Speed of onset of bronchodilator response to salbutamol inhaled via different devices in asthmatics: a bioassay based on functional antagonism

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Aims

To evaluate the speed of onset of bronchodilation following salbutamol administered via a metered-dose inhaler with a spacer (pMDI + Volumatic) and a dry-powder inhaler (Diskus), as well as the relative potencies of these devices in asthmatic patients with methacholine-induced bronchoconstriction.

Methods

Eighteen patients inhaled methacholine (MCh) until FEV₁ decreased by 35% of control. Following administration of placebo, 200 µg salbutamol or 400 µg salbutamol through the pMDI + Volumatic or the Diskus, we calculated the time elapsed from drug administration and the appearance of a 90% increase in post-MCh forced vital capacity (FVC), FEV₁ and volume-adjusted mid-expiratory flow (recovery times). The salbutamol doses to be delivered by the two inhalation devices to achieve similar recovery times and the relative potencies of the devices were calculated by using the 2-by-2 Finney parallel regression method.

Results

For all functional variables, recovery times were significantly ($P < 0.01$) shorter in pMDI + Volumatic than Diskus trials. The salbutamol doses to be delivered by the Diskus to achieve recovery times for FVC, FEV₁ and volume-adjusted mid-expiratory flow similar to those obtained with 200 µg salbutamol administered via the pMDI + Volumatic were 558 (95% CI 537, 579) µg, 395 (95% CI 388, 404) µg and 404 (95% CI 393, 415) µg, respectively, and corresponded to relative potencies of 2.79 (95% CI 2.68, 2.90), 1.98 (95% CI 1.94, 2.02), and 2.02 (95% CI 1.96, 2.07).

Conclusions

Administration of salbutamol via the pMDI + Volumatic provides faster reversal of induced bronchoconstriction than via the Diskus. The salbutamol dose targeting the lungs with the pMDI + Volumatic is approximately twice that with the Diskus.

Introduction

Short-acting β_2 -adrenoceptor bronchodilators are widely used for the treatment of acute asthma attacks [1]. Compared with oral administration, inhaled bronchodilators provide a more rapid onset of action, greater

airway response and reduced risk of systemic side-effects [1]. The pressurized metered-dose inhalers (pMDIs) and dry-powder inhalers (DPIs) are commonly used for administering β_2 -adrenergic bronchodilators, such as salbutamol. Studies performed in asthmatic

patients with spontaneous [2] or induced bronchoconstriction [3] have shown that salbutamol administration via a DPI or a pMDI in conjunction with a spacer provides faster bronchodilation than the pMDI alone. Whether a difference exists in the speed of the bronchodilator response to bronchodilator agents administered via either a DPI or a pMDI with a spacer in asthmatic patients with natural or induced bronchoconstriction remains to be established. Rapid onset of action is an important feature of bronchodilator drugs in patients with asthma, as prompt relief from bronchoconstriction will give reassurance of effect and may be a key factor in patient compliance [1]. Furthermore, because rapidity of action of inhaled bronchodilators depends on the amount of drug delivered to the appropriate receptors in the airways [4], it is important that clinicians know whether different inhalation devices, such as the DPI and the pMDI with a spacer, are bioequivalent, i.e. if they are able to deliver essentially the same drug amount to the relevant receptor.

One of the most commonly used methods for the *in vivo* assessment of bioequivalence between different formulations (e.g. powdered or aerosolized) of the same bronchodilator drug is based on the measurement of the degree of bronchodilation in patients with spontaneous bronchospasm. With this method, however, the results are often difficult to interpret, especially in patients with different degrees of baseline airway obstruction. To overcome this problem, some authors have evaluated the magnitude of bronchodilation in patients with mild degrees of induced airway narrowing [3, 5], usually corresponding to a 20% decrease in baseline FEV₁. In such experimental conditions, however, even low doses of bronchodilators are likely to result in near maximal bronchodilation [6–9], so that differences in the magnitude of responses to different agents or to varied formulations of the same agent may be difficult to detect.

Evidence from *in vitro* [10] and *in vivo* [11] studies in animals, and from dose–response studies in humans [12–14], indicate that the dose of β_2 -adrenoceptor agonists required to overcome induced bronchoconstriction is related not only to the degree of airway narrowing, but also to the type of bronchoconstrictor used (e.g. methacholine or histamine) and the dose required to achieve the desired level of bronchoconstriction [15–17]. With methacholine (MCh), the larger the dose required to obtain a given level of airway narrowing, the larger the dose of β_2 -adrenoceptor agonist needed to reverse it: a phenomenon called functional antagonism [17]. With this in mind, we have hypothesized that a bioassay based on a strong functional antagonism

between MCh and salbutamol might be particularly suitable to evaluate differences in the amount of salbutamol that reaches β -adrenergic receptors in the airways when the drug is administered via a pMDI with a spacer or via a DPI. Therefore, we have used a clinical bioassay based on a moderate [18] degree of MCh-induced bronchoconstriction to compare the speed of the bronchodilator response following salbutamol inhaled via either a DPI (the Diskus) or a pMDI in conjunction with a large-volume spacer (pMDI + Volumatic) in asthmatic patients. In addition, we have calculated the relative potency of the Diskus and the pMDI + Volumatic, i.e. the ratio of the salbutamol doses administered by the two inhalation devices producing an equal response. An attempt has also been made to ascertain which pulmonary function variable is most sensitive to detect differences in the speed of bronchodilation following salbutamol administered via the two inhalation devices. The present investigation is a *posthoc* analysis performed on data collected in a previous study that investigated the magnitude of the bronchodilator response following salbutamol inhaled via these different inhalation devices in asthmatic patients [19].

Methods

Patients

We studied 18 nonsmoking outpatients (seven females, mean age 29 years, range 18–35 years) with intermittent asthma [1]; their baseline FEV₁ value corresponded (mean 95% confidence interval, CI) to 100.4% (93.4, 107.4) of predicted [20]. None of the patients had suffered from recent (<4 weeks) respiratory tract infections and all were steroid naïve and were not receiving regular medications for their asthma. All patients had first been tested for bronchial hyper-responsiveness, and the mean (95% CI) value of provocative MCh concentration causing a 20% fall in FEV₁ was 1.64 mg ml⁻¹ (1.27, 2.01). This study was approved by the local ethics committee and individual informed consent was obtained.

Methacholine-induced bronchoconstriction

Patients tidally inhaled for 2 min doubling MCh concentrations (acetyl- β -methylcholine chloride, Lofarma, Milano, Italy) ranging from 0.06 to 16 mg ml⁻¹ through a DeVilbiss no. 646 nebulizer (DeVilbiss Co, Somerset, PA, USA) driven by a constant airflow (8 l min⁻¹) and preceded by 0.9% saline as a control solution. The bronchial response was evaluated by measuring the FEV₁ 90–120 s after administration of either saline or each MCh concentration by means of a water-sealed spirometer (Collins Survey II, Braintree, MA, USA) connected

to an X-Y recorder to obtain volume-time tracings of the forced vital capacity (FVC).

Protocol

This was a double-blind, double-dummy, six-way cross-over study, randomized for different treatments and inhalation devices. On each study day, MCh inhalation was preceded by baseline assessments of FVC, FEV₁ and mean forced expiratory flow rate measured between 25 and 75% of the FVC (FEF₂₅₋₇₅). The latter variable is considered an expression of small airway patency [21]. When FEV₁ had fallen by $\approx 35\%$ of the postsaline (control) value following MCh inhalation, patients inhaled placebo or 200 or 400 μg of salbutamol, either through the Diskus DPI (Diskus 200 μg salbutamol per actuation, GlaxoSmithKline Inc., Ware, Hertfordshire, UK) or the pMDI (HFA134a salbutamol sulphate, 100 μg salbutamol per actuation, Glaxo Operations, Speke, UK) in conjunction with the Volumatic spacer device (Volumatic, Allen & Hanbury's, Uxbridge, Middlesex, UK), while keeping the inspiratory flow rate as close as possible to 30 l min^{-1} [19]. Values of FVC, FEV₁ and FEF₂₅₋₇₅ were determined at 3-min intervals for 15 min, with further assessments performed 30, 45 and 60 min after drug administration. Additional details regarding the techniques for salbutamol and placebo administration, as well as the blinding procedures, have already been described [19].

Data analysis

Power calculations indicated that 12 patients were required to provide a greater than 80% chance of detecting a 20% difference between treatments in the speed of onset of bronchodilation (as assessed by FEV₁ changes) at the 5% level of significance [3, 19]. The largest of three reproducible ($\pm 5\%$ coefficient of variation) FVC, FEV₁ and FEF₂₅₋₇₅ measurements, the latter obtained from the single best test curve, was used for calculations [18]. Because of the dependence on changes in lung volumes, values of FEF₂₅₋₇₅ recorded following placebo and salbutamol administration were corrected for baseline FVC and expressed as isoFEF₂₅₋₇₅ [3, 22]. As all placebo trials attained overlapping results, the latter were averaged and considered as a whole in all subsequent analyses. Functional changes over time induced by placebo and salbutamol administration were evaluated by linear and nonlinear regression analysis, respectively [19].

Scrutiny of results revealed that, in some trials, the decay in lung function provoked by MCh inhalation could not be fully reversed by salbutamol within the 60-min observation period. For instance, with 200 μg salb-

utamol the increases in FVC, FEV₁ and isoFEF₂₅₋₇₅ values turned out to be only slightly greater than 90% of the corresponding control values (Figure 1). Therefore, recovery times were always calculated as the time elapsed from salbutamol administration and the appearance of a 90% increase [23] in post-MCh FVC, FEV₁ and isoFEF₂₅₋₇₅ values. Recovery times were subsequently compared by repeated measures analysis of variance (ANOVA) followed by the Bonferroni's test for multiple comparisons. We also noted that placebo administration caused relatively small increases in post-MCh lung function variables; as these increases consistently failed to reach the 90% level (Figure 1), recovery times were not calculated in placebo trials. Conceivably, salbutamol-induced increases in lung function values over time result from summation of the pharmacological effect by salbutamol plus a modest bronchodilator effect by placebo. Therefore, to calculate the net bronchodilator response to salbutamol, the post-MCh FVC, FEV₁ and isoFEF₂₅₋₇₅ increases induced by placebo administration were subtracted, at each considered time interval, from the corresponding increases observed following salbutamol administration. Recovery times for placebo-corrected changes in lung function variables over time were subsequently calculated with the same procedure as that described above. To evaluate differences in recovery times between FVC, FEV₁ and isoFEF₂₅₋₇₅, the recovery times calculated in each patient were pooled, and compared by ANOVA followed by the Bonferroni's test for multiple comparisons. Comparisons between dose-response curves obtained in pMDI + Volumatic and Diskus trials were performed by using the Finney 2-by-2 parallel regression bioassay analysis [24]. In essence, after having demonstrated a significant dose-response relationship and the absence of deviation from parallelism [24], this statistical method constructs a two-point dose-response curve for each of the two inhalation devices studied. Based on these curves, we calculated, for each considered pulmonary function variable, the salbutamol doses to be delivered by the two inhalation devices to achieve equal recovery times, as well as the ratio between these two doses, i.e. the relative potency [24]. In addition, to evaluate which pulmonary function variable was most sensitive in detecting differences in recovery times observed in trials performed with the two different inhalation devices, we calculated the ratio of the root mean squared error (s) to the slope (b) of the previously determined dose-response curves [25, 26]. The lower the s : b ratio below 1, the greater the sensitivity [25, 26]. Because in the present study all slope (b) values of the dose-response curves were negative (i.e. decreases in recovery times with increasing salbutamol

Figure 1

Time course of mean (18 patients) percentage changes in postmethacholine (MCh) forced vital capacity (FVC), forced expiratory volume in 1 s (FEV₁) and volume-adjusted forced expiratory flow rate between 25 and 75% of the FVC (isoFEF₂₅₋₇₅) following administration of placebo (mean values of two different preparations, X symbols), 200 (open circles) and 400 (filled circles) µg of salbutamol through the pressurized metered dose inhaler with the Volumatic spacer (solid lines) and the Diskus inhaler (dotted lines). In each panel, the thick dotted line marks the level of a 90% increase in postmethacholine value used to calculate the recovery times (see Methods). C, control (premethacholine) conditions

doses), the calculated *s* : *b* values were more conveniently expressed as absolute values. Comparisons between *s* : *b* values for FVC, FEV₁ and isoFEF₂₅₋₇₅ were performed by repeated measures ANOVA followed by the Bonferroni's test. Data analyses and representation were performed using statistical and graphic software (Graphpad Prism release 3.01; Graphpad Software, San Diego, CA, USA), and sample size and power calculations were performed using dedicated software (PS Power and Sample Size Calculations, release 2.1.30, Vanderbilt University Medical Center, Nashville, TN, USA). In all instances, a *P* value less than 0.05 was taken as significant.

Results

In all trials, MCh inhalation induced comparable FEV₁ decreases up to a level that closely approached -35% of the corresponding postsaline value (mean maximum FEV₁ fall 35.33%, 95% CI 34.90, 35.77). The mean (95% CI) MCh concentration required to produce a 35% fall in post-saline FEV₁ was 4.83 mg ml⁻¹ (3.89, 5.77). Reductions in FEV₁ were always accompanied by decreases in FVC (-20.27%, 95% CI -18.37, -22.17) and isoFEF₂₅₋₇₅ (-55.33%, 95% CI -52.2, -56.05). Methacholine-induced bronchoconstriction was well tolerated by all patients, and no adverse effects were observed.

Salbutamol administration via either inhalation device caused a rapid rise (within 3–6 min) in lung function variables, followed by further, slower increases up to a plateau level that was achieved within 30 min for FVC, and FEV₁, and within 45 min for isoFEF₂₅₋₇₅ (Figure 1). Individual regression coefficients for post-salbutamol changes in lung function variables over time ranged from 0.90 to 0.99 for FVC, from 0.88 to 0.99 for FEV₁, and from 0.96 to 0.99 for isoFEF₂₅₋₇₅ (*P* < 0.01). Following placebo, all variables displayed a slower, linear increase that consistently failed to achieve the corresponding control value (Figure 1).

For all lung function variables, mean recovery times were dose-dependently shortened by salbutamol inhalation (Figure 2); independently of the salbutamol dose, mean recovery times for each considered variable were significantly (*P* < 0.01) shorter in pMDI + Volumatic than in Diskus trials (Table 1). As shown in Figure 3, recovery times for FVC were significantly shorter than those for FEV₁ and isoFEF₂₅₋₇₅ (*P* < 0.01).

The demonstration of dose-response relationships and the absence of a significant deviation from parallelism allowed us to evaluate the relative potencies between the two inhalation devices [24]. Assessment of relative potencies revealed that, in Diskus trials, twice the salbutamol dose was required to obtain, for each

Figure 2

Parallel regression analysis for forced vital capacity (FVC), forced expiratory volume in 1 s (FEV₁) and volume-adjusted forced expiratory flow rate between 25 and 75% of the FVC (isoFEF₂₅₋₇₅) recovery times observed in pMDI + Volumatic (filled circles) and Diskus (empty circles) trials. The arrowed dotted lines indicate the calculated salbutamol dose to be delivered by the Diskus to achieve recovery times equal to those observed following 200 µg salbutamol administered via the pMDI + Volumatic

Table 1

Mean (95% confidence interval) recovery times (in min) for FVC, FEV₁ and isoFEF₂₅₋₇₅ following administration of 200 and 400 µg salbutamol via the pressurized metered-dose inhaler with Volumatic spacer (pMDI + Volumatic) and the Diskus dry-powder inhaler. The corresponding placebo-corrected (PL-corrected) recovery times are also indicated. See Methods for further details

	FVC	FEV ₁	isoFEF ₂₅₋₇₅
200 µg salbutamol via pMDI + Volumatic	6.58 (6.14, 7.42)*	19.06 (18.20, 19.52)*	12.50 (12.02, 13.38)*
PL corrected	8.52 (8.12, 9.36)*	32.10 (30.48, 33.36)*	14.20 (13.50, 14.50)*
400 µg salbutamol via pMDI + Volumatic	2.24 (2.12, 2.45)*§	4.42 (4.10, 5.14)*§	2.46 (2.27, 3.05)*§
PL corrected	4.20 (4.07, 4.33)*§	7.40 (7.1, 8.10)*§	3.05 (2.45, 3.20)*§
200 µg salbutamol via Diskus	18.43 (17.40, 19.45)	35.27 (33.19, 37.35)	29.24 (27.42, 31.06)
PL corrected	29.50 (27.47, 31.53)	42.15 (40.07, 44.23)	44.38 (42.08, 47.00)
400 µg salbutamol via Diskus	12.38 (10.56, 14.18)§	18.42 (17.37, 19.46)§	13.07 (11.32, 14.42)§
PL corrected	16.50 (14.38, 19.12)§	30.15 (-29.10, 31.20)§	16.12 (14.02, 18.22)§

FVC, forced vital capacity; FEV₁, forced expiratory volume in 1 s; isoFEF₂₅₋₇₅, volume-adjusted forced expiratory flow rate between 25 and 75% of the FVC. Within each column **P* < 0.01 comparing the same salbutamol dose administered via different inhalation devices, §*P* < 0.01 comparing different salbutamol doses administered via the same device.

considered variable, recovery times equal to those achieved in pMDI + Volumatic trials (Table 2).

The *s* : *b* ratios for FVC, FEV₁ and isoFEF₂₅₋₇₅ were 0.11 (95% CI 0.09, 0.12), 0.05 (95% CI 0.04, 0.06) and 0.06 (95% CI 0.05, 0.07), respectively. Analysis of variance showed that *s* : *b* ratios for FEV₁ and isoFEF₂₅₋₇₅

were similar and significantly (*P* < 0.05) lower than that for FVC.

Discussion

The results show that, in patients with intermittent asthma, administration of salbutamol via the pMDI

Figure 3

Comparison of recovery times for forced vital capacity (FVC), forced expiratory volume in 1 s (FEV₁) and volume-adjusted forced expiratory flow rate between 25 and 75% of the FVC (isoFEF₂₅₋₇₅) performed on pooled data. Each bar represents the mean (95% confidence interval) FVC, FEV₁, and isoFEF₂₅₋₇₅ values ($n = 18$) obtained by pooling the corresponding values recorded during all salbutamol trials. * $P < 0.01$

Table 2

Bioequivalence between salbutamol administered via the pMDI+Volumatic and via the Diskus

Variable	Salbutamol dose (μ g) via Diskus equivalent to 200 μ g via pMDI + Volumatic	Relative potency (pMDI + Volumatic vs Diskus)
FVC	558 (537, 579)	2.79 (2.68, 2.90)
FEV ₁	395 (388, 404)	1.98 (1.94, 2.02)
IsoFEF ₂₅₋₇₅	404 (393, 415)	2.02 (1.96, 2.07)

Values are means $\pm$ 95% confidence interval. See Table 1 for abbreviations.

+Volumatic causes a more rapid reversal of MCh-induced bronchoconstriction than via the Diskus. The results also indicate that it would require approximately twice the dose of salbutamol administered via the Diskus to obtain the same recovery times as those recorded when salbutamol is administered via the pMDI + Volumatic.

A demonstration of clinical bioequivalence and assessment of the relative potency of inhaled medications are required before the marketing of new types of delivery systems [6, 7]. Bioequivalence ensures that

equal doses of two different formulations (e.g. powdered or aerosolized) of the same drug produce equivalent pharmacodynamic effects [6, 7]. The principal *in vivo* methods currently available for the assessment of bioequivalence between different formulations of the same drug are plasma pharmacokinetic studies, radio-aerosol drug deposition studies, and pharmacodynamic clinical efficacy studies [6, 7]. Pharmacokinetic studies are of limited value for inhaled bronchodilators because the doses administered are small and the resulting serum concentrations are often too low to assay accurately; in addition, they may not correlate with the dose delivered to the lungs [6, 7]. Indeed, regulatory authorities do not consider pharmacokinetic studies reliable reflections of the amount of bronchodilator drug delivered to the lungs [27]. Radioaerosol studies provide useful information about lung deposition, but their ability to predict the clinical efficacy of β_2 -adrenoceptor agonists is controversial [7]. Pharmacodynamic clinical efficacy studies are considered the most useful tool for assessing both the effectiveness and bioequivalence of inhaled bronchodilators [6, 7]. Generally, such studies rely on the measurement of clinically relevant pharmacodynamic responses that are thought to reflect the amount of drug delivered to the lungs by the tested preparation and the reference preparation [6, 7]. Many of these studies have assessed bioequivalence between different formulations of β_2 -adrenoceptor agonists by evaluating their ability to prevent induced bronchoconstriction [13, 14, 26]. A potential disadvantage of this method is that it is time-consuming and requires considerable investigator expertise [8]; in addition, it has been questioned whether bronchoprotection represents a situation amenable to the clinical world [8]. Other pharmacodynamic clinical efficacy studies of bronchodilators, including salbutamol, are carried out by measuring bronchodilation in patients with spontaneous [2] or induced bronchoconstriction [3, 5]. Although relatively quick and easy to perform, evaluation of salbutamol-induced changes in airway calibre in patients with spontaneous bronchoconstriction may be difficult, due to the potentially confounding effects related to the variability of the levels of spontaneous airway narrowing, especially when such variability is not adequately accounted for in patient selection. With induced bronchoconstriction, a common problem arises from the magnitude of the provoked effect. Indeed, if the level of induced airway narrowing is mild, even low doses of β_2 -adrenoceptor agonists delivered by virtually any inhalation device may produce near maximal bronchodilator responses [8]. Therefore, in these experimental conditions, detection of differences in the bronchodilator response to different formulations (e.g.

powdered and aerosolized) of the same agent may be difficult [15]. The bioassay used in our study is based upon the firmly established pharmacological principle of functional antagonism between muscarinic and β -adrenergic agonists, which, working through different receptors, produce opposite responses [17]. According to this principle, the airway response to β_2 -adrenergic agonists depends on the MCh dose [11, 16]; the greater the amount of MCh needed to cause bronchoconstriction, the greater the concentration of β_2 -adrenergic agonist needed to reverse it [17]. Conceivably, administration of higher MCh concentrations with subsequently more marked levels of bronchoconstriction probably increases the functional antagonism between MCh and salbutamol. A strong functional antagonism shifts the dose-response curve to the right and may produce differences that are not apparent in bronchodilator studies performed in patients with a mild level of MCh-induced bronchoconstriction. Thus, we believe that the present clinical bioassay, based on the induction of a more marked level of bronchoconstriction (FEV_1 fall -35%) offers a valuable alternative to conventional bronchodilator studies [2] or to studies using bronchoprotection [13, 14, 26].

Admittedly, such a level of induced bronchoconstriction raises important safety issues. However, reductions in FEV_1 ranging from 30 to 75% of baseline have been achieved in several previous studies [28–30] involving asthmatic patients, and have been reported to be well tolerated and devoid of significant side-effects [28–30]. In keeping with this, the MCh-induced 35% fall in baseline FEV_1 was always well tolerated by our patients, all having normal baseline FEV_1 values (mean FEV_1 100.4%, 95% CI 93.4, 107.4%). Thus, once the desired level of induced bronchoconstriction had been achieved, their FEV_1 fell to approximately 75% of predicted, a value corresponding to only a mild level of airway obstruction [18]. Based on these considerations, we believe that the extent of MCh-induced bronchoconstriction employed in our study is safe and tolerable.

We compared the dose-response curves obtained in pMDI + Volumatic and Diskus trials by using the Finney linear bioassay statistical method [24]. This procedure calculates the salbutamol doses required by the two inhalation devices to achieve the same recovery times. We found that approximately twice the salbutamol dose administered via the Diskus is needed to obtain the same recovery times as those obtained with a single salbutamol dose inhaled via the pMDI + Volumatic. Thus, it seems logical to conclude that the pMDI + Volumatic is preferable for administra-

tion of aerosol medications, especially when the fastest bronchodilator response is required.

It has been shown that, in asthmatic patients, administration of bronchodilator drugs in a cumulative-dose regimen causes greater bronchodilator responses than with the equivalent single-dose regimen [31, 32]. In consequence, it could be argued that the differences in recovery times observed in our study are also due to differences in the scheme of salbutamol administration. We believe that this is unlikely in the light of the following considerations. First, at variance with studies [31, 32] in which the time elapsed from administration of multiple bronchodilator doses was at least 10 min, we have always performed multiple actuations within 30 s, i.e. probably in the absence of any prominent effect by the first administered dose [33]. Second, and more importantly, if one compares the recovery times of trials performed with 200 μ g salbutamol via the pMDI + Volumatic (two actuations) and 400 μ g salbutamol via the DPI (two actuations), i.e. in conditions where the number of actuations is not influential, the FEV_1 response obtained with 400 μ g via DPI is the same as that obtained with 200 μ g salbutamol via the pMDI + Volumatic. Therefore, the key factor accounting for the overlapping recovery times despite twice as much the salbutamol dose must have been the inhalation device and not the drug administration scheme.

Recovery times for FVC turned out to be significantly shorter than those for FEV_1 and $isoFEF_{25-75}$ (Figure 3). We have previously shown [19] that, following a 35% fall in FEV_1 induced by MCh, hyperinflation occurs, as documented by parallel reductions in both the inspiratory capacity and vital capacity. Hyperinflation could be promptly abolished by salbutamol administration [19]. Given the similar functional meaning of the slow and forced vital capacity in patients with airflow limitation [34], we believe that the reductions in FVC observed here after MCh-induced bronchoconstriction also reflect dynamic hyperinflation. It seems possible that even submaximal bronchodilation may have promptly abolished, at least to a considerable extent, the dynamic hyperinflation induced by MCh inhalation, thus explaining the shorter recovery times for FVC compared with those of flow-dependent variables such as the FEV_1 and the $isoFEF_{25-75}$.

In the present study we have also aimed to evaluate which pulmonary function variable was most sensitive in detecting differences in the recovery times observed in pMDI + Volumatic and Diskus trials. This was done by calculating the ratio of the variability of the response (commonly symbolized as *s*) to the slope of the dose-response curve (commonly symbolized as *b*). We found

that, although all considered pulmonary function variables displayed high *b* values and low variability of the response, the *s* : *b* values for FEV₁ and isoFEF_{25–75} were significantly lower than that calculated for FVC, thus suggesting that the former are more sensitive than FVC in assessing differences in the recovery times. The sensitivity of a given clinical bioassay study for evaluating the bioequivalence between different drugs or different inhalation devices is related to the slope of the dose–response (*b*) and the variability of the response (*s*) [25, 26]. Thus, we believe that our clinical bioassay has sufficiently steep dose–response slopes and low variability of the response to provide a clinically useful assessment of the bioequivalence of the two inhalation devices being compared and therefore sufficient sensitivity to identify which inhalation device delivers the greater amount of salbutamol to the lungs.

Bronchoactive drugs are optimally deposited in the small airways when the aerosol particle size is in the respirable range, that is, a mass median aerodynamic diameter <5 µ [35]. To the best of our knowledge, however, no studies have specifically compared lung deposition of bronchoactive drugs obtained by using the pMDI + Volumatic with that obtained with the Diskus inhaler. Pharmacokinetic and radioaerosol studies have shown a correlation between the amount of particles with a mass median aerodynamic diameter <5 µ (commonly referred as fine particles) and lung deposition [36]. It has also been shown that the proportion of fine particles obtained by using the pMDI + Volumatic is significantly higher than that obtained with the Diskus [37]. Furthermore, physiological studies have shown that low FEF_{25–75} values reflect significant obstruction in peripheral airways, even in patients with FEV₁ values >80% predicted [21, 38]. Thus, we are confident that the rapid isoFEF_{25–75} improvements observed in salbutamol pMDI + Volumatic trials actually reflect peripheral airway dilation, possibly due to a higher percentage of fine particle produced by using the pMDI + Volumatic than the DPI. This may be important not only to reverse airway obstruction rapidly, but also to prevent future asthma attacks [39] and the progression to airway fibrosis and remodelling [40].

In conclusion, this study shows that salbutamol administered via the pMDI + Volumatic determines more rapid bronchodilation than via the Diskus, and that the pMDI + Volumatic is approximately twice as efficient as the Diskus inhaler in relative lung delivery of salbutamol in asthmatic patients with induced bronchoconstriction. However, it should be emphasized that large differences in drug delivery can be observed among spacers of different size and shape, as well as when the

same device is used with different drugs. Furthermore, inhalation of sensitizing allergens may induce, in patients with allergic asthma, late asthmatic reactions [41] that begin 3–4 h after allergen inhalation, may last 24 h or longer, and are not easily reversed by doses of β-adrenergic agonists such as those employed in the present study. For these reasons, care should be taken in extrapolating our results to inhalation devices, bronchodilator drugs and clinical conditions other than those examined here.

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