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The daily variability of bronchial responsiveness to methacholine

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The daily variability of bronchial responsiveness to methacholine. C.J. Trigg, N. Jhalli, M.J. Herdman, D.R. Cundell, J.M. Thomas, R.J. Davies.
ABSTRACT: Ten mild atopic asthmatics on inhaled beta$_2$-agonists alone were studied in order to determine the repeatability of methacholine inhalation provocation tests at 24 h intervals over a period of 5 days. Such patients are most frequently studied in therapeutic trials of anti-asthmatic medications. There were no significant differences in results obtained on any of the days and no evidence for the development of tolerance to methacholine in this group of patients at one day intervals. The 95% confidence interval for repeatability of the results was ±1.05 doubling doses of methacholine, and 95% range ±2.36 doubling doses, comparable to the results obtained by other investigators on similar patients. Some investigators have produced more highly repeatable results but these have generally been obtained using highly selected groups of patients.
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Individuals with current symptomatic asthma can be demonstrated to have a higher degree of nonspecific airways responsiveness than nonasthmatics [1], and the degree of this bronchial hyperresponsiveness (BHR) correlates well with symptoms and medication requirements [2]. Methods of challenge with agents such as histamine and methacholine have been technically standardized [3], and a high degree of repeatability of results has been established in the short-term, both over a period of days and within the same day. Most investigators express their results in terms of provocation dose or concentration: the dose or concentration at which a 20% fall in forced expiratory volume in one second (FEV_1) is provoked ($PD_{20}FEV_1$ or $PC_{20}FEV_1$), the most reproducible parameter for measurement of bronchial responsiveness [4]. JUNIPER *et al.* [2] produced highly repeatable results. We have calculated from their figures that the 95% range for mean difference in $PC_{20}FEV_1$ was ±0.93 doubling concentrations of methacholine and ±0.45 doubling concentrations of histamine when the test was repeated, at the same time of day on two days during the same week. Such highly repeatable results have led to the use of serial measurements of bronchial responsiveness in time course studies, investigating the effects of therapeutic agents in asthma [5–7]. However, the results of other investigators suggest that the test may not be so highly repeatable [8].

Several factors affect the repeatability of results. The true biological fluctuations in bronchial responsiveness in any group of subjects may be affected by the time interval between tests, allergen exposure or other environmental influences and patient selection for the study. Additionally, the methodology employed may affect the magnitude of the technical random error.

It is important to consider the time of day and the number of days between repeated tests. During a 24 h period, DEVRIES *et al.* [9] showed that a nocturnal drop of 1–2 doubling concentrations of histamine occurred when bronchial provocation tests were repeated 4 hourly. However, RUFFIN *et al.* [10] showed that $PC_{20}FEV_1$ recordings were highly repeatable with histamine at a time interval of as little as 30 min and up to 4 times at 40 min intervals during normal working hours, within the same day. It has been recommended that tests can be compared within the same day between the hours of 09.00h–17.00h and between days at the same time of day [11]. However, more recently, tachyphylaxis using histamine [12] at 3 h intervals and tolerance to methacholine at intervals up to 24 h [13] have been reported and these factors may confound the results of studies investigating short-term time trends in bronchial responsiveness.

Repeatability may be affected by patient selection. Application of stringent inclusion criteria such as highly stable resting airway function may select for a less severe group of asthmatics [2, 14]. The inclusion of patients on inhaled steroids [2] may reduce lability as such medication is known to diminish the late response to allergen inhalation [15].

Some studies have included nonasthmatics, a large proportion of whom require higher doses of agonist, introducing the possibility of dose-related effects [2, 13]. Inclusion of atopic individuals increases the influence of allergen exposure on bronchial responsiveness [16].

Methodology should be carefully standardized to minimize the technical random error [1]. Protocols for inhalation challenge that minimize technical variables

such as aerosol generation and inhalation, method of inhalation, measurement and expression of response have been well described [1, 17–20]. Methacholine is the preferred agonist for bronchial provocation testing as it is better tolerated than histamine, having a wider dose range without unpleasant side-effects [2]. However, most studies of repeatability have tended to use histamine as the agonist, because of its shorter duration of action [21].

Most of the previous short-term repeatability studies have not specified the number of days between tests and have recorded results at varying intervals between subjects of up to 14 days. More recent studies have tended to concentrate on other aspects of repeatability [12, 13, 20], and it is not clear what the actual day to day variability of the test is, without the intervention of other factors such as repeated tests within the same day. Stable mild atopic asthmatics are most frequently studied in evaluation of therapeutic agents for asthma, yet some studies have included nonasthmatics [2, 13] and patients on inhaled steroids [2, 4, 8] and have imposed artificial limits on repeatability of forced expiratory volume in one second (FEV_1) [2, 14]. In this study, we have re-evaluated the repeatability of results using methacholine in stable mild atopic asthmatics on inhaled salbutamol alone at 24 h intervals over 5 consecutive days.

Subjects

Six female and four male nonsmokers with mild asthma aged 18–55 yrs (mean 28.6 yrs) were studied. All patients were atopic as defined by the presence of one or more positive skin tests to common inhalant allergens (weal diameter ≥2 mm and at least 1 mm greater than the negative control). All patients were able to record an FEV_1 of >60% of predicted for age and height when all medication was withheld. The mean FEV_1 was 3.08 *l* (range 1.60–3.35 *l*) and the geometric mean $PD_{20}FEV_1$ was 0.43 (range 0.04–3.06). Two of the patients were on regular inhaled salbutamol (200 μg *qid*) and eight on intermittent doses (100–200 μg daily). None of the patients were on other medication nor had they received vaccinations or suffered respiratory tract infection within one month of the study. None of the patients were occupationally exposed to allergens or sensitizers nor was there intermittent exposure to environmental allergens during the study. All patients gave informed consent to take part in the study.

Methods

Patients attended the laboratory at the same time of day on five consecutive days, having withheld all medication for 4–6 h. FEV_1 was recorded 10–15 min after arrival at the laboratory (Vitalograph dry bellows spirometer, Vitalograph Ltd, Buckingham, UK). Bronchial provocation testing was carried out by a standard tidal breathing method [17], using a DeVilbiss 646 nebulizer (DeVilbiss Health Care Ltd, Feltham, UK) driven by air at a flow rate of 6.5 $l \cdot min^{-1}$.

Solutions were inhaled by tidal breathing for 2 min, with the nose clipped. FEV_1 was recorded at 30, 90 and 180 s after inhalation as the best of two reproducible attempts. The lowest reproducible value of the three time points was recorded as the response. A control solution of phenol saline (0.4% phenol, 0.5% NaCl, 0.275% $NaHCO_3$) was followed by doubling concentrations of methacholine (acetyl-beta methacholine chloride, Sigma Chemicals, Poole, UK) in phenol saline from 0.03–16 $mg \cdot ml^{-1}$ at 5 min intervals.

The test was terminated when the FEV_1 had fallen by 20% from the lowest value obtained after inhalation of the control solution, or when the highest concentration of methacholine was reached. None of the patients reacted with a ≥15% fall in FEV_1 on the control solution.

The percentage fall in FEV_1 was plotted noncumulatively against logarithmic concentration of methacholine and the $PC_{20}FEV_1$ determined by linear interpolation of the last two points on the curve. Cumulative $PD_{20}FEV_1$ was calculated from the sum of the doses required to provoke a 20% fall in FEV_1. Dose (μmols) delivered by the nebulizer was determined from the equation:

$$\text{Dose} = \frac{\text{Concentration} \times \text{nebulizer output}}{195.7^*}$$

*: molar weight of methacholine

Nebulizer output was determined by weighing before and after the operation for two min when starting with a volume of 5 ml phenol saline and repeating the procedure 20 times. The mean nebulizer output was 0.855 ±0.007 ml in 2 min.

Analysis of results

The intraclass correlation coefficient (Rho) was calculated for both $PD_{20}FEV_1$ and FEV_1, the equation being derived from a one-way analysis of variance:

$$\text{Rho} = \frac{a - b}{a + (n - 1)b}$$ (SNEDECOR and COCHRAN [22])

where a = between subject mean square; b = error mean square (within subject mean square); n = number of days. A confidence interval for Rho was also calculated [22].

Cumulative $PD_{20}FEV_1$ results were analysed, in terms of change over five days, by two-way analysis of variance of logarithmically transformed data [23]. A normal probability plot of residuals from the analysis of variance showed no significant departure from normality (r=0.98) after logarithmic transformation for $PD_{20}FEV_1$ data. Any deviations were probably a function of sample size. Thus, the use of parametric statistics was considered appropriate.

A 95% confidence interval [24] for the difference between the mean values on any two days was calculated using the following equation:

$$t_{36} \sqrt{s^2\left(\frac{1}{n} + \frac{1}{n}\right)}$$ [22]

Table 1. – Two-way analysis of variance for day to day variability of $PD_{20}FEV_1$ (log_{10} data)

Source	Degrees of freedom	Sum of squares	Mean squares	F	p
Between subjects	9	11.175	1.242	10.18	<0.01
Between days	4	0.684	0.171	1.42	>0.10
Error	36	4.387	0.122		
Total	49	16.245			

Two way analysis of variance for day to day variability of resting FEV_1

Source	Degrees of freedom	Sum of squares	Mean squares	F	p
Between subjects	9	45.9439	5.1049	106.80	<0.01
Between days	4	0.0696	0.0174	0.36	>0.10
Error	36	1.7202	0.0478		
Total	49	47.7337			

$F_{4,36}$=2.65·$PD_{20}FEV_1$: provocation dose producing a 20% fall in forced expiratory volume in one second.

t_{36} = value for Student's t for (10-1) (5-1) degrees of freedom; s^2 = error mean square (pooled variance from analysis of variance); n = number of patients.

The confidence interval was then converted into units of doubling dose (DD), by division of the logarithmic value by $\log_{10} 2$ (=0.3010).

The 95% range (including 95% of the intra-individual differences recorded) was calculated from the product of t_{36} and $\sqrt{s^2}$ and converted into units of doubling dose as above.

The same techniques were applied to raw FEV_1 data which was normally distributed. (Normal probability plot, r=0.94). FEV_1 results were also analysed in terms of percentage change from the initial value in order to compare with the ranges for other studies.

Results

The intraclass correlation coefficient for $PD_{20}FEV_1$ was 0.637 (95% confidence interval 0.375–0.870). For FEV_1, it was 0.958 (95% confidence interval 0.901–0.988).

Results of two-way analysis of variance are shown in table 1. There was no significant difference in $PD_{20}FEV_1$ (within subject) between days on any of the five days. Between subject differences were present as expected and these are taken into account in calculation of the confidence interval using the pooled variance. The 95% confidence interval for repeatability of $PD_{20}FEV_1$ was ±1.05 doubling doses of methacholine, 95% range was ±2.36 doubling doses. Resting FEV_1 also showed no significant change over the five days, with a 95% confidence interval for repeatability of ±0.198 *l*, 95% range ±0.444 *l* ±16.2% in percentage terms). Within subject mean and SD are shown in table 2.

Table 2. – Intrasubject variability in $PD_{20}FEV_1$ over 5 days

Subject	Mean µmols	SD DD
1	0.36	0.749
2	0.93	2.181
3	0.34	0.916
4	0.05	1.116
5	0.36	1.951
6	0.69	0.337
7	1.17	0.561
8	3.05	0.349
9	2.01	0.526
10	0.79	1.399

Intrasubject variability in resting FEV_1 over 5 days

Subject	Mean *l*	SD *l*
1	1.63	0.042
2	2.16	0.094
3	2.98	0.036
4	2.47	0.209
5	3.31	0.079
6	2.28	0.268
7	5.17	0.137
8	3.94	0.143
9	3.36	0.457
10	2.98	0.256

$PD_{20}FEV_1$: provocation dose producing a 20% fall in forced expiratory volume in one second; DD: doubling doses.

Discussion

In this study, we have shown that the results obtained from bronchial provocation testing using methacholine have an acceptable range of repeatability at 24 h intervals, over five consecutive days, in stable mild asthmatics on inhaled beta$_2$-agonists alone, a group of patients commonly studied in therapeutic trials of anti-asthmatic treatments. The 95% confidence interval for repeatability (±1.05 DD) was comparable with the results of other investigators using the tidal breathing method (DEHAUT *et al.* [4] ±1.6 doubling concentrations (DC); MADSEN *et al.* [8] ±1.72 DC) but less repeatable than those calculated from JUNIPER *et al.* [2] (±0.26 DC) and RYAN *et al.* [14] ±0.34 DC; this paper erroneously quoted 95% range). It is notable that the latter two studies used a somewhat different group of patients. JUNIPER *et al.* [2] studied 14 patients, 3 of whom were not asthmatic, none of whom showed greater than 5% variability in resting FEV_1 recordings. This study also included a predominance of patients on inhaled steroids in whom the severity of BHR is reduced [7]. RYAN *et al.* [14] only studied patients with less than 10% variability in resting FEV_1. Such restrictions may well be physiologically valid in assessing the technical repeatability of the test. However, such results do not reflect the degree of variability in the group of patients we studied: mild atopic asthmatics, none of whom were on steroids and who showed a ±16.2% variability in resting FEV_1.

It is important to know the expected range of variability of $PD_{20}FEV_1$ in such patients: ±2.36 DD in this study with an intraclass correlation coefficient of 0.643. The confidence interval of the intraclass correlation coefficient was very wide because some of the patients showed much greater variability than others. This variability was not accounted for by day to day influences over the whole group. It is a function of true fluctuations in bronchial responsiveness particular to the patients studied and the technical variables inherent in this complex test. We have aimed in this study to minimize the factors that might affect bronchial responsiveness (for instance recent respiratory infection and time of day). Allergen exposure was not known to vary but it is impossible to be sure that environmental influences are controlled in a group of allergic individuals such as those we studied who may be exposed to many possible allergens.

We followed a standardized protocol for inhalation provocation testing [17] which is modified from the widely accepted protocol of COCKCROFT *et al.* [3]. The DeVilbiss 646 nebulizer used had an acceptably repeatable output (0.855±0.007 ml in 2 min) and is the standard nebulizer used in our laboratory for both the tidal breathing and dosimeter techniques because $PD_{20}FEV_1$ values obtained using these two techniques are directly comparable [17]. The particle size produced is suitable for distal airway deposition and does not vary significantly with airflow [18]. In fact, aerosol output has been shown to be the major factor affecting the resulting $PD_{20}FEV_1$ when different nebulizers are compared in the same patients [18]. The influence of minor variations in aerosol mass median diameter (AMMD) has not been shown to be significant [18, 19].

In a recent study, MADSEN *et al.* [20] were able to improve repeatability to some extent by controlling the pattern of tidal breathing during the test and such modifications of the protocol may increase the sensitivity. However, for the currently accepted tidal breathing protocol there is a large degree of intrasubject variability which may obscure minor changes in bronchial responsiveness in therapeutic trials unless sufficiently large numbers are studied to show a significant difference. It is thus advisable that all such trials include an assessment of repeatability in the protocol to document the range of individual variability in the particular patients studied.

We did not show any evidence of tolerance occurring to repetitive methacholine challenges at 24 h intervals in this group of mild asthmatics. However, the study by BECKETT *et al.* [13] assessed tolerance in terms of the change in airways resistance and thus may have detected more subtle changes. Also, in the latter study, only nonasthmatics with higher $PD_{20}FEV_1$ were studied and, as these authors suggested, the effect may be dose-related and not detectable in asthmatics.

This study has documented for the first time the expected day to day variability of $PD_{20}FEV_1$ in a group of mild stable asthmatics on inhaled bronchodilators alone. The results are comparable with those obtained in other studies at varying time intervals over 1–14 days but not with those using selected patients on inhaled steroids and with highly stable resting airway function. There was no evidence that repetition of tests at 24 h intervals resulted in tolerance to methacholine in this group of patients.

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Variabilité de la réactivité bronchique à la methacholine d'un jour à l'autre. C.J. Trigg, N. Jhalli, M.J. Herdman, D.R. Cundell, J.M. Thomas, R.J. Davies.

RÉSUMÉ: Dix asthmatiques atopiques légers, soumis à un traitement isolé par bêta2-agonistes, ont été étudiés pour déterminer la reproductibilité de la provocationn par methacholine en inhalation à des intervalles de 24 h. pendant une période de 5 jours. Ces patients sont les plus fréquemments étudiés dans les essais thérapeutiques de médications antiasthmatiques. Il n'y avait pas de différence significative entre les résultats obtenus à n'importe quel des jours, et aucune preuve du développement d'une tolérance à la methacholine dans ce groupe de patients à des intervalles d'un jour. L'intervalle de confiance de 95% pour la reproducibilité des résultats était de ±1.05 doses de doublement de la methacholine, et les écarts à 95% de ±2.36 doses de doublement, comparable aux résultats obtenus par d'autres investigateurs chez des patients semblables. Certains investigateurs avaient montré des résultats plus reproductibles encore, mais ces résultats ont généralement été obtenus en utilisant des groupes de patients fortement sélectionnés.
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