

Comparison of histamine and methacholine for use in bronchial challenge tests in community studies

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ABSTRACT Measurement of bronchial reactivity is widely used in epidemiological surveys. Histamine has been compared with methacholine inhalation challenge in two samples of adults from a small town to determine which is the better agent for use in community studies. Increasing doses of histamine and methacholine were given, up to a maximum of 4 and 12 μmol respectively, according to the method of Yan *et al*, the provocative dose of agonist causing a 20% fall in FEV_1 (PD_{20}) being measured. More subjects had a measurable PD_{20} with methacholine than with histamine, both in a random sample of 108 subjects (25 v 11 subjects, $p < 0.01$) and in an additional 95 subjects selected because of wheeze in the last 12 months (67 v 48 subjects, $p < 0.01$). Side effects were mild with both agents but histamine caused voice change in more subjects (21% v 11%). Repeatability was assessed in a further group of subjects with wheeze in the last year. The 95% range for a single estimation of PD_{20} in subjects with a measured PD_{20} on at least one occasion was ± 2.5 doubling doses for histamine ($n = 25$) and ± 2.1 doubling doses for methacholine ($n = 33$). Thus methacholine has advantages over histamine for community studies of bronchial reactivity as it is possible to use doses that produce more PD_{20} measurements with fewer side effects.

Introduction

Bronchial reactivity has been used as an objective marker of asthma in epidemiological surveys, and there is growing interest in the distribution of this measurement in the population.¹⁻⁸ The importance of using a standardised bronchial challenge test is well recognised, but which of the available tests is best suited to epidemiological work has not been determined. For community studies a test must be quick, repeatable, and readily acceptable. It should also provide a measure of reactivity in as large a proportion of the general population as possible. With tests such as exercise or cold air hyperventilation the maximum stimulus that can be achieved is inadequate to produce bronchoconstriction in most non-asthmatic individuals. With pharmacological agents such as histamine and methacholine the dose that can be given is limited by side effects.

Histamine and methacholine are the two agonists

used most widely for inhalation challenge. In laboratory comparisons in patients with asthma there is close agreement between the provocative doses causing a 20% fall in FEV_1 (PD_{20}) for the two agents and repeatability has usually been similar, although there has been a suggestion that methacholine may produce fewer side effects for a given degree of bronchoconstriction.⁹ If this is true in a community setting methacholine would have an important advantage over histamine for epidemiological surveys. We have therefore compared histamine and methacholine inhalation challenge in a community study.

The study also evaluated serial peak flow measurements as an alternative to bronchial challenge tests. This is mentioned in the "Methods" section because it was incorporated into the study design, but the results will be described separately. This report is confined to the comparison of histamine and methacholine challenge tests.

Methods

SUBJECTS

The study was conducted in a small town of some 6500 adults, situated about 10 km from Nottingham. The

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only general practice in the town has 7800 patients aged 18–75 years on its list, and this includes nearly all the town's residents. Six copies of a simple questionnaire on respiratory symptoms were delivered to each household in the town with a request that every adult should complete and return one copy.

Two groups of subjects were recruited. Group A consisted of 199 subjects aged 18–75 years, drawn from the general practice age–sex register as a systematic random sample. The subjects were sent a letter countersigned by their own general practitioner explaining the purpose and nature of the study, asking them to take part, and enclosing an appointment time and a stamped, addressed envelope for reply. It was made clear that one of the practitioners was available to answer questions about the project. Subjects who did not respond were sent a second invitation. If this produced no reply an attempt was made to contact the subject by telephone or with a visit to his or her home. Subjects who were found to have left the town were replaced by new ones chosen at random from the age–sex register.

Group B consisted of the first 400 people replying to the questionnaire who admitted to wheeze or whistling in the chest within the last year or to having a diagnosis of asthma. Appointments were made in the same way as for group A, but a second approach was not made to non-responders. Informed consent was obtained from all subjects. The study was approved by the Nottingham City Hospital ethics committee.

STUDY DESIGN

Group A subjects were randomly allocated either to undergo a bronchial challenge test or to record peak flow readings for a week. Subjects allocated to bronchial challenge were further randomised to undergo either a histamine or a methacholine test. On completion of the first challenge test the subjects were asked to return at the same time on another day to perform a second test with the alternative agonist; after this was completed they were asked to record peak flow readings for a week. Subjects who were initially allocated to the group making peak flow recordings were asked on completing these to undergo a bronchial challenge test, and subsequently a repeat test with the alternative agonist.

The procedure for group B was the same except that after the first bronchial challenge test the subjects were again randomised and half received the same agonist, so that repeatability could be assessed.

BRONCHIAL CHALLENGE TESTS

The invitation to take part in the study included a request to abstain from cigarettes for six hours, bronchodilator inhalers for six hours, and antihistamines for 24 hours before attending the test centre.

On arrival subjects rested for 5–10 minutes while the test procedure was explained, and a questionnaire on respiratory symptoms and recent illness was completed.

Baseline FEV₁ and forced vital capacity (FVC) were measured with a dry bellows spirometer (Vitalograph), the higher of two successive readings within 5% of each other being used. Challenge testing was not performed in subjects whose baseline FEV₁ was less than 60% predicted, in subjects who were pregnant, or in those who had had a recent serious illness.

The bronchial challenge test was based on that described by Yan *et al*¹⁰ and used De Vilbiss No 40 nebulisers that had been shown by prior testing to have an output in the range 0.0025–0.0035 ml per activation. After baseline spirometric measurements subjects inhaled normal saline followed by increasing doses of histamine or methacholine. FEV₁ was measured one minute after each inhalation, the higher of two successive readings within 5% of each other being taken, and this was followed immediately by administration of the next dose. In subjects with asthma or recent wheeze the test started with 0.03 μmol histamine or 0.048 μmol methacholine, followed by doubling dosage increments. All other subjects started with 0.06 μmol histamine or 0.096 μmol methacholine and continued with quadrupling increments, changing to doubling increments if the FEV₁ fell by 10–19%. The test was stopped if the FEV₁ fell by 20% or more, or when the subject had received a maximum cumulative dose of 4 μmol histamine or 12 μmol methacholine (doses rounded to the nearest integer). These doses were chosen because they produced approximately equal side effects in a small pilot study. At the end of the test any side effects experienced by the subjects were elicited by means of a standard questionnaire.

SKIN TESTS

Skin prick tests for *Dermatophagoides pteronyssinus*, grass pollen, and cat dander, with histamine and saline controls, were performed on the ventral aspect of the forearm at the time of the first challenge test. Weal size was measured as described previously.¹ Subjects were regarded as atopic if the mean weal diameter was 2 mm or more with any allergen and 1 mm or less with saline.

ANALYSIS OF RESULTS

The provocative dose of histamine (PD₂₀H) or methacholine (PD₂₀M) producing a 20% fall in FEV₁ from the post-saline value was determined by a curve fitting method,¹¹ with extrapolation to one doubling dose above the maximum dose administered. PD₂₀ values above this dose are described as “censored,” and assigned a value at the upper limit of the estimated

range (8 μmol histamine or 24 μmol methacholine). Base 10 logarithmic transformations of PD_{20} were used in all analyses.

Among subjects who completed both challenge tests the number of measurable $\text{PD}_{20\text{H}}$ and $\text{PD}_{20\text{M}}$ values were compared by McNemar's test. To allow the two agents to be compared over an approximately equipotent dose range a second $\text{PD}_{20\text{M}}$ was derived from data up to the 6 μmol dose only, with extrapolation to 12 μmol (these figures for relative potency are based on a previous laboratory study⁹ suggesting that 4 μmol histamine is similar in its bronchoconstrictor activity to 6 μmol methacholine).

Side effects from the two agents among subjects completing both challenge tests were compared by χ^2 analysis. Side effects were also analysed from the first test on each subject, to avoid potential bias from subsequent non-attenders.

Repeatability was expressed in terms of the 95% range for a single measurement. The standard deviations of the differences between repeat results with histamine and with methacholine were used to calculate the expected standard deviation of the within person difference between $\text{PD}_{20\text{H}}$ and $\text{PD}_{20\text{M}}$ results, and this was compared with the observed difference.

Results

PARTICIPATION RATES

The numbers of subjects participating in the two groups are shown in figure 1.

The total response rate in group A was 73% (145 subjects), with 15 subjects only recording peak flow rates. Of the 130 subjects who attended for at least one

challenge test, 108 completed both tests and 22 had only one because spirometric readings were too low or because of subsequent non-attendance. In group B, the group with wheeze in the last year, 257 subjects (64%) kept at least one appointment and 234 attended for at least one challenge test. Ninety five completed challenge tests with histamine and methacholine, 50 completed repeat challenge tests with histamine and 46 repeat methacholine tests. One repeat histamine study was not included in the analysis because the subject's spirometry technique was unsatisfactory. There were 39 atopic subjects and 26 current smokers in group A (36% and 24% of the total); the corresponding figures in group B were 109 and 41 (57% and 22%).

ESTIMATES OF PD_{20}

The numbers of subjects from groups A and B with a measurable PD_{20} are shown in the table. Of the 108 subjects in group A who completed both tests, 11 (10%) had a $\text{PD}_{20\text{H}}$ below 8 μmol compared with 25 (23%) with a $\text{PD}_{20\text{M}}$ below 24 μmol ($p < 0.01$) and 16 with a $\text{PD}_{20\text{M}}$ below 12 μmol ($p > 0.1$). Of the 95 subjects in group B who carried out both tests, 48 had a $\text{PD}_{20\text{H}}$ below 8 μmol and 67 a $\text{PD}_{20\text{M}}$ below 24 μmol (50% v 70%, $p < 0.01$).

RELATION BETWEEN $\text{PD}_{20\text{H}}$ AND $\text{PD}_{20\text{M}}$

The relation between $\text{PD}_{20\text{H}}$ and $\text{PD}_{20\text{M}}$ values for the 78 subjects from group A and group B who had a measurable PD_{20} on at least one occasion is shown in figure 2. The regression coefficient (SE) of $\log_{10}(\text{PD}_{20\text{M}})$ on $\log_{10}(\text{PD}_{20\text{H}})$ was 0.72 (0.10) and the intercept was 0.15. This was not altered significantly when smokers or atopic subjects were considered separately (regression coefficients (SE) = 0.72 (0.16)

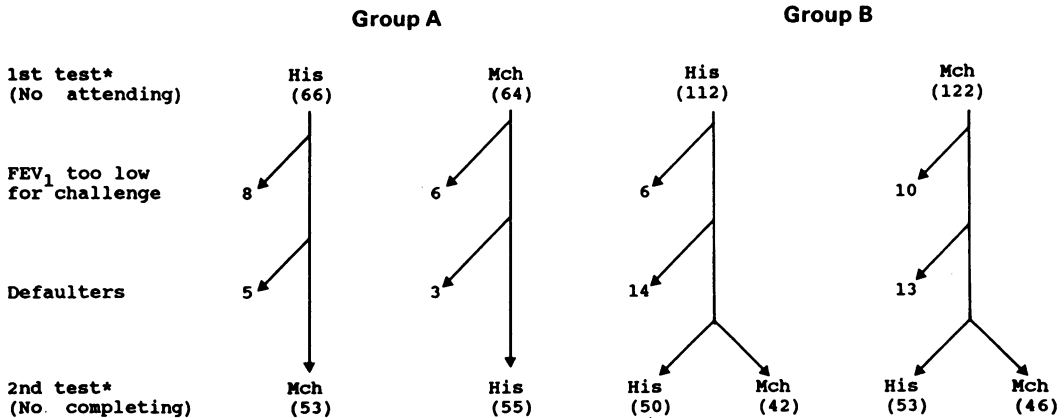


Fig 1 Number of subjects attending and completing bronchial challenge tests. *His—histamine challenge test; Mch—methacholine challenge test.

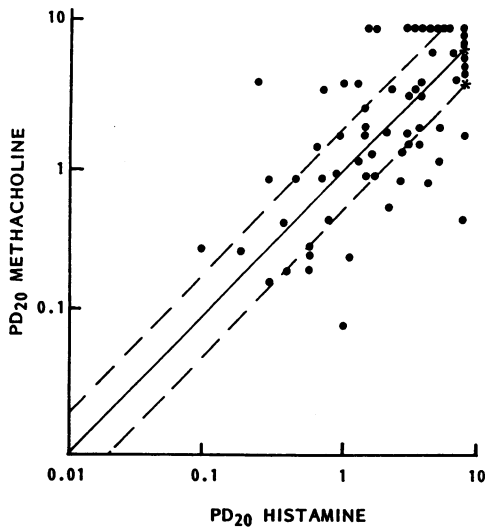


Fig 2 Relation between $PD_{20}H$ and $PD_{20}M$ (provocative dose of histamine and of methacholine causing a 20% fall in FEV_1). The solid line is the line of identity and the broken lines indicate one doubling dose from the line of identity. *Multiple data points superimposed.

for smokers, 0.78 (0.18) for atopic subjects). The mean within subject difference between $PD_{20}H$ and $PD_{20}M$ values was $0.034 \log_{10} \mu\text{mol}$ and the standard deviation of the difference was $0.472 \log_{10} \mu\text{mol}$. When the 48 subjects who had a measurable PD_{20} with both agents were considered separately the regression coefficient (SE) was 0.56 (0.14) ($p < 0.001$).

REPEATABILITY OF HISTAMINE AND METHACHOLINE PD_{20} VALUES

Of the 96 subjects who attended for repeat tests with the same agent, 52 had a measurable PD_{20} on at least one occasion (25 with histamine and 27 with methacholine—table and figs 3 and 4). The standard deviation for the difference between repeat PD_{20} measurements was $0.55 \log_{10} \mu\text{mol}$ for histamine and

Number of PD_{20} estimates obtained in group A (random sample) and group B (subjects with wheeze in last year)

Test	Group	$PD_{20}H$ < 8 μmol	$PD_{20}M$	
			< 12 μmol	< 24 μmol
Histamine/ methacholine	A (n = 108)	11	16	25
	B (n = 95)	48	51	67
Histamine/ histamine	B (n = 50)	25* (15)	—	—
Methacholine/ methacholine	B (n = 46)	—	27* (22)	33* (27)

*Uncensored PD_{20} on at least one occasion; () indicates uncensored PD_{20} on both occasions. $PD_{20}H$ ($PD_{20}M$)—provocative dose of histamine (methacholine) causing a 20% fall in FEV_1 .

$0.45 \log_{10} \mu\text{mol}$ for methacholine, from which the 95% range for a single estimation of PD_{20} was ± 2.5 doubling doses in the 25 subjects receiving histamine and ± 2.1 doubling doses in the 27 subjects receiving

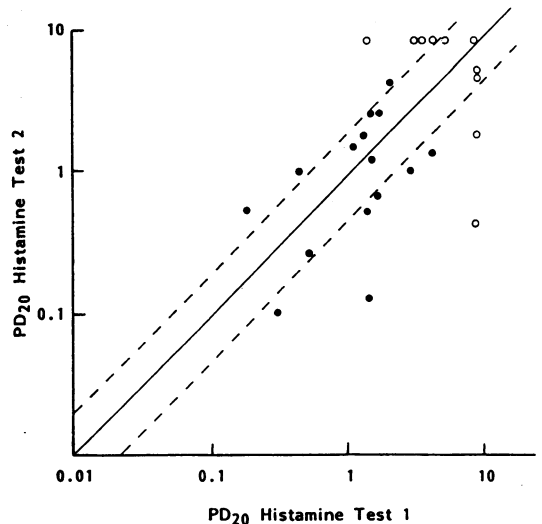


Fig 3 Relation between PD_{20} values from repeat histamine tests. Closed circles represent subjects having a measurable PD_{20} on two occasions and open circles subjects with one measurable and one censored PD_{20} ; the solid line is the line of identity and the broken line indicates one doubling dose from the line of identity.

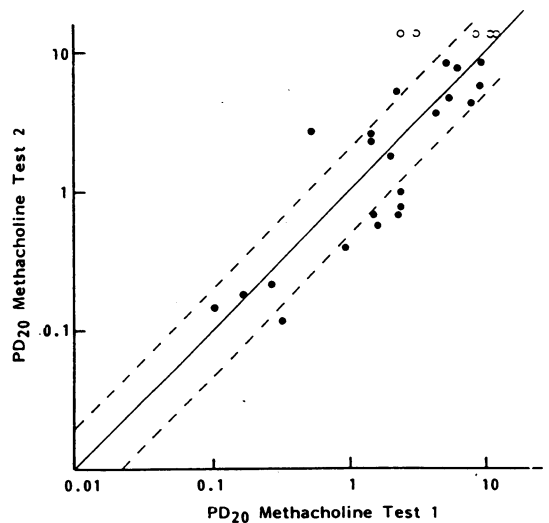


Fig 4 Relation between PD_{20} values from repeat methacholine tests. Closed circles represent subjects having a measurable PD_{20} on two occasions and open circles subjects with one measurable and one censored PD_{20} ; the solid line is the line of identity and the broken lines indicate one doubling dose from the line of identity.

methacholine. An uncensored PD_{20} value was obtained on both occasions in 15 subjects with histamine and 22 subjects with methacholine, and in these subjects the 95% range for a single estimation was ± 2.4 doubling doses for histamine and ± 1.9 doubling doses for methacholine. Repeatability with methacholine did not differ significantly from that with histamine, whether censored values were included (variance ratio = 1.49, $p > 0.1$) or excluded (variance ratio = 1.46, $p > 0.1$).

From the standard deviations of the differences between repeat PD_{20} measurements the expected within subject standard deviation of the difference between PD_{20H} and PD_{20M} results was calculated as 0.50 \log_{10} μmol .

Eleven subjects undergoing repeat histamine tests and 10 undergoing repeat methacholine tests developed symptoms of a cold or a change in the frequency of wheeze, or both, between tests. Excluding values from these subjects did not alter the repeatability figures for either agent significantly. The interval between tests was similar for the two agents (median 7 (range 1–23) days for histamine and 6 (1–32) days for methacholine).

ADVERSE EFFECTS

Side effects were mild with both agents. Histamine caused more voice change than methacholine, both in the first test of all subjects (21% v 11%; $p < 0.05$) and in the subjects who had both agents (18% v 10%; $p < 0.05$). Of the other adverse effects, only cough occurred with any frequency, and this was similar with histamine and methacholine (30% v 34%, NS), as was the incidence of any other side effect (17% v 16%, NS).

Discussion

The aim of this study was to determine whether histamine or methacholine is the better agent for use in epidemiological surveys of bronchial reactivity. The assessment was made by comparing the number of PD_{20} estimates, the incidence of side effects, and the repeatability of PD_{20} values with the two agents. The number of PD_{20} estimates and the incidence of side effects are both dose dependent and therefore need to be considered together.

With any test designed for field studies it is important that side effects are kept to a minimum. We chose 4 μmol histamine and 12 μmol methacholine as maximum doses because they produced a similar number of mild side effects in a pilot study. In the community study methacholine produced a significantly greater number of PD_{20} estimates but a lower incidence of adverse effects than histamine in the subjects who completed tests with both agents. As some subjects may have failed to keep their second

appointment because of side effects experienced at the first visit we also compared symptoms reported in the first test by all subjects who attended at least once, and methacholine was again the more acceptable agent. A similar conclusion was reached in one previous laboratory study,⁹ in which most of the subjects were asthmatic. We have been able to show in a larger field study that this permits a significantly greater number of PD_{20} measurements to be made with methacholine, a major advantage in community studies.

We had predicted that 24 μmol of methacholine would produce more PD_{20} estimates than 8 μmol of histamine as the two agents have previously been shown to cause a similar degree of bronchoconstriction for a given concentration in mg/ml.⁹ Thus, after correction for molecular weight, 8 μmol histamine was expected to produce the same effect as 12 μmol methacholine. In our study, however, the potency of methacholine is very close to that of histamine (fig 2) with a mean difference between PD_{20H} and PD_{20M} values of only 0.034 \log_{10} μmol ; the 95% confidence interval for this difference suggests that the PD_{20M} is likely to be from 0.85 to 1.38 times the PD_{20H} .

As only 10–20% of a random population are likely to have a 20% fall in FEV_1 with acceptable doses of bronchoconstrictor agonist,¹⁸ we recruited an additional group of subjects who had recently experienced an episode of wheezing and who were therefore more likely to have a measurable PD_{20} , so that enough PD_{20} estimates were obtained to enable us to assess repeatability. Whether analysis of repeatability should include subjects who have one censored PD_{20} or be confined to those with two measurable PD_{20} values is debatable.¹¹ We have analysed both and found methacholine to have slightly better repeatability in both cases, although the difference is not significant. In most laboratory comparisons the repeatability of histamine and methacholine has been similar,^{9,12} although one author has reported better repeatability with methacholine in subjects recovering from an exacerbation of asthma.¹³ The two agents have not been compared in a community based population before, and there are no published measures of repeatability with either agent in a randomly selected community population. The 95% ranges for a single estimation are inevitably less good than those seen in the laboratory in selected subjects familiar with the measurement techniques.^{9,12,14,15} It may be possible to reduce measurement error in epidemiological studies by altering the challenge test technique, but any modification would have to be assessed in a similar randomly selected population. In the mean time the designs of studies in the community need to take account of the repeatability of the method used.

Histamine challenge tests might be expected a priori

to be less repeatable than methacholine challenge tests because, in addition to a direct action on bronchial smooth muscle, histamine appears to be more likely than methacholine to cause mucosal oedema, activate irritant receptors, and increase vagally mediated bronchoconstriction.¹⁶ Despite these possible differences histamine and methacholine challenges are often described interchangeably as tests of non-specific bronchial reactivity, with the implication that they are measuring the same underlying pathophysiological process. If this assumption is true the agreement between PD₂₀H and PD₂₀M values should be as close as the agreement between repeat measurements with the same agent. When account is taken of the repeatability of each method the predicted standard deviation for the difference between PD₂₀H and PD₂₀M values in the same subject is 0.50 log₁₀ μmol. This is almost identical to the observed standard deviation of the difference between the two measurements (0.47 log₁₀ μmol) in the subjects undergoing both tests (the findings are similar if censored values are excluded, the predicted value being 0.44 log₁₀ μmol and the observed value 0.50 log₁₀ μmol). Thus the methods agree as closely as would be expected from a knowledge of the repeatability of each agent. Furthermore, if histamine and methacholine are not measuring the same underlying abnormality the relationship between PD₂₀H and PD₂₀M might be expected to differ between atopic and non-atopic subjects and between smokers and non-smokers, as increased reactivity in these groups may be produced by different mechanisms.^{17,18} In fact, the regression of PD₂₀M on PD₂₀H values was virtually identical in smokers, non-smokers, and atopic and non-atopic subjects. Thus our findings do not provide support for the idea that histamine and methacholine are measuring different phenomena.

We have shown that when used in an epidemiological study methacholine produces more measurements of non-specific bronchial reactivity than histamine, with less unwanted effects. Methacholine results were also slightly more repeatable. We believe that methacholine is the preferred agent for community studies of bronchial reactivity.

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