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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