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Protection against methacholine-induced bronchospasm: salbutamol pMDI *versus* Clickhaler® DPI

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Protection against methacholine-induced bronchospasm: salbutamol pMDI versus Clickhaler® DPI. M.T. Newhouse, P. Patel, M. Parry-Billings. ©ERS Journals Ltd 2003.

ABSTRACT: Passive dry-powder inhalers (DPIs) have been developed as an alternative to pressurised metered-dose inhalers (pMDIs) to improve aerosol delivery on inhalation and eliminate the need for propellants. However, new DPI formulations of generic drugs must be rigorously compared with conventional pMDI therapy.

This randomised, double-blind, double-dummy, placebo-controlled, seven-way cross-over study evaluated bronchoprotection from methacholine challenge in order to compare a novel salbutamol DPI (Clickhaler®) with a reference salbutamol pMDI (Ventolin®). Adult asthma patients with airway hyperresponsiveness to methacholine (provocative concentration of methacholine causing a 20% fall in the forced expiratory volume in one second (PC_{20}) <4 mg·mL^{-1}) were treated on separate days with 0, 100, 200 or 400 µg of salbutamol *via* the DPI or pMDI. Methacholine challenge was performed before and after salbutamol treatment and the PC_{20} ratios analysed by Finney's bioassay to test for therapeutic equivalence of the inhalers.

Eighteen patients completed the study and showed significant dose-related responses to salbutamol. The relative potency of DPI:pMDI was 1.29 (90% confidence interval 1.04–1.63). There were no treatment differences in safety (cardiac frequency, blood pressure, adverse events).

Methacholine-challenge methodology provides a sensitive bioassay and has demonstrated therapeutic equivalence of the salbutamol Clickhaler® dry-powder inhaler with the conventional salbutamol pressurised metered-dose inhaler.

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Salbutamol, the β_2-agonist bronchodilator most commonly used in the treatment of asthma, has been administered using pressurised metered-dose inhalers (pMDIs) and pressurised chlorofluorocarbon (CFC) propellants for the past 30 yrs. Substituting "ozone-friendly" alternatives puts the onus on the pharmaceutical industry to produce cost-effective replacement inhalers and establish that the switch-over from CFC pMDIs does not compromise patient care [1]. Each new device/formulation is a unique combination that must satisfy strict regulatory requirements [2].

Pharmaceutical research and development has followed two major pathways: upgrading pMDIs with hydrofluoroalkanes (HFAs) as chlorine-free propellants, and innovative design of efficient dry-powder inhalers (DPIs) [3, 4]. The activity of inhaled medications in the lung is determined by the amount of drug entering the lower respiratory tract [5], which is dependent on the mass of particles of ~1–5 µm diameter and inspiratory flow velocity through the device [6]. Changes of formulation, humidity, device or handling may alter drug mass or deposition, with implications for both safety and efficacy [7–9]. Rapid systemic absorption of inhaled salbutamol occurs mainly at the vascular lung surface and the risk:benefit ratio can change with improved deposition [10]. *In-vitro* data therefore need the support of clinical studies to elucidate drug bioavailability [11, 12].

Sequential measurement of the forced expiratory volume in one second (FEV_1) in mild, stable asthmatics is commonly used to assess bronchodilator efficacy and compare new inhalers with existing standards [13]. Valid comparison requires two or more doses of each drug since using single doses gives no information as to whether the bronchodilator effect has reached a plateau [14]. Parallel dose/response curves can then be used to determine doses with an equivalent pharmacodynamic effect and the potency ratio calculated, with a value of 1.0 indicating bioequivalence [15]. However, with available clinical formulations of salbutamol it is often difficult to achieve submaximal responses and bronchoconstrictor challenge methods are increasingly being used to improve the sensitivity of bioequivalence testing [16].

Adult patients with moderate, stable asthma, who screened positive for hyperresponsiveness to inhaled methacholine, showing dose/response to salbutamol bronchoprotection, were recruited accordingly to compare the clinical effectiveness of a new salbutamol DPI with a widely used pMDI.

Materials and methods

Patients

Male and female patients (≥18 yrs) with moderate stable asthma were enrolled at the initial screening visit if they showed a baseline FEV_1 of ≥70% predicted and hyperresponsiveness to inhaled methacholine, evidenced by a provocative concentration causing a 20% fall in FEV_1 (PC_{20}) at a concentration of ≤4 mg·mL^{-1}. Patients were additionally screened for PC_{20} dose/response to 100 and 200 µg of salbutamol *via* pMDI, and had to demonstrate correct use of the

DPI and pMDI, using open-label placebo devices according to the manufacturers' instructions, achieving an inspiratory flow of 30 $L \cdot min^{-1}$ (±25%). Inhalation was from functional residual capacity (FRC) to total lung capacity (TLC), the flow being confirmed by a computerised monitor, which provided a target rate and range for the patient to achieve [17]. Eligible patients had been nonsmokers for ⩾6 months, with a maximum smoking history of 5 pack-yrs. Use of inhaled corticosteroids, sodium cromoglycate or nedocromil sodium was permitted if the dose had been stable for ⩾4 weeks.

Exclusion criteria included any current or past medical condition other than asthma that might significantly affect the pharmacodynamic response to salbutamol or any condition within the last 6 weeks that could affect airway reactivity to methacholine. Further reasons for exclusion included: hypersensitivity to salbutamol, lactose, methacholine or any component of the pMDI or DPI and hospitalisation for acute asthmatic symptoms, or oral steroid treatment, within the previous 3 months. Females who were breastfeeding, pregnant or likely to become pregnant were also excluded. Patients with a history of seasonal asthma exacerbations were not excluded if they were studied outside the relevant allergen season. The study was carried out in accordance with the ethical principles of the Declaration of Helsinki and the protocol was reviewed and approved by the Independent Ethics Committees associated with each centre. All patients gave written informed consent at the start of the first study visit.

Study design

This was a randomised, double-blind, double-dummy, placebo-controlled seven-way crossover study, carried out at two hospital physician centres in Canada. The study consisted of 2 screening days (visits 1 and 2) and 7 test days (visits 3–9). At visit 1, demographics, medical history, drug therapy and vital signs were recorded (table 1). Hyperresponsiveness to methacholine and dose/response to salbutamol were established by methacholine PC20 measurements at visits 1 and 2 (table 2). Patients who met all the eligibility criteria at screening were randomised to study treatment and received three doses of salbutamol and a placebo from each inhaler during visits 3–9.

Table 1. – Patient characteristics at entry to the study (visit 1)

Parameter	Patients	
	Randomised	Completed
Patients n	26	18
Age yrs	31±10 (18–56)	31±11 (18–56)
Sex M:F	6:20	5:13
Weight kg	80±24 (48–169)	77±17 (57–115)
Height cm	168±9 (152–184)	169±8 (152–184)
Blood pressure mmHg		
Systolic	108±10 (85–130)	110±10 (90–130)
Diastolic	67±9 (50–82)	68±9 (50–82)
Cardiac frequency beats·min^{-1}	80±15 (46–126)	83±16 (56–126)
Respiratory rate breaths·min^{-1}	18±2 (14–20)	18±2 (14–20)
Asthma medication % patients		
Inhaled β_2-agonist	100	100
Inhaled corticosteroid	23	28
Inhaled chromone	8	6

Data are presented as mean±SD (range), unless otherwise stated. M: male; F: female.

Table 2. – Baseline lung function (visit 1) and salbutamol dose/response in the methacholine- challenge test at screening (visits 1 and 2)

	Randomised	Completed
Patients n	26	18
FEV1		
L	3.0±0.8 (2.0–4.9)	3.0±0.7 (2.1–4.7)
% predicted	86±11 (72–110)	88±11 (74–110)
Methacholine challenge		
Baseline PC20 mg·mL^{-1}	1.0±0.9 (0.1–3.0)	0.8±0.8 (0.1–3.0)
Salbutamol dose/response		
PC20 post 100 µg	4.7±5.1 (0.2–18.9)	4.0±3.3 (0.8–11.9)
PC20 post 200 µg	13.3±18.1 (0.9–93.9)	10.4±7.3 (1.8–27.0)
Ratio PC20 post 200:100 µg	3.9±3.3 (1.1–12.5)	3.4±2.8 (1.1–12.5)

Data are presented as mean±SD (range), unless otherwise stated. FEV1: forced expiratory volume in one second; PC20: provocative concentration of methacholine causing a 20% fall in FEV1.

Study days were separated by ⩾48 h and all visits fell within a 60-day period for each patient. Routine asthma medication was continued between visits, but before each study day the patients had to discontinue short-acting inhaled β_2-agonists for 6 h, long-acting β_2-agonists for 48 h, short-acting oral β_2-agonists for 12 h, long-acting oral β_2-agonists for 24 h and theophylline for 48 h. They were also not to take an antihistamine within 96 h. Caffeine-containing beverages were discontinued for 8 h and alcohol for 24 h before study visits. Visits started at the same time of day for each patient, generally between 08:30–10:00 h. A light meal was allowed ⩾1 h before the first challenge but only water (and a light snack if required) during the testing session. Patients refrained from exercise or exposure to cold air throughout the visit.

At each visit, stability of the patient's asthma was assessed by a pulmonologist, who determined whether they had experienced deterioration since the last visit in exercise tolerance, nocturnal awakening, morning chest tightness and bronchodilator use. Baseline FEV1 and methacholine PC20 values were determined. If baseline FEV1 was not within 85–115% or baseline PC20 within 50–200% of visit-1 values, the treatment visit was rescheduled (up to three times). Continued baseline instability led to the patient being withdrawn. Adverse events and changes in concomitant medication or coexistent diseases were also recorded at each visit.

Methacholine challenge

A baseline FEV1 was measured at each visit, taking the lowest of three reproducible (±5%) recordings, followed by a standard methacholine challenge test to determine the baseline PC20. Methacholine solutions, diluted in 0.9% saline and sterilised, were prepared by the study pharmacist in doubling concentrations from 0.03–256 $mg \cdot mL^{-1}$. Aerosols were inhaled by tidal breathing for 2 min from an English Wright Jet Nebuliser (Aerosol Medical Ltd, Colchester, UK) at an output of 0.13 $mL \cdot min^{-1}$, using the methodology established by JUNIPER *et al.* [16] and SIERSTED *et al.* [18]. Starting concentrations of methacholine (0.03–0.125 $mg \cdot mL^{-1}$) were determined by the investigator for each patient, based on inhaled corticosteroid therapy and the response to 0.9% sterile saline. FEV1 was measured 30, 90 and 180 s after each inhalation, then at 2-min intervals until it began to recover.

A single FEV1 recording was made each time (if technically unsatisfactory, this was repeated after 10 s). If no decrease in FEV1 was observed within 5 min after one inhalation of methacholine, the patient moved on to inhale a higher concentration. The test ended when FEV1 decreased by $\geqslant$20% from baseline, allowing calculation of the PC20.

Salbutamol dose/response

At visits 1 and 2, patients were treated with one (100 μg) or two actuations (200 μg) from an open-label salbutamol pMDI (Ventolin®; Allen and Hanburys Ltd, Uxbridge, UK), 2 h 45 min after a baseline methacholine test, followed by a second methacholine challenge 15 min later. Dose order for each patient was randomised. Sixty minutes after the post-treatment challenge, all patients received 200 μg of salbutamol *via* a standard pMDI and FEV1 was measured after 15 min to check that it had recovered to near baseline level before the patient was allowed to leave the clinic. For study eligibility, PC20 after two actuations of the salbutamol pMDI had to be more than the PC20 after one actuation (a dose:response ratio of $\geqslant$2.0 was specified initially, but this proved too stringent in a large number of the patients screened).

Study treatments

Patients eligible at screening were randomised to seven treatment visits on separate days, during which they received salbutamol (100, 200 or 400 μg) *via* the DPI or standard pMDI, or placebo. They also received placebo from the alternative device at each visit. Since both devices delivered 100 μg per actuation, each study treatment required the patient to inhale once from eight coded inhalers (four DPIs and four pMDIs). The DPIs (Innovata Biomed Ltd, St. Albans, UK) contained either micronised salbutamol sulphate and lactose or lactose only. pMDI salbutamol was administered from Ventolin® pMDIs. Placebo pMDIs, having a similar content minus the salbutamol, were manufactured by Miza Ltd (Runcorn, UK), who also code-labelled and packaged all inhalers used in the study. In order to standardise the conditions of the methacholine challenge test and for patient safety, the investigators (but not the subjects) were aware of which dose of salbutamol patients received, although they were not aware of which device it was delivered from. At each treatment visit, patients confirmed their correct technique with placebo devices before using the study inhalers. pMDIs were primed by shaking and discharge to waste (not into the clinic air) five times, at 30–60-s intervals, and were then placed in the valve-up position for 1–30 min before dosing. DPIs and pMDIs were shaken immediately before each actuation. Patients inhaled (30 $L \cdot min^{-1}$) from FRC to TLC and held their breath for a few seconds after each inhalation.

After 2 h 45 min from methacholine challenge, patients received the test medication, followed by a further methacholine challenge 15 min later. Post-treatment methacholine concentrations started at visit-1 baseline levels following placebo or 100 μg salbutamol, and at two concentrations below previously screened PC20 values for 100 and 200 μg salbutamol, respectively, following treatment with 200 and 400 μg salbutamol. Vital signs (blood pressure, respiratory rate and cardiac frequency by 3-lead electrocardiogram (ECG) rhythm strip) were recorded 15 min before and 5 and 15 min after the first challenge test, 15 min after study treatment, immediately before the second challenge test and 5, 15 and 60 min after the second challenge test. Patients then received 200 μg salbutamol *via* standard pMDI and FEV1 was measured 15 min later to check recovery to near baseline value before they left the clinic.

Statistical analysis

PC20 values were determined by linear interpolation of methacholine concentration (log $mg \cdot mL^{-1}$) against percentage decrease in FEV1 from baseline. The response of FEV1 to each methacholine concentration was calculated by the investigator as:

$$\%\ \text{decrease in FEV}_1 = 100 \times \frac{(\text{baseline FEV}_1 - \text{lowest FEV}_1\ \text{postmethacholine})}{\text{baseline FEV}_1} \quad (1)$$

The percentage decrease in FEV1 was then plotted against the concentration ($mg \cdot mL^{-1}$) of methacholine on a logarithmic scale and the PC20 determined as follows:

$$PC_{20} = \text{antilog log C1} + \frac{(\log C2 - \log C1)(20 - R1)}{(R2 - R1)} \quad (2)$$

where R1 is the percentage decrease in FEV1 (<20%) due to penultimate concentration (C1) and R2 is the percentage decrease in FEV1 (>20%) due to last concentration (C2).

The primary efficacy end-point was the drug activity ratio (PC20 post-treatment:PC20 pretreatment) for each dose of salbutamol from each device. The dose/response relationship of drug activity ratio with salbutamol dose was compared for the two inhalers using Finney bioassay analysis of variance methods to calculate relative potency and 90% confidence interval (CI) based on Food and Drug Administration (FDA) guidance for generic drugs [1, 15, 19]. This required per protocol analysis of completed patients, with standard tests for validity (linearity, parallelism and regression) of the dose/response comparison [20]. Power calculations based on published data for drug activity ratios were used to estimate a minimum sample size of 16 patients [21, 22].

Safety parameters (any adverse events, vital signs and changes in concomitant medication) were summarised for all screened patients and considered on a case-by-case basis, as appropriate.

Results

Sixty-three patients were screened, of whom only 26 were eligible for randomisation to treatment and 18 completed the study. Screening data for eligible and completing patients are summarised in tables 1 and 2. No important differences were apparent between the randomised group and those who completed. Of the eight patients who failed to complete study treatment, five were withdrawn due to instability of baseline PC20, one due to the onset of the allergy season, one because of a spirometer malfunction and one because they were unable to complete the study in time.

The dose-related effects of salbutamol on PC20 complied with Finney bioassay validity criteria, showing linear dose/responses with no significant deviation from parallelism or overlap between the two devices [15]. Drug activity ratios for treatment with 100, 200 and 400 μg salbutamol *via* DPI or pMDI, or placebo, are shown in table 3. The potency ratio of the study treatments based on the drug activity ratio was 1.29 (90% CI: 1.04–1.63), *i.e.* one actuation of the DPI is equivalent to ~1.3 actuations of the pMDI.

The overall incidence of adverse events was very low, the most common event (incidence 0.1%) being headache during the screening period. No serious adverse events or clinically

Table 3. – Drug activity ratio (post:pretreatment provocative concentration of methacholine causing a 20% fall in the forced expiratory volume in one second (PC_{20})) for treatment with 100, 200 and 400 μg salbutamol *via* a dry-powder inhaler (DPI) or pressurised metered-dose inhaler (pMDI), or placebo

	Methacholine PC_{20} drug activity ratio	
Salbutamol dose μg	DPI	pMDI
100	9.5	5.7
200	15.6	12.5
400	23.8	23.7
Placebo	1.2	1.2

Overall potency ratio DPI:pMDI=1.29; 90% confidence interval 1.04–1.63. n=18.

important changes in vital signs occurred during the study and there were no treatment-associated differences in safety parameters.

Discussion

This pharmacodynamic study followed FDA guidelines in establishing therapeutic equivalence of the salbutamol Clickhaler®, a breath-actuated reservoir DPI [23], with the conventional Ventolin® pMDI in adult patients with stable, moderate asthma [1, 15]. Validated methods for bronchodilator bioequivalence studies have only recently been clearly defined as requiring at least two doses of each treatment and adequate group sizes to establish a potency ratio [14, 19]. Challenge methods are increasingly used to help achieve these conditions. For example, crossover studies of salbutamol Turbuhaler® and pMDI required 23 patients to assess bronchoprotective effects against methacholine [24], whereas direct assessment of bronchodilation required $\geqslant$40 evaluable patients [7]. Methacholine-induced bronchoconstriction is now widely accepted as a method of increasing the sensitivity of the efficacy end-point in assessing inhaled β_2-agonists, and was recently validated in a bioequivalence study of HFA and CFC pMDIs [25]. This placebo-controlled crossover study required 18 asthmatic patients to compare 100, 200, and 400 μg doses of salbutamol from the two devices and Finney bioassay showed a potency ratio of 1.08, confirmed using the nonlinear E_{max} model [15, 25].

The crossover, single-dose/separate-day study design has the potential disadvantage of inaccuracies resulting from variations in baseline FEV_1 and PC_{20}. Methodological studies suggest that inter-occasion variability should be accounted for in the statistical evaluation of the β_2-agonist dose/response and potency ratio, whether using linear or nonlinear mixed effects models [26]. Variability can be reduced by imposing limits on the baseline measurements for each treatment day, as observed in the present study, for which a minimum requirement of 16 evaluable patients was estimated, based on validated histamine challenge bioassays of salbutamol [21, 22]. Although 20% of the randomised patients had to be withdrawn owing to variation in baseline PC_{20}, 18 patients remained to provide per protocol data for three doses of each treatment [1, 15]. In recruiting sufficient subjects, it was found that the FDA recommendation for PC_{20} responsiveness to 200:100 μg salbutamol (a response ratio of $\geqslant$2.0) was too stringent [1]. Moderation of this entry criterion was justified by achievement of significant salbutamol dose/responses with both inhalers, showing no impact on the study outcome. The potency ratio of 1.29 suggests a slightly improved lung deposition of salbutamol from the Clickhaler® compared with the pMDI. This difference is not considered clinically relevant but may support the increased sensitivity of bronchoprotective assessments.

Recent Turbuhaler® studies have confirmed that pulmonary deposition data obtained by pharmacokinetic methods provide a useful surrogate for measuring the overall clinical response to inhaled β_2-agonists [13, 27]. However, the practical relevance of the greater deposition and bronchoprotective potency of terbutaline *via* Turbuhaler® is obscured by the abnormally high inspiratory flow (90 $L \cdot min^{-1}$) used with the pMDI [27]. In this study clinically relevant inspiratory flows for Clickhaler® and pMDI were used, consistent with previous bronchodilator studies [17, 28]. In a cumulative dose/response study, these devices showed an FEV_1 efficacy ratio close to 1.0 when comparing a submaximal nominal dose of salbutamol (100 μg) in patients with mild, moderate or severe asthma [28]. The maximum change in FEV_1 following inhalation of 200 μg salbutamol at 15, 30 or 60 $L \cdot min^{-1}$ from the Clickhaler® again matched the optimally used (30 $L \cdot min^{-1}$) pMDI [17], whilst supportive pharmacokinetic data demonstrate similar relative lung bioavailability of salbutamol from the Clickhaler® at fast (60 $L \cdot min^{-1}$) or slow (30 $L \cdot min^{-1}$) inhalation rates [29].

Breath-actuated DPIs have an environmental advantage over pMDIs in avoiding potential hazards of ozone destruction and global warming associated with propellant gases [30]. Breath actuation also obviates difficulties in coordinating actuation and inhalation, which many patients experience with the "press and breathe" pMDI [31]. A disadvantage associated with some DPIs is the rapid inhalation manoeuvre required for optimal actuation [3, 7, 27]. This contrasts with the pMDI, which is more effective using slower inhalation [32]. It also raises concerns over the effective use of DPIs by paediatric asthma patients aged <6 yrs and others who may be unable to achieve sufficient flow rates. DPI development is addressing these problems. The Clickhaler® requires a deep inhalation for optimal lung delivery but is driven by patient effort alone, relying on design features and optimised formulations to minimise flow-rate dependence [17, 29, 32]. Asthmatic children aged >6 yrs are able to generate sufficient inspiratory flow and can reliably inhale initially, following appropriate instruction, to operate the DPI [33], which as a potential replacement mimics the familiar shape and handling of the pMDI [32]. Simplicity and acceptability may influence a patient's compliance with inhaled treatments and are important factors in cost-effectiveness.

To conclude, salbutamol is the world's most widely used bronchodilator but environmental concerns are pressing for the provision of bioequivalent replacements for the chlorofluorocarbon-driven pressurised metered-dose inhalers [30]. This study has employed validated methods to demonstrate that the Clickhaler® dry-powder inhaler delivers comparable amounts of salbutamol to the lungs of asthmatic patients to a correctly used chlorofluorocarbon-pressurised metered-dose inhaler.

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