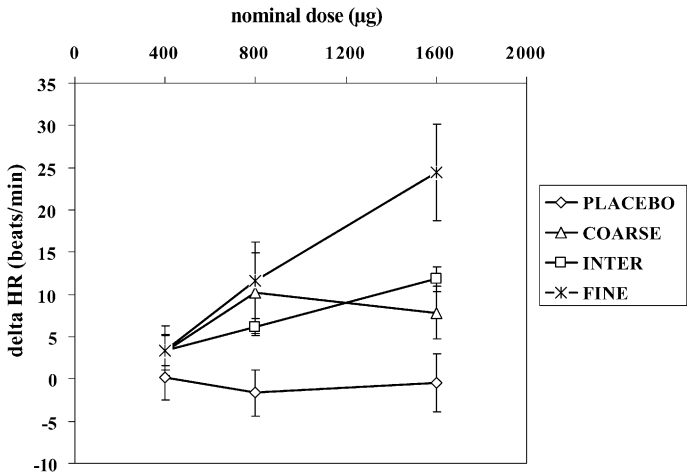

Fig. 4. HR 15 min after administration of nominal doses of 400, 800, and 1600 μg placebo and three salbutamol inhalation powders with different aerodynamic particle size distribution, including standard errors of the mean. The HR is expressed as difference vs. baseline values.

Table 2
Slope, intercept, and correlation coefficients (r) of the IVIVCs between the amount of salbutamol deposited on the various impactor stages of the MSLI and delta K^+-serum

Stages	Intercept	Slope ($\times 10^{-4}$)	r
M up to 5	0.119	−4.38	−0.660
MA up to 5	0.104	−4.56	−0.638
T up to 5	0.099	−5.10	−0.634
1 up to 5	0.051	−4.96	−0.553
2 up to 5	0.012	−9.82	−0.803
3 up to 5	0.003	−11.7	−0.798
4 and 5	0.032	−21.9	−0.808
5	0.126	−67.2	−0.791

MSLI ($p < 0.03$). For all IVIVCs, significant correlations were seen ($p < 0.0001$).

4. Discussion

For the in vitro experiments, the two multistage impactors described in the EP were used, i.e., the MSLI and the ACI. The MSLI was fully operated according to the instructions for dry powder inhalers included in the EP, applying a pressure drop of 4.0 kPa across the inhaler. Due to the relatively high resistance of the ACI, it was not possible to obtain a pressure drop of 4.0 kPa.

Table 3
Slope, intercept, and correlation coefficients (r) of the IVIVCs between the amount ofsalbutamol deposited on the various impactor stages of the ACI and delta K^+-serum

Stages	Intercept	Slope ($\times 10^{-4}$)	r
M up to 8	0.107	−3.83	−0.633
MA up to 8	0.105	−4.06	−0.632
T up to 8	0.131	−5.07	−0.684
0 up to 8	0.111	−5.16	−0.642
1 up to 8	−0.016	−7.17	−0.795
2 up to 8	−0.012	−7.45	−0.796
3 up to 8	−0.001	−8.28	−0.800
4 up to 8	0.015	−10.1	−0.804
5 up to 8	0.033	−19.0	−0.805
6 up to 8	0.035	−55.7	−0.811
7 and 8	0.107	−242	−0.787
8	0.096	−382	−0.775

The maximum achievable flow rate of 60 l/min was therefore used. This difference in flow rate between the MSLI and ACI is not considered relevant, because the purpose of the in vitro study was not to compare the aerodynamic particle size distributions obtained with the MSLI and ACI, but to compare the three preparations within one impactor and to evaluate the capability of both impactors to predict clinically relevant differences between these preparations.

For the in vivo study, a starting dose of 400 μg was used, since it is expected that below this dose the K^+-serum and HR will not noticeably be influenced (Kleerup et al., 1996; Seppala et al., 2001). The maximum dose was set at 1600 μg, because this is the maximum daily dose registered in the Netherlands. The K^+-serum level and the HR were selected as response parameters for safety, reflecting the systemic β_2-response of salbutamol. The study has not been carried out with asthmatic patients, but with healthy volunteers, because there are indications regarding the development of tolerance with respect to β_2-responses in asthmatic patients when using β_2-agonists during longer periods (Hancox et al., 2002; Woude et al., 2001). However, the development of tolerance is not applicable to asthmatic patients using a β_2-agonist for the first time. If tolerance is an issue, any differences in systemic β_2-response detected with healthy volunteers will therefore also be relevant for asthmatic patients in general. The discriminatory power of the study was thus maximized by selecting healthy volunteers.

The in vivo data show that the three preparations are significantly different with respect to the decrease in K^+-serum. On the other hand, COARSE and INTER do not produce differences in HR. The difference in HR with FINE is significant, but only a dose of 1600 μg results in a considerable HR increase. At 800 μg, the difference in HR versus baseline is below 10 bpm for all three preparations. Apparently, the HR is a less sensitive parameter for safety evaluation of salbutamol inhalation powders. This is confirmed by other studies (Janssen et al., 2002; Fowler and Lipworth, 2001). Summarizing the in vivo data, we conclude that differences in the aerodynamic particle size distribution of the aerosols generated from different formulations result in significant differences in side effects of salbutamol. The clinical relevance of impaction data for the development and the quality control of salbutamol inhalation powders with respect to side effects is thus confirmed. Furthermore, the decrease in K^+-serum appears to be a more sensitive parameter to detect differences in safety. For determination of the IVIVCs only this parameter is therefore taken into account.

The in vitro results show large differences between the three active preparations with both the MSLI and ACI. The results of the adhesive mixtures are in line with other studies investigating the effect of carrier size on the dispersion of salbutamol sulphate (Zeng et al., 2000; Louey et al., 2003). Smaller lactose particles result in higher fine particle doses of salbutamol sulphate.

The relationship between the impactor measurements and the K^+-serum was investigated by determination of IVIVCs. Cumulative amounts of salbutamol deposited on the impactor stages were plotted against the K^+-serum and the correlation coefficients were calculated. For the construction of these IVIVCs, the assumption was made that the amount of salbutamol absorbed via the gastrointestinal tract was negligible for all three preparations, since this fraction could theoretically contribute to the systemic side effects. It is known that after inhalation of salbutamol the fraction absorbed from the gastrointestinal tract contributes only 0.3% to the overall bioavailability over the first 30 min post inhalation (Chrystyn et al., 1996). Since all three salbutamol doses were administered within 40 min and each session was completed within 60 min, our assumption is reasonable. The data of all three preparations and all three dose levels were therefore combined in one plot. The results given in Tables 2 and 3 show a breaking

point for the IVIVCs at stage 2 of the MSLI (cut-off diameter 11 μm) and at stage 1 of the ACI (cut-off diameter 5.9 μm), with correlation coefficients higher than 0.78.

It is generally accepted that particles with an aerodynamic diameter below 5–7 μm have the highest probability of reaching the deep or peripheral lungs (Heyder et al., 1986). In this respect, it is remarkable that a good correlation is obtained with the cumulative amount of drug on the stages 2–5 of the MSLI, since particles between 7–11 μm are included in this range. It is however conceivable that for the preparations used in our study the amount of salbutamol deposited at stage 2 mostly consists of particles with an aerodynamic diameter that approaches the cut-off diameter of 5.9 μm of this stage. This explanation is supported by the fact that the micronised salbutamol sulphate batch used for the production of the preparations contains more than 98% particles smaller than 6.0 μm. If a larger fraction of particles with a size close to 11 μm would be present, the correlation coefficient might be less favourable. In view of the broad range of particles that are likely to deposit at stage 2 and taking into account the fact that only particles below 5–7 μm will reach the lungs, it is sensible not to use stage 2–5 for comparison of different formulations of a product or for quality control of product batches. In addition, the advantage of including the mass on stage 2 is negligible in terms of a higher correlation coefficient compared with stage 3–5. So, for salbutamol the FPD range is the most relevant impactor part for comparison of systemic side effects.

Unexpectedly, no difference in the correlation coefficients is seen between the various impactor stages within the FPD range. The MMAD of the preparations decreases in the ranking order of COARSE > INTER > FINE. When particles get smaller the deposition at the alveoli increases at the expense of the bronchial deposition (Heyder et al., 1986). In view of the large surface area of the alveolar epithelium, the thin air-blood barrier, and the high blood flow, it is conceivable that the alveolar fraction will be rapidly absorbed into the systemic circulation. This fraction no longer contributes to the desired clinical effect, but may cause systemic side effects. Indeed, efficacy studies with monodisperse aerosols indicate that the size of the particles should not be too small (Zanen et al., 1994; Usmani et al., 2003). A safety study comparing monodisperse fenoterol particles of 2.8 μm and a pMDI with valved holding chamber showed that the latter preparation caused more systemic side effects, suggesting that the very small particles (0.5–2 μm) of the polydisperse pMDI could be responsible for this difference (Zanen and Lammers, 1999). Our data demonstrate that a lower MMAD results in more systemic side effects of salbutamol. The fact that within the entire FPD range the same correlation coefficient is found, is probably due to the polydisperse nature of the preparations. Taking into consideration the GSD there will be a considerable overlap in the particle size distributions. It is possible that a comparable study with monodisperse aerosols would reveal a better correlation for the lower impactor stages compared to the upper stages. Our correlation data are therefore only valid for polydisperse aerosols. It is remarked that all salbutamol products that are currently on the market are polydisperse.

The results of this study also allows us to consider the definition of the FPD. The EP requires the FPD to be calculated by interpolation as the mass of active ingredient less than 5.0 μm from the cumulative mass versus stage cut-off diameter plot. Our results show no clinical evidence that the exact definition adopted in the EP is necessary for development and quality control, since the correlation coefficients of our IVIVCs do not change much around 5.0 μm. We, therefore conclude that our definition of the FPD as being the amount of drug deposited on the stages below the stage with the cut-off diameter with the closest value to 5.0 μm is adequate.

Summarizing the in vivo and in vitro data, it is concluded that measuments obtained with the MSLI as well as the ACI are clinically relevant with respect to systemic side effects of inhaled salbutamol. The FPD range is the most suitable impactor fraction to investigate.

5. Conclusion

We showed that impactors are capable of predicting clinically relevant differences in systemic side effects between salbutamol inhalation powders. Good IVIVCs were found in the FPD range for both the MSLI and the ACI. The MSLI and ACI are equally predictive. In a previous study the same impactors were shown to be overdiscriminating with respect to efficacy, because large differences in the aerodynamic particle size dis-

tribution did not lead to clinically relevant differences in efficacy. The value of these apparatuses for the development and the quality control as well as the bridging of post-approval changes in the formulation and/or production of salbutamol inhalation powders is thus confirmed.

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