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Methacholine dry powder inhaler as a new tool for bronchial challenge test

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Abstract

Background: The methacholine (MCH) challenge test is performed to detect bronchial hyperresponsiveness in subjects suffering from asthma. It is conducted by inhaling spasmogen substances at increasing doses and measuring FEV1-PD20 variation following the bronchoconstriction evoked.
Aim: This paper describes a new method for MCH challenge test using pre-metered respirable powders of MCH at different doses for facilitating test execution. The availability of a series of pre-metered doses gives higher control over aerosolized dose and fine particle fraction (respirable dose), improving the accuracy and repeatability of the test. Dosimetric tests with MCH solution and pre-dosed powder challenge tests were clinically compared.
Methods and materials: The inhalation powders were prepared by spray drying of solutions of methacholine, mannitol and hydroxypropylmethylcellulose in which different concentrations of MCH were included. The methacholine powders prepared were carefully characterized in terms of aerodynamic properties.
Results: Inhalation powders containing methacholine from 12.5 to 200 μg per metered dose, having a fine particle fraction between 40 and 60%, were prepared using mannitol and cellulose polymer. Eighteen subjects (12 hyperresponsive and six normal) were subjected to both the MCH solution and powder tests in random sequence. No significant differences in FEV1 and PD20 values were found between the challenge tests performed with liquid and powder formulations of methacholine.
Conclusions: Powders of MCH having high respirability of the delivered doses can be prepared by spray drying. They allow for the performance of a challenge test using a dry powder inhaler. The powder dose series can be an alternative to the current dosimetric test with MCH solutions.

Keywords: Respirable particles; Hyperresponsiveness; Bronchoconstriction; Dry powder inhaler; Methacholine; Challenge test

1. Introduction

Asthma is a disease in which airway inflammation causes the airflow in the lung to be reduced. Chronically inflamed airways are hyperresponsive and bronchoconstriction may be produced by a variety of exogenous stimuli. The evaluation of bronchial hyperresponsiveness is a tool for identifying asthma either in epidemiological studies or preventive medicine. The methacholine challenge test is a method for assessing airway responsiveness.

Methacholine chloride (MCH), a derivative of acetylcholine, shows greater duration and selectivity of action than the parent compound and is well tolerated without producing systemic effects (Parker et al., 1965; Chatham et al., 1982; Yan et al., 1983; Hopp et al., 1984; O'Connor et al., 1987). Owing to stability constraints, methacholine is distributed as a crystalline powder in sterile and sealed vials. The powder is deliquescent (Windholz et al., 1983) and must be stored refrigerated in desiccators. The solutions to be nebulized are prepared with sterile saline and must be used immediately or stored at 4 °C to avoid contamination and decomposition.

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Bronchial challenge testing with methacholine chloride entails the inhalation of an aerosol of one or more ascending concentrations of the solution. Results of pulmonary function tests (spirometry) performed at baseline and after each inhaled concentration are used to quantitate the response. The target level for a positive challenge is defined as a decrease of 20% from the baseline forced expiratory volume in the first second (FEV1) or of the postdiluent FEV1 value (Scanlon and Beck, 1994; Elsasser et al., 1996; Spence et al., 1996). However, the use of dosimeters or nebulizers producing different aerosol size distributions represents a key variable in the response obtained at the airway level. In fact, this can affect the sensitivity to the stimulus, the level of bronchoconstriction or the maximum attainable effect (American Thoracic Society, 1995).

Therefore, the possibility to produce a bronchial challenge test based on the inhalation of methacholine powder formulations could significantly improve the test performance, as it would assure the accuracy of the delivered and respirable doses by means of a reliable dry powder inhalation technique. Unfortunately, the unfavorable physico-chemical properties of MCH and the microgram dose range have hindered the preparation of a respirable powder of pure methacholine.

Inhalation powders have to be prepared with excipients able to modify the unfavorable characteristics of MCH in order to obtain reliable metering, aerosolizing and deposition of the powder dose (Murakoshi et al., 2005; Nakate et al., 2005).

The aim of this work was the preparation by spray drying technique of methacholine inhalation powders capable of remaining stable at normal storage conditions and exhibiting reproducible delivered doses and fine particle doses when used in a dry powder inhaler (DPI). The powder respirability was studied by means of the Turbospin® DPI, using a Twin Stage Impinger, which is considered a suitable apparatus for development studies.

A dosimetric challenge test, comparing MCH pulmonary dry powder and MCH solution for asthma detection, was performed in 12 patients.

2. Materials and methods

2.1. Materials

Methacholine hydrochloride was purchased from Sigma–Aldrich (I-Milan); hydroxypropylmethylcellulose (HPMC, Methocel E3) was obtained from Colorcon Ltd. (Orpington, UK). Mannitol was Eur. Pharm. Grade. Solvents and reagents were of analytical grade.

2.2. Preparation of methacholine powders by spray drying

Solutions in purified water containing methacholine hydrochloride (0.06–1 parts), mannitol (98.0–98.94 parts) and 0.5–1 part of hydroxypropylmethylcellulose were prepared. Spray drying was performed on a "Mini Spray-Dryer Büchi" mod. 191 (BÜCHI Labortechnik AG, Flawil, Switzerland) in the following conditions: nozzle diameter 0.7 mm, air flow 600 Nl/h, aspiration 35 m^3/h, inlet temperature 130 °C, solution feed rate 6.5 ml/min, outlet temperature 45–65 °C.

2.3. Characterization of methacholine powders

SEM photographs of the powders were taken using a scanning electron microscope (JSM-6400, Jeol, Japan) and the volume diameter was determined by laser diffraction (Mastersizer®, Malvern Instruments Ltd., Malvern, UK) upon dispersion of the microparticles in acetonitrile (Fluka, UK) and using a 45 mm lens.

The methacholine content of the spray-dried powders was measured by HPLC on an LC 10AS (Shimadzu, Japan) in the following conditions: column C18 Bondapak® 3.9 mm × 300 mm (Waters, Milford, MA, USA); mobile phase 0.02 M sodium heptansulphonate:methanol (60:40), flow rate 1 ml/min; detector wavelength 210 nm. Mannitol content was measured by periodate titration (Higuchi and Bronchmann-Hanssen, 1961).

2.4. Aerodynamic assessment

Since different pre-metered doses of methacholine are required for the test, several inhalation powders had to be prepared containing 12.5–200 μg of methacholine dispersed in 20 mg of powder.

For the aerodynamic assessment, 20 mg of powder were metered in type 2 gelatin capsules. A suitable passive dry powder inhaler (Turbospin®, PH&T, I-Milan) and Apparatus A of European Pharmacopoeia 5th Ed. (Glass Impinger) were employed (air flow 60 ± 5 l/min). The pump was operated for 5 s. Fractions deposited, respectively, in the upper chamber, lower chamber and inhaler adapter and capsule together were quantified by measuring mannitol content in order to determine the mass balance and the fine particle fraction (FPF). Six tests were completed for each DPI formulation.

2.5. Clinical study

Twelve subjects with a history of hyperresponsiveness and six normal subjects as controls were enrolled in a clinical study (Table 1). Written informed consent was obtained and the Ethics Committee approved the trial (Azienda Policlinico Umberto I, University of Roma "La Sapienza"; 14/9/2006; prot.550/06). Each patient underwent both the conventional dosimetric challenge test with MCH solution and the test with methacholine

Table 1
Patient characteristics

Hyperresponsive subjects ($n = 12$)	
Sex	4M, 8F
Age (years)	27 ± 8.5
Mean basal FEV1 (L)	3.25 ± 0.5
Normal subjects ($n = 6$)	
Sex	4M, 2F
Age (years)	27.3 ± 7.5
Mean basal FEV1 (L)	3.53 ± 0.4

Table 2
Aerosol characteristics of MCH solutions delivered by the Mefar MB3 dosimeter and standard MB3 ampoule

	MMAD (μm)	GSD (μm)
Diluent	2.15	1.45
MCH 0.125%	1.31	1.50
MCH 0.25%	1.38	1.49
MCH 1%	1.76	1.49

Mass median aerodynamic diameter and geometric standard deviation.

dry powder (7 days apart) in random sequence. Basal FEV1 was >80% of that predicted according to international protocol (Crapo et al., 2000). Spirometric parameters were measured at time 0 and then at each cumulative dose step (0, 12.5, 25, 50, 100, 200, 400, 800, 1600 μg of methacholine).

MCH solution was obtained by reconstituting lyophilized methacholine (Lofarma, I-Milan) in distilled water (dilutions at 1, 0.25, and 0.125%) and then delivered by means of a Mefar MB3 dosimeter (I-Bovezzo) and MB3 ampoule (3 ml). The operating conditions were set as follows: compressed air 1.75 atm; air flow 9 l/min; nebulization time 1 s; output 10 ± 0.2 μl. Aerosol characteristics of MCH solutions were measured with Aerosizer® (Table 2). Methacholine powder was administered at the same dose steps as those considered for solution. Dry powder inhalers loaded with mannitol or with one of five formulations of methacholine powder were used (step 1: spray-dried mannitol 20 mg; subsequent steps: 12.5, 25, 50, 100, 200 μg of methacholine dispersed in approximately 20 mg of spray-dried powder). When doses higher than 200 μg were required, 2 or more 200 μg capsules were used. Patients were instructed as to how to use the inhaler.

A salbutamol metered-dosed inhaler ($2\,\mu g \times 100\,\mu g$) was administered at the end of the tests in hyperresponsive subjects and spirometry was performed to assess functional recovery. FEV1 was measured using a spirometer (Quark PFT1, Cosmed s.r.l., I-Pavona di Albano). Hyperresponsiveness was defined as the PD20 value, i.e., the dose of methacholine (μg) that causes a 20% reduction of basal FEV1.

GraphPad was used for statistical analysis of the results. Student's *t*-test for the direct comparison and ANOVA were performed. Statistical significance was accepted at $p < 0.05$.

3. Results and discussion

3.1. Spray-dried methacholine powder formulations and characteristics

The micronization of methacholine to obtain a respirable powder was performed by spray drying water solutions containing MCH together with suitable excipients. Several spray-dried powders were prepared and carefully tested especially with respect to their aerodynamic properties. It is well known that the aerodynamic performance of a powder depends on three main particle characteristics – size, density and shape (Hinds, 1982). Thus, in order to find the most appropriate composition of methacholine and excipients, these particle characteristics were used as reference parameters to further progress in the formulation.

Several attempts were made to formulate inhalation MCH powders suitable for dosimetric application. Since methacholine as it is could not be used in the dry powder inhaler, owing to its hygroscopic nature, the first step was to identify an adjuvant capable of protecting the substance from moisture. Mannitol was chosen in consideration of its inertness and non-hygroscopicity and because it is itself a bronchoconstrictor used to assess airway hyperresponsiveness (Glover et al., 2006). In addition, mannitol is often present in pulmonary dry powder formulations. Preliminarily, a mannitol solution was spray-dried having a total solid content of 9% (w/v), from which smooth particles were obtained with a median volume diameter around 9.0 μm and a spherical shape (Fig. 1A). Considering the 20 mg total amount of powder metered in the gelatin capsule, the percentage emitted by the Turbospin® inhaler was $94 \pm 1\%$ and the percentage recovered from the lower chamber of the impinger was around $24.4 \pm 3.1\%$, despite the large geometric size of particles.

When methacholine was dissolved in the mannitol solution (2%, w/w of the solid content, i.e., the Mannitol/MCH ratio was 98:2), the percentage of the spray-dried powder recovered from the impinger's lower stage decreased to below 5%. The result was attributed to the observed tendency of these particles to agglomerate and stick to the capsule wall. This mannitol/methacholine powder had a particle size around 8.0 μm, with quite a rough surface and a spheroid shape (Fig. 1B).

Several successive modifications were introduced (spray drying conditions, reduction in mannitol content, addition of isoleucine), although these resulted as being ineffective (data not shown). The powder fraction deposited in Stage 2 rose to $15.5 \pm 1.3\%$ when the MCH content was reduced to 1% (w/w) with respect to the total amount of solid (Mannitol/MCH 99/1). In this case the mean particle size was around 6 μm, but the shape was more spherical and the tendency to agglomerate disappeared (Fig. 1C).

Then, with the aim of further increasing the fine particle fraction, the solution containing mannitol and MCH in the ratio 99:1 was sprayed at a more diluted solid content, i.e., 1% (w/v) instead of 9% (w/v). The dilution of the sprayed solution resulted in an evident reduction in size (Fig. 1D) and a higher fine fraction deposited in Stage 2 (from 15.5 to $21.7\% \pm 2.8\%$).

Maintaining this concentration of the sprayed solution, low viscosity hydroxypropylmethylcellulose (HPMC) was added to the Mannitol/MCH (99/1) solution as a particle shaper at a concentration of 0.5% (w/w) of the total solid content. HPMC was chosen since it had previously been used as particle stabilizer in fluticason-17 propionate microparticles manufactured by spray drying (Steckel et al., 2003). The fine particle fraction of the powder obtained (Mannitol/MCH/HPMC 98.5/1/0.5) increased to $34 \pm 3.0\%$ (Fig. 1E). When HPMC was further increased to 1% (w/w), the percentage of powder deposited in the lower chamber of the impinger jumped to $44.4 \pm 2.4\%$ and the particles exhibited a round shape and dented surface (Fig. 1F). This morphology was attributed to the effect of HPMC on the drying of the droplet during spray drying. In fact, particles prepared from the mannitol/methacholine solution without HPMC appeared perfectly smooth and round-shaped. The shrinking of the droplets during

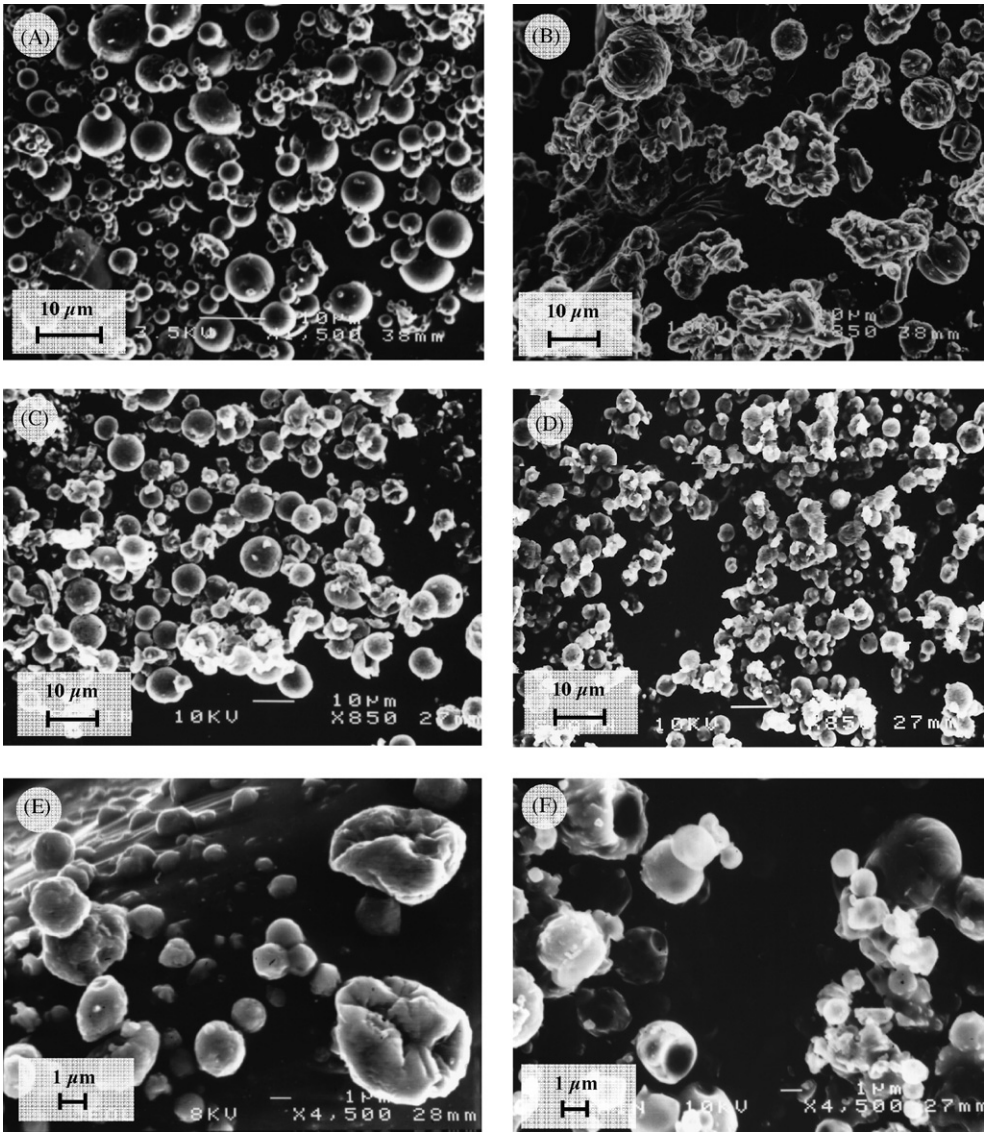

Fig. 1. SEM images of spray-dried powders: Mannitol 100 from 9% (w/v) solution (A), Mannitol/MCH 98/2 from 9% (w/v) solution (B), Mannitol/MCH 99/1 from 9% (w/v) solution (C), Mannitol/MCH 99/1 from 1% (w/v) solution (D), Mannitol/MCH/HPMC 98.5/1/0.5 from 1% (w/v) solution (E) and Mannitol/MCH/HPMC 98/1/1 from 1% (w/v) solution (F).

spray drying was likely caused by the presence of the polymer in the solution.

Therefore, we selected the Mannitol/MCH/HPMC 98/1/1 formulation as the reference for preparing the series of test powders to be used in the challenge test with MCH powder. The compositions and size characteristics of all test powders, which contained increasing amounts of methacholine dispersed in a total mass of approximately 20 mg, are reported in Table 3. The true density of these spray-dried powders (1.5 g/cm^3) was not different from mannitol density, owing to the preponderant amount of this component in the formulation. The drying procedure was very efficient and the residual water content in the powders ranged between 0.5 and 1.4% (w/w).

These spray-dried powders consisted of microparticles having a round shape characterized by an irregular dented surface. All powders exhibited a bimodal particle size distribution and the geometric mean diameter ranged between 1.56 and 2.47 μm. The maximum amount of methacholine in the series of spray-dried powder was 1% and the minimum was 0.06% (w/w).

In summary, the spray drying procedure and excipients selected made feasible the preparation of MCH microparticles useful for inhalation. Mannitol was capable of protecting MCH from humidity, in particular when a small amount of HPMC as particle shaping adjuvant was employed. Owing to the particle size distribution, density and shape of the Mannitol/MCH/HPMC 98/1/1 spray-dried powder, this was selected for the challenge test and further studied for aerosol formation and pulmonary deposition.

3.2. Aerodynamic assessment

Several parameters can be measured when assessing the aerodynamic behavior of a powder by means of the Twin Stage Impinger (TSI) (Lucas et al., 1998). This apparatus, correspond-

Table 3
Composition, dimensional characteristics (mean geometric diameter, d_g, and geometric standard deviation, σ_g,) FPF% and FPD of spray-dried test powders prepared for the challenge test

Code	Composition % (w/w)	MCH metered dose (μg)	d_g (μm)	σ_g	FPF (%)	FPD (μg)
Test powder #1	Mannitol 98.94 MCH 0.06 HPMC 1	12.5	1.57	3.08	53.8 ± 3.2	6.7 ± 0.4
Test powder #2	Mannitol 98.87 MCH 0.13 HPMC 1	25	1.56	2.94	48.3 ± 3.9	12.1 ± 1.0
Test powder #3	Mannitol 98.75 MCH 0.25 HPMC 1	50	2.24	3.15	40.8 ± 0.4	20.5 ± 0.2
Test powder #4	Mannitol 98.5 MCH 0.5 HPMC 1	100	2.47	2.81	41.2 ± 2.8	41.2 ± 2.8
Test powder #5	Mannitol 98 MCH 1 HPMC 1	200	1.71	3.05	43.9 ± 0.7	87.8 ± 1.4

ing to Apparatus A of European Pharmacopoeia 5th Ed., was used for the aerodynamic assessment of fine particles. The amounts of powder deposited in the upper and in the lower stage were measured. In addition, in order to calculate the mass balance, the amounts of powder remaining inside the capsule and inside the inhaler adapter after actuation were measured as well. The amount of powder deposited in the lower chamber, expressed as a percentage of the labeled dose, yields the Fine Particle Fraction (FPF). This is the actual fraction of the powder that is capable of entering and depositing in the lower respiratory region (respirability). The cut-off value of the lower chamber in the operating conditions adopted (air flow through the apparatus of 60 ± 5 l/min measured at the inlet to the throat) was 6.4 μm. The present cut-off value for FPF has been fixed by the European Pharmacopoeia at 5 μm as aerodynamic diameter.

Turbospin® was used as a dry powder inhaler passive device since it is a medium resistance device (0.09 $(cmH_2O^{1/2})/(l\,min^{-1})$; Meakin et al., 1996) limiting the effect of flow rate differences and breathing volume between the patients.

Fig. 2 shows a comparative view of the deposition of the spray-dried powders in the impinger, highlighting the influence of the formulation on the aerodynamic behavior of the powders. The spray-dried powder of mannitol alone was mainly deposited in the upper chamber (Stage 1), with a fine particle fraction of about 20%. In contrast, the spray-dried powder Mannitol/MCH/HPMC 98/1/1 was mainly deposited in the lower chamber (Stage 2). Low MCH content and low solid concentration in the solution to be sprayed, together with the presence of HPMC as shaper, were the formulation parameters improving powder respirability. The changes in particle shape and size observed from SEM pictures clearly underline the influence of these parameters on the aerodynamic behavior of the powders (Fig. 1).

Therefore, methacholine 1% (w/w) was considered as being the maximum concentration of the active substance in the preparations constituting the challenge series. The five test powders of Table 3 were metered in gelatin capsules in an amount of 20 ± 2 mg to obtain a series of five capsules containing the challenge doses of methacholine from 12.5 to 200 μg for the dosimetric administration with Turbospin®. For the sake of clarity, we made available a series of gelatin capsules already dosed to be used in sequence with the Turbospin® inhaler at a flow rate of 60 l/min. A capsule containing approximately 20 mg of spray-dried mannitol was prepared to be used in Step 1 of the challenge test. Fig. 3 shows the aerodynamic characteristics of the five test powders used. As can be observed, all the powders showed high values of fine particle fraction, higher than the usual values found with powder inhalers.

The sequence of doses expressed as metered dose and fine particle dose are summarized in Table 3 together with the dimensional characteristics of the powders. There was a very good linear relationship between the metered dose and the dose deposited in Stage 2 of the Twin Stage Impinger (FPD), as illustrated in Fig. 4. This linearity facilitates the set-up of the series of methacholine doses to be used in the challenge test.

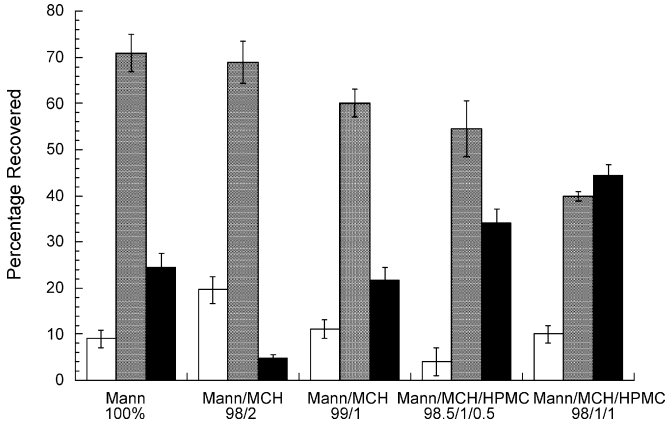

Fig. 2. Aerodynamic assessment by TSI during formulation development: percentage of inhalation powder recovered in Stage 1 (grey), in Stage 2 (black) and in capsule and inhaler adapter (white); mean value and standard deviation; $n = 6$.

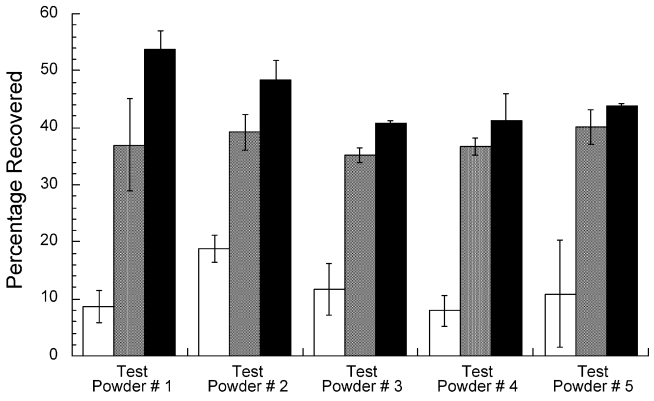

Fig. 3. Aerodynamic assessment by TSI of test powders: percentage of powder recovered in Stage 1 (grey), in Stage 2 (black) and in capsule and inhaler adapter (white); mean value and standard deviation; $n = 6$.

The amount of methacholine deposited in Stage 2 estimates the amount of drug available for deposition in the lower respiratory tract, hence capable of evoking the bronchoconstrictive response. The combination of the label MCH dose with the FPF allows the experimenter to construct the relationship between the amount administered and the response evoked, leading to the calculation of the PD20 value.

Summarizing, the *in vitro* delivered and deposited doses indicated a promising respirability of the prepared MCH pulmonary powders. The linear relationship between the metered dose and fine particle dose is the basis for setting the dosing sequence. A guided procedure for delivering methacholine during bronchial challenge tests was established.

3.3. Clinical assessment of MCH powders in comparison with MCH solutions

All 18 participants in the comparative trial underwent both types of challenge test, with MCH solution and MCH powder, respectively. In Fig. 5, PD20 values for each of the 12 hyperresponsive subjects are reported. The values obtained with methacholine solution (MCH-S) and dry powder (MCH-P) in the hyperresponsive subjects were not significantly different ($p = 0.91$). In fact, in these hyperresponsive subjects the mean FEV1 values at each dose step did not show significant differences for the two tests performed with different formulations (Fig. 6) ($p = 0.72$).

In the normal subjects (i.e., not hyperresponsive) used as controls, neither the FEV1 values at each dose step of the test nor the percentage of reduction at 1600 μg of methacholine (end of the test) were significantly different from the initial values (data not shown) ($p = 1.0$).

Spirometry performed 15 min after inhalation of salbutamol showed a complete recovery of FEV1 after the test with both MCH solution and MCH powder.

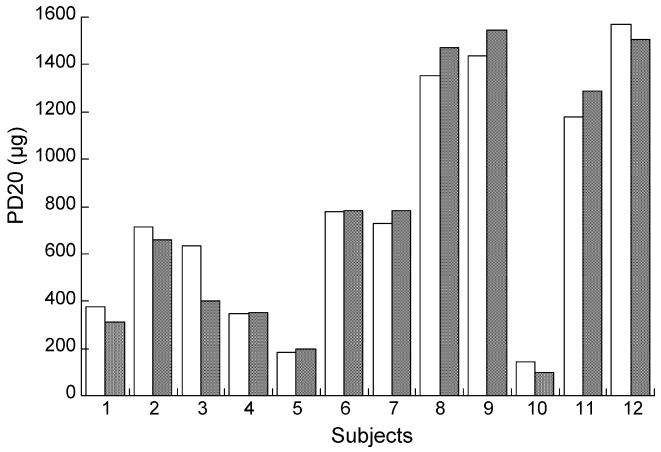

Fig. 5. PD20 values in 12 hyperresponsive subjects after administration of methacholine solution (white bar) and dry powder (grey bar), ($p = 0.91$).

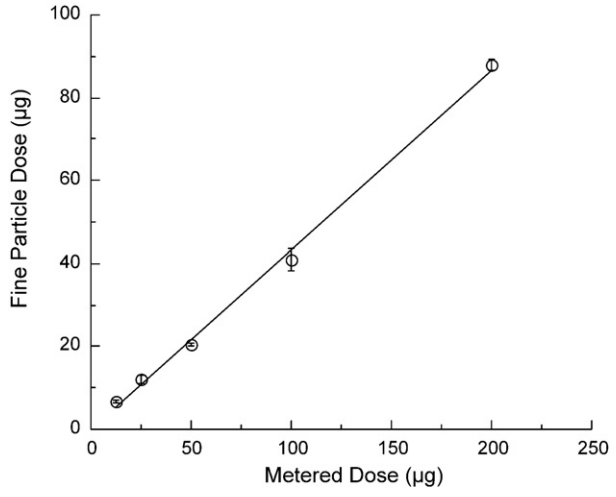

Fig. 4. Linear regression ($y = 0.1 + 0.43303x$ $R^2 = 0.9973$) between the MCH metered dose and the dose deposited in Stage 2 of the Twin Stage Impinger (FPD); mean value and standard deviation; $n = 6$.

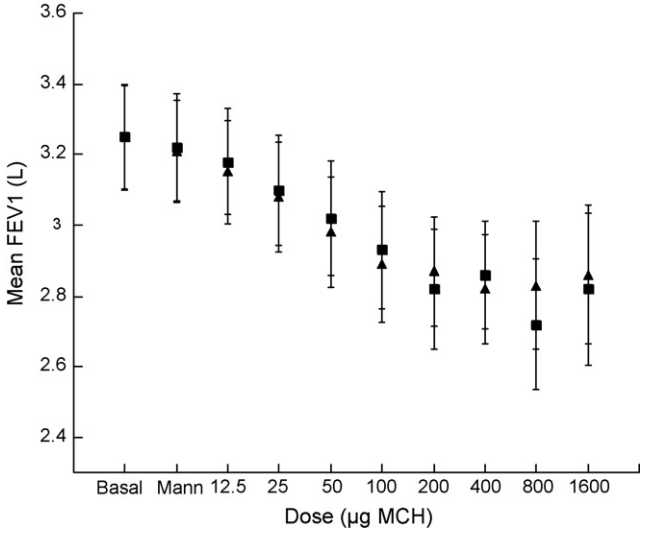

Fig. 6. Mean FEV1 measured in the 12 hyperresponsive subjects basally, after mannitol administration and after the dose sequence from 12.5 to 1600 μg of methacholine solution (■) and methacholine powder (▲); mean value and standard deviation.

Finally, no significant side-effects were observed during or after the test. Coughing was reported in seven out of the 12 hyperresponsive subjects during the MCH-S test and in 10 out of the 12 subjects during the MCH–P test.

Clinically, spray-dried methacholine powder showed adequate safety and performance in diagnosing airway hyperresponsiveness as conventionally done by dosimetric test with solutions. In fact, the methacholine powder led to results similar to MCH solution in identifying hyperresponsive and normal subjects, with no unexpected side-effects. Combining each metered dose with the measured fine particle fraction, the amount of methacholine available for deposition is more reliably predicted than in the case of nebulized solution. Therefore, the relationship between MCH dose and the response in terms of FEV1 is stronger.

4. Conclusions

From the results obtained it can be concluded that the spray drying procedure allows for the preparation of respirable microparticles of MCH dispersed in mannitol to be usefully applied in the dosimetric challenge test with methacholine powder. Hence, a series of MCH doses metered in gelatin capsules to be used with a dry powder inhaler has been provided for clinicians.

Methacholine inhalation powders, overcoming the unfavorable characteristics of the substance, in particular its deliquescent nature, were prepared by spray drying. Mannitol and HPMC low viscosity were found to be essential in structuring the particles in view of aerosol formation and pulmonary deposition. Furthermore, the respirability of the aerosol improved when the particles were produced by spray drying of diluted solutions.

Finally, the dry powder inhalation technology with premetered doses was found to be a reliable technique for the execution of the bronchoprovocative test with methacholine. This new tool, which could improve the clinical procedure as it relies on precise and accurate MCH metered doses, has been brought to the attention of physio-pathologists. In fact, the metered, delivered and deposited doses at each step of the sequence of administrations were known, thus avoiding the evaluation of the performance of dosimetric apparatuses, as has to be done with MCH solutions. Hence, the dry powder inhalers could be used for delivering methacholine during bronchial challenge tests if these results are confirmed on larger clinical studies in patients.

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References

American Thoracic Society, 1995. Standardization of Spirometry, 1994 Update. Am. J. Respir. Crit. Care Med. 152, 1107–1136.

Chatham, M., Bleecker, E.R., Norman, P., Smith, P.L., Mason, P., 1982. A screening test for airways reactivity. An abbreviated methacholine inhalation challenge. Chest 82, 15–18.

Crapo, R.O., Casaburi, R., Coates, A.L., Enright, P.L., Hankinson, J.L., Irvin, C.G., MacIntyre, N.R., McKay, R.T., Wanger, J.S., Anderson, S.D., Cockcroft, D.W., Fish, J.E., Sterk, P.J., 2000. Guidelines for methacholine and exercise challenge testing-1999. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. Am. J. Respir. Crit. Care Med. 161, 309–329.

Elsasser, S., Donna, E., Demirozu, C.M., Danta, I., Wanner, A., 1996. Metaproterenol responsiveness after methacholine\and histamine-induced bronchoconstriction. Chest 110, 617–623.

Glover, W., Chan, H.K., Eberl, S., Daviskas, E., Anderson, S., 2006. Lung deposition of mannitol powder aerosol in healthy subjects. J. Aerosol Med. 19, 522–532.

Higuchi, T., Bronchmann-Hanssen, E., 1961. Pharmaceutical Analysis. Interscience Publishers, New York.

Hinds, W., 1982. Aerosol Technology: Properties, Behavior, and Measurement of Airborne Particles. Wiley Interscience, New York.

Hopp, R.J., Bewtra, A.K., Nair, N.M., Townley, R.G., 1984. Specificity and sensitivity of methacholine inhalation challenge in normal and asthmatic children. J. Allergy Clin. Immunol. 74, 154–158.

Lucas, P., Anderson, K., Staniforth, J.N., 1998. Protein deposition from dry powder inhalers: fine particle multiplets as performance modifiers. Pharm. Res. 15, 562–569.

Meakin, B.J., Woodcock, P.M., Segu, A., Niccolai, F., Vecchio, C., 1996. Evaluation of the resistance to airflow through Turbospin in comparison with dry powder inhalers available on the market. Boll. Chim. Farmaceutico-Anno 135, 107–109.

Murakoshi, H., Saotome, T., Fujii, Y., Ozeki, T., Takashima, Y., Yuasa, H., Okada, H., 2005. Effect of physical properties of carrier particles on drug emission from a dry powder inhaler device. J. Drug Deliv. Sci. Technol. 15, 223–226.

Nakate, T., Yoshida, H., Ohike, A., Tokunaga, Y., Ibuki, R., Kawashima, Y., 2005. Improvement in the variation of inhalation properties of an FK888 dry powder inhaler using an E-haler, by adding coarse lactose in spherical soft agglomerates of fine drug particles, and its absorption in healthy volunteers. J. Drug Deliv. Sci. Technol. 15, 167–175.

O'Connor, G., Sparrow, D., Taylor, D., Segal, M., Weiss, S., 1987. Analysis of dose-response curves to methacholine. An approach suitable for population studies. Am. Rev. Respir. Dis. 136, 1412–1417.

Parker, C.D., Bilbo, R.E., Reed, C.E., 1965. Methacholine aerosol as test for bronchial asthma. Arch. Intern. Med. 115, 452–458.

Scanlon, P.D., Beck, K.C., 1994. Methacholine inhalation challenge. Mayo. Clin. Proc. 69, 1118–1119.

Spence, D.P., Graham, D.R., Jamieson, G., Cheetham, B.M., Calverley, P.M., Earis, J.E., 1996. The relationship between wheezing and lung mechanics during methacholine-induced bronchoconstriction in asthmatic subjects. Am. J. Respir. Crit. Care Med. 154, 290–294.

Steckel, H., Rasenack, N., Villax, P., Müller, B.W., 2003. In vitro characterization of jet-milled and in-situ-micronized fluticasone-17-propionate. Int. J. Pharm. 258, 65–75.

Windholz, M., Budavari, S., Blumetti, R.F., et al. (Eds.), 1983. The Merck Index, 10th ed., p. 850. Merck and Company Rahway, NJ.

Yan, K., Salome, C., Woolcock, A.J., 1983. Rapid method for measurement of bronchial responsiveness. Thorax 38, 760–765.