Preliminary Report

Metered-Dose Inhaler To Deliver Methacholine in Bronchial Provocation Testing*

A Pilot Study

Nicolas Roche, MD; Fayssal El Husseini, MD; Sylvie Labrune, MD; Violaime Giraud, MD; Thierry C. Chinet, MD; and Gérard J. Huchon, MD, FCCP

**Background:** Nonspecific bronchial provocation tests may be simplified by the use of hand-held devices to deliver methacholine.

**Objective:** To study the feasibility of using a metered-dose inhaler (MDI) to administer methacholine in bronchial provocation tests, and the ability of such a device to diagnose bronchial hyperresponsiveness (BHR) accurately.

**Methods:** In an open randomized crossover pilot study, we compared the provocative dose that induces a 20% fall in FEV₁ (PD_{20} FEV₁) obtained with the methacholine MDI with that obtained using a conventional nebulizer in 20 hyperresponsive and 20 nonhyperresponsive subjects. The MDI delivers 400 doses of 100 μg of methacholine, and was used via a spacer. Bronchial hyperresponsiveness (BHR) was defined as a PD_{20} FEV₁ <2,000 μg with the conventional test using the nebulizer. The tests were performed in each subject in a randomized order, 1 to 7 days apart.

**Results:** Of the subjects who had a nebulizer PD_{20} FEV₁ <2,000 μg, all but one had an MDI PD_{20} FEV₁ <500 μg. When 500 μg was taken as the threshold for the diagnosis of BHR with the MDI test, the accuracy of this test to diagnose BHR was 97.5%, and the two tests were highly concordant for the diagnosis of BHR (Pearson χ², 36.19; p<0.0001).

**Conclusion:** A hand-held device may be suitable for delivery of methacholine during bronchial provocation tests, if these results are confirmed in large samples. *(CHEST 1998; 113:1684-88)*

Key words: asthma; bronchial hyperresponsiveness; bronchial provocation tests; metered-dose inhaler; methacholine

Abbreviations: BHR = bronchial hyperresponsiveness; MDI = metered-dose inhaler; PD_{20} = provocative dose causing a 20% fall in FEV₁

Bronchial responsiveness is measured for clinical assessment of patients with chronic airways diseases—especially asthma—for research purposes and in epidemiologic studies. Pharmacologic agents used to assess nonspecific bronchial hyperresponsiveness (BHR) are mainly histamine or acetylcholine and its derivatives, carbachol and methacholine (acetyl-β-methyl choline chloride). These agents are delivered by nebulizers, either during tidal breathing or by dosimeter methods. To assess the bronchial response, the most reproducible and frequently used variable is FEV₁. The results are plotted in dose-response curves from which several indexes can be calculated; dose of agent that induces a 20% fall of FEV₁ from baseline (PD_{20}) is the most reproducible.

Non-specific bronchial provocation tests could be simplified by use of methacholine metered-dose.
inhaler (MDI), because it is portable and easy to handle, and because there would be no need to purchase a nebulizer and to spend time preparing solutions. Moreover, the use of a methacholine MDI may obviate the need to perform bronchial provocation tests in special rooms with increased ventilation. To assess the feasibility of the administration of methacholine with an MDI in nonspecific pharmacologic bronchial provocation tests, and the ability of such a device to discriminate between hyperresponsive and nonhyperresponsive subjects, we performed an open randomized crossover pilot study comparing the results of a test using a methacholine MDI with those of a reference test using a conventional nebulizer to deliver methacholine.

MATERIALS AND METHODS

Subjects

We studied 20 subjects with BHR and 20 subjects without BHR. BHR was defined by a PD_{20} FEV_1 <2,000 μg of methacholine. Demographic characteristics of all subjects are shown in Table 1. Among the subjects with BHR, 18 had either diagnosed asthma or symptoms consistent with asthma. The two others were free of respiratory symptoms. Nonhyperresponsive subjects had no history of chronic respiratory disease. None of the subjects had a history of exposure to occupational sensitizers, smoking, or acute respiratory illness within 3 months before the study. The patients with asthma had stable mild-to-moderate asthma as defined by the criteria of the international consensus report on asthma. None had been treated with long-acting β-agonists, anticholinergic agents, or methylxanthines. Other medications (corticosteroids, antihistamines, cromones) were permitted, but the dose had to be stable beginning 3 weeks prior to the study and for the duration of the study. All subjects had a baseline FEV_1 >70% of predicted value and did not respond to saline solution inhalation (see below). All subjects signed an informed consent form. The protocol was approved by the ethics committee of our institution.

Study Design

This was an open randomized crossover pilot study. In each subject, bronchial provocation test with the methacholine MDI was compared with a reference test that uses a conventional nebulizer to deliver methacholine. For each subject, tests were performed in a randomized order at the same time of the day to limit the consequences of spontaneous diurnal variations of bronchial reactivity.7,8 Nebulizer and MDI tests were separated by 1 to 7 days (1) to avoid tachyphylaxis to methacholine, which has been demonstrated in nonasthmatic subjects and lasts <24 h, and (2) to ensure reproducibility for each subject, since the level of BHR has been shown to remain stable over a period of 8 days in clinically stable asthmatic subjects.9-12

Bronchial Provocation Testing

Treatment with short-acting β-agonist drugs was withdrawn 6 h before each test. Spirometry was performed with a spirometer (Minijhardt Volulograph Spirometer; MSR; Paris, France). After baseline measurement, FEV_1 was measured 60 and 120 s after each aerosol, the lowest technically satisfactory value being used in the analysis.1 In each subject, a bronchial challenge with nebulized normal saline solution (NaCl 0.9%) was performed on the day of the first bronchial provocation testing. Subjects were not included if postsaline solution FEV_1 was <90% of baseline value. Tests ended either when a fall in FEV_1 of 20% from baseline was obtained or when the maximal cumulative dose of methacholine was reached (see below). BHR was defined by a PD_{20} FEV_1 at <2,000 μg of methacholine with the nebulizer test.3

Nebulizer Test

Solutions were administered with a nebulizer (Aerosolan; MSR; Paris, France). The nebulizer generates heterodisperse droplets with a mass median aerodynamic diameter of 5 μm and a geometric SD of 1.2 μm, as measured by cascade impactor. The aerosol was delivered through a mouthpiece during quiet tidal breathing at spontaneous frequency and using a nose clip. The mouthpiece was connected to the nebulizer by an inspiratory valve and to a spirometer (Aerotest; MSR) by an expiratory valve. The methacholine solution was prepared the day of each test by diluting 100 mg of lyophilized acetyl-β-methyl choline chloride (Pharmacie Centrale des Hôpitaux; Paris, France) in 10 mL of normal saline solution. Delivered doses of aerosol were determined by the inhaled volume measured by the spirometer and were 100, 200, 400, 800, 300, 300, and 300 μg (maximal cumulative dose: 2,400 μg of methacholine).

MDI

The methacholine MDI (Pharmacie Centrale des Hôpitaux; Paris, France) contains 20 mL of solution and propellant, consisting of the following: acetyl-β-methyl choline chloride, 40 mg; sorbitol sesquioleate, 20 mg; ethanol 100%, 1 mL; and dichlorodifluoro methane, 19 mL. It generates heterodisperse droplets with a mass median aerodynamic diameter of 2.74 μm and a geometric SD of 1.58 μm, as measured by laser-Doppler flowmetry. Each dose (0.05 mL) contains 100 μg of methacholine. The MDI can deliver 400 doses. Aerosol was delivered through a conic 750-mL extension device (Nebulhaler; Astra Draco; Lund, Sweden) and using a nose clip. Each inhalation of the solution was preceded by a quiet expiration, and followed by an apnea of 10 s. Delivered doses were 100, 200, 200, 200, 200, 100, and 100 μg (maximal cumulative dose, 1,100 μg of methacholine). Doses of 200 μg were given by two separate actuations at a 30-s interval, each preceded by a quiet expiration, and followed by a 10-s apnea.

Table 1—Demographic and Baseline Characteristics of Subjects

<table>
<thead>
<tr>
<th>Subjects</th>
<th>With BHR</th>
<th>Without BHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>28.7±1.7</td>
<td>25.6±0.5</td>
</tr>
<tr>
<td>Male/female ratio</td>
<td>7/13</td>
<td>16/4</td>
</tr>
<tr>
<td>Baseline FEV_1 (% of predicted value)</td>
<td>92.25±2.11</td>
<td>100.45±3.00*</td>
</tr>
<tr>
<td>MDI test</td>
<td>90.65±2.35</td>
<td>100.15±2.92*</td>
</tr>
<tr>
<td>Postsaline solution FEV_1 (% of baseline)</td>
<td>100.46±1.57</td>
<td>99.84±0.87</td>
</tr>
</tbody>
</table>

*p<0.001 when compared with hyperreactive subjects.
Data were plotted in cumulative dose-response curves, and the bronchial response was assessed by FEV$_1$ (percent of baseline value). When a 20% fall of FEV$_1$ was reached, the value of PD$_{20}$ FEV$_1$ (cumulative dose of methacholine provoking a 20% fall of FEV$_1$) was obtained by interpolation from the dose-response curve. When it was not reached after the highest cumulative dose of methacholine (ie, 1,100 µg with the MDI and 2,400 µg with the nebulizer), PD$_{20}$ FEV$_1$ was calculated by extrapolation from the dose-response curve; the calculated value was accepted only when it was less than [(2×last administered dose) + administered cumulative dose], ie, 3,000 µg with the nebulizer test and 1,300 µg with the MDI test. The ratio (MDI—PD$_{20}$ FEV$_1$)/(nebulizer—PD$_{20}$ FEV$_1$) was calculated for each subject in whom PD$_{20}$ FEV$_1$ was obtained with both tests.

**Statistical Analysis**

Baseline FEV$_1$, postsaline solution FEV$_1$ and PD$_{20}$ FEV$_1$ were compared between tests and between subjects with paired or unpaired t tests, as appropriate. Correlation between MDI PD$_{20}$ FEV$_1$ and nebulizer PD$_{20}$ FEV$_1$ was calculated. Concordance between the two tests for the diagnosis of presence or absence of BHR was analyzed using Pearson $r^2$.$^{13}$ Statistics were performed with statistical software (BMDP Statistical Software Inc; Los Angeles). All results are given in mean±SEM.

**Results**

The demographic and baseline characteristics of subjects are shown in Table 1. Baseline FEV$_1$ was higher in nonhyperreactive subjects than in hyperreactive patients in both nebulizer and MDI tests. In hyperreactive and in nonhyperreactive subjects, there was no difference in baseline FEV$_1$ between the two tests. Postsaline solution FEV$_1$, as a percentage of baseline FEV$_1$, was similar in nonhyperreactive subjects and in hyperreactive subjects.

For subjects with BHR, individual values of PD$_{20}$ FEV$_1$ are shown in Figure 1. With the nebulizer test, mean PD$_{20}$ FEV$_1$ was 821±141 µg in hyperreactive patients. PD$_{20}$ FEV$_1$ could be calculated in only three nonhyperreactive subjects, with values of 2,500, 2,750, and 2,800 µg of methacholine. In other nonhyperreactive subjects, PD$_{20}$ FEV$_1$ could not be calculated and was therefore >3,000 µg. With the MDI test, mean PD$_{20}$ FEV$_1$ was 293±53 µg in hyperreactive patients. PD$_{20}$ FEV$_1$ could be calculated in only one nonhyperreactive subject and was 1,000 µg. In other nonhyperreactive subjects, PD$_{20}$ FEV$_1$ could not be calculated (>1,300 µg).

There was no correlation between values of PD$_{20}$ FEV$_1$ and baseline FEV$_1$ in MDI and in nebulizer tests ($r=0.23$ and $r=0.24$, not significant).

MDI PD$_{20}$ FEV$_1$ and nebulizer PD$_{20}$ FEV$_1$ correlated ($r=0.64$, p<0.01) (Fig 1). The ratio (nebulizer PD$_{20}$ FEV$_1$)/(MDI PD$_{20}$ FEV$_1$) was 3.93±1.11 (median, 2.43), ranging from 0.75 to 18.31.

Of the subjects who were hyperreactive according to the nebulizer test, the MDI PD$_{20}$ FEV$_1$ was <800 µg in all but one. When taking this value as a threshold for the diagnosis of BHR with the MDI test, the overall accuracy of this test was 97.5% when the nebulizer test was taken for reference (sensitivity, 95%; specificity, 100%), and the two methods were concordant for the diagnosis of presence or absence of BHR (p<0.0001).

**Discussion**

We studied the relationship between PD$_{20}$ FEV$_1$ obtained with a bronchial provocation test using a methacholine MDI and PD$_{20}$ FEV$_1$ obtained with a reference test using a nebulizer to deliver methacholine in 20 hyperreactive subjects (ie, PD$_{20}$ FEV$_1$ <2,000 µg) and 20 nonhyperreactive subjects. Results of the two tests correlated, but PD$_{20}$ FEV$_1$ was lower with the MDI test than with the nebulizer test. When taking a threshold of 800 µg to define BHR with the MDI test, the accuracy of this test was 97.5% for diagnosis of BHR when the nebulizer test was taken for reference. The two tests were concordant for diagnosis of BHR, except in one subject.

To establish the precise relationship between the values of PD$_{20}$ FEV$_1$ obtained with the two methods, we increased doses very gradually instead of doubling the dose at each administration, as it is usually done. For this reason and since successive doses of methacholine can be considered as cumulative only during approximately 10 min, we could study only bronchial hypersensitivity—defined as a bronchoconstriction in response to a stimulus that has no...
effect in normal subjects—but not maximal airway narrowing—defined as excessive intensity of the bronchial response.\textsuperscript{15}

Since the main purpose of this preliminary study was to assess the ability of the methacholine MDI to discriminate between hyperresponsive and nonhyperresponsive subjects, we included patients on the basis of their bronchial responsiveness status and studied a group of hyperresponsive subjects and a group of nonhyperresponsive subjects. Further studies should be directed at comparing the results of the methacholine MDI test with those of the reference test in unslected populations of patients with various lung diseases or respiratory symptoms who are referred for bronchial provocation testing.

The mean ratio between nebulizer PD\textsubscript{20} FEV\textsubscript{1} and MDI PD\textsubscript{20} FEV\textsubscript{1} was 3.78. We expected PD\textsubscript{20} FEV\textsubscript{1} with nebulizer and MDI to be different, because pulmonary deposition of aerosols appears to be lower with nebulizers than with an MDI plus spacer (5 to 12% vs 14 to 16%).\textsuperscript{16-20}

However, this ratio was highly variable between subjects, ranging from 0.75 to 18.31; this is probably unrelated to tachyphylaxis to methacholine, because this phenomenon lasts <24 h, which was the minimal interval between the two tests in our study.\textsuperscript{9,21} Intraindividual variations of the degree of BHR are also unlikely, since the two tests were performed within 7 days and at the same time of the day for all subjects and since hyperresponsive patients were all in clinically stable condition during the study.\textsuperscript{10-12} Another possible explanation for these variations may be interindividual differences of reactivity to the diluents of methacholine, i.e., isotonic saline solution for nebulizer and propellant gas, oleic acid and ethanol for MDI. Hyperreactivity to saline solution can be ruled out since it was an exclusion criterion, but we cannot exclude the possibility of BHR to MDI additives, even if none of the subjects responded only to MDI and not to nebulizer-administered methacholine. A more likely explanation would be that there is a difference between subjects in their mode of inhalation with the two devices, inducing differences of methacholine lung deposition from one subject to another; during the MDI test, a spacer was used to limit variations in deposition related to misuse of the MDI, which is observed in about 50% of asthmatic patients, even after they have been taught how to use it;\textsuperscript{22} however, tidal volume and ventilatory frequency were not controlled during nebulizer tests in our study; further studies will be necessary to ensure that the ratio between nebulizer PD\textsubscript{20} FEV\textsubscript{1} and MDI PD\textsubscript{20} FEV\textsubscript{1} is reproducible within subjects. Finally, we noted that the MDI did not work properly in some cases (approximately 20%), the puff being obviously less than normal.

When such abnormalities were detected, the spacer was removed before the subject began to inhale and the dose of methacholine was delivered with a new one. However, some mild abnormalities may have been missed by the investigators, leading to errors in calculation of MDI PD\textsubscript{20} FEV\textsubscript{1} and thus causing interpersonal variations.

Only one subject in the hyperresponsive group had BHR with one test (nebulizer PD\textsubscript{20} FEV\textsubscript{1}, 1,500 \textmu g) but not with the other (MDI PD\textsubscript{20} FEV\textsubscript{1} >1,300 \textmu g). Like all subjects in our population, he was not responsive to the administration of saline solution. Since we could not explain this result, both tests were repeated in this subject, with the same results. In all other subjects, the two tests were in agreement for the diagnosis of presence or absence of BHR. However, the correlation between the two tests was rather low (r=0.64), which suggests that the MDI and nebulizer tests may not be interchangeable to grade severity of BHR.

We observed no side effects with the MDI test (or with the nebulizer test). Similarly, two uncontrolled studies of a methacholine MDI concluded that the device was easy to use and safe in cohorts of 643 and 143 patients, respectively; neither of these studies compared the MDI test with a reference test.\textsuperscript{23,24}

In conclusion, the high concordance between the MDI test and the nebulizer test for the diagnosis of BHR suggests that the methacholine MDI may be useful for nonspecific bronchial provocation testing, at least for assessment of bronchial hypersensitivity. When compared with nebulizers, the MDI is a simpler way to administer methacholine; it is less cumbersome and there are no nebulizer or supplies to purchase and no solutions to prepare. Such a portable and easy-to-handle device may be particularly suitable for epidemiologic studies.

However, several points remain to be addressed before considering clinical use of the methacholine MDI: first, the results of this pilot study need to be confirmed in larger populations, including subjects with various lung diseases, and the intrapersonal reproducibility of the results needs to be studied further. Second, improvement of the device is desirable, even if the use of a spacer remains necessary to avoid decreases in bronchial penetration of methacholine because of poor coordination; in addition, considering the limitation in the use of chlorofluorocarbons, it will be necessary to reformulate the methacholine solution with a chlorofluorocarbon-free propellant. Third, the addition of an integrated system for counting doses would also be useful. Finally, an MDI containing a lower methacholine dosage should also be developed, since 100 \textmu g may be a dangerous unitary dose in highly hyperresponsive subjects.