Comparison of the dose-response curves obtained by forced oscillation and plethysmography during carbachol inhalation


ABSTRACT: We compared the cumulative dose-response curves obtained during carbachol inhalation by simultaneous measurements of airway specific conductance (SGaw) and respiratory conductance, in 23 subjects with or without bronchial hyperresponsiveness. The SGaw was measured by a body plethysmograph, whilst the random noise forced oscillation technique (FOT) was used to determine respiratory conductance. The SGaw was compared to respiratory conductance extrapolated to zero frequency (GRs0). Bronchial sensitivity was assessed by the threshold dose of carbachol (TD) that induced a decrease in SGaw and GRs0 of twice the baseline coefficient of variation. Bronchial responsiveness was assessed by the slopes (S) of the individual dose-response curves. The TD and S values obtained by FOT and by plethysmography were closely correlated (p < 0.001). The carbachol doses inducing a 50% reduction in SGaw were equivalent to those causing a 42% reduction in GRs0 (r = 0.90; p < 0.001). During bronchial challenge testing, FOT provides comparable information in terms of bronchial sensitivity and responsiveness to that supplied by plethysmography.

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The forced random noise oscillation technique (FOT) enables the measurement of respiratory resistance with a minimum of co-operation from the subject. This technique has proved to be sensitive in detecting airway obstruction [1, 2]. Several authors have reported that it is useful in detecting changes in bronchial tone during provocation tests in children [3, 4] and adults [5], or after administration of a bronchodilator agent in cases of pre-existing airflow limitation [6]. PIMMEL et al. [7] found that airway resistance correlated with forced random noise resistance during methacholine inhalation. Bronchial reactivity is usually evaluated by analysing the dose-response curves during bronchial challenge, with parameters such as the provocative dose (PD), the threshold dose (TD) or the slope (S) of the dose-response curve [8]. Plethysmography is an accepted standard for bronchial challenge and does not require forced expiratory manoeuvres. The aim of this study was therefore to compare, in terms of bronchial sensitivity and reactivity, the dose-response curves obtained by plethysmography and FOT; these techniques were performed simultaneously during bronchial challenge with inhaled carbachol in normal subjects and patients with bronchial hyperresponsiveness.

Materials and methods

Subjects
We studied 23 adults; fourteen men and nine women between 17 and 63 yrs of age. They included eight normal non-smoking subjects with no history of respiratory disease or atopy, recruited from our medical and paramedical hospital staff. The remaining subjects included asthmatic patients who met the American Thoracic Society's criteria for asthma [9] and had documented bronchial hyperresponsiveness to carbachol, as well as out-patients with a recent history suggestive of asthma, referred to our laboratory for a bronchial challenge test for the purpose of diagnosis. In all subjects, total lung capacity (TLC), vital capacity (VC), one second forced expiratory volume (FEV1) and maximum mid-expiratory flow (FEF25-75) were at least 80% of the predicted values. TLC, VC and FEV1 were measured with a water-sealed spirometer (Gauthier, France) and FEF25-75 was obtained during flow measurement using a Fleisch No. 3 pneumotachograph and a Validyne pressure transducer connected to a microcomputer (Apple Ile). FEV1 manoeuvres were performed at least 30 min before the bronchial challenge test. Bronchodilator therapy, if any, was withdrawn at least 12 h before the test. Research Ethics Committee approval for the study was obtained and all subjects gave their informed consent.
Measurements of specific airway conductance (sGaw)

The sGaw was measured with a flow pressure-compensated body plethysmograph according to the method of Du Bois et al. [10], modified by Lorino et al. [11]. The subjects panted at a thoracic volume close to their functional residual capacity (FRC). After each manoeuvre, sGaw was calculated and displayed on the visual unit of an Apple IIe microcomputer connected to the plethysmograph.

Measurements of respiratory impedance

Respiratory impedance was determined by FOT as described previously [12, 13] (EMA, Plaisir, France). Briefly, the seated subjects supported their cheeks with their hands and breathed quietly through a screen pneumotachograph (Jaeger, Würzburg, Germany). A random noise signal containing all harmonics from 2–25 Hz was applied to the mouth by means of loudspeakers. Mouth pressure and flow signals were measured by two identical differential pressure transducers (Sensym LDX 06001) and fed into an Apple IIe microcomputer. Recorded pressure and flow signals were processed by spectral analysis to calculate the impedance of the respiratory system at all oscillatory frequencies. To increase the signal to noise ratio, we modified our previous method of computation [12] which consisted of analysing each acquisition of data (lasting 16 s), and selecting the manoeuvre with the highest coherence function; instead, two data sets lasting 16 s each were obtained successively and these were processed together in order to provide a single value for respiratory impedance. Only impedance values with a coherence function of at least 0.8 were retained. According to Miller et al. [14], the normalized standard error of impedance for a limiting value of coherence of 0.8 is a function of the number of data ensembles that are averaged to calculate the impedance; since 8 ensembles were used in this study, the normalized error is about 0.2.

Respiratory impedance was separated into its real part or resistance (Rrs) and imaginary part or reactance. The following parameters were computed: Rrs0, an estimate of Rrs at zero frequency (the intercept of the Rrs-frequency regression line), the slope (A) of the Rrs-frequency regression line (Rrs = A • f + Rrs0, f being the oscillatory frequency) and the resonant frequency (Fr). For comparison with sGaw, Rrs0 was converted into its reciprocal Grs0. In this study, we chose to quantify the frequency dependence of resistance by means of a linear regression. Such a procedure might seem inappropriate since the relationship between resistance and frequency has been found to be non-linear, particularly in obstructive patients [15]. However, we obtained high linear coefficients of correlation (over 0.90) in all subjects. It is possible that more sophisticated methods, such as fitting multiple parameter models, could give more information on the mechanical behaviour of the respiratory system. However, the slope of the regression line of resistance over frequency is a simple and descriptive index of frequency dependence.

Carbachol dose-response curves

Baseline values for sGaw and respiratory impedance were obtained after inhalation of a aerosol of saline. In ten subjects (five normal and five patients), the challenge was preceded by five consecutive measurements of baseline sGaw and Grs0 in order to calculate their coefficient of variation (CV).

For all subjects, individual dose-response curves were constructed using a nebulized solution of 0.2% (weight per unit volume) carbachol (Sigma) in saline buffered to pH 7.0 with sodium bicarbonate for hyperresponsive patients, and 2% (weight per unit volume) carbachol in the same solution for normal subjects. A nebulizer (3M Bird products, St Paul, Minn; mass median diameter of particles: 3 μm) was used to fill a spirometer bell with fresh aerosol before each inhalation. The subjects were instructed to inspire slowly from their FRC a fixed volume of air (560 ml) containing the aerosol, and to hold their breath for 4 s to ensure maximal particle deposition. Three to five doses were administered to construct the dose-response curve. The initial dose of carbachol was 0.04 mg for hyperresponsive patients and 0.20 mg for normal subjects. The subsequent doses were determined according to the intensity of the bronchial response. Each dose consisted of a variable number of consecutive inhalations of the 560 ml of aerosol, from the subject's FRC. The test was stopped after sGaw had dropped by half. For four of the normal subjects, however, it was not possible to induce this drop despite their inhalation of large doses of carbachol, and the test was stopped after 30 min. At the end of the test, two puffs from a canister of albuterol aerosol (100 μg per puff) were administered to relieve airway obstruction. After each inhalation, sGaw (mean of two measurements) and respiratory impedance (one determination) were obtained in the same sequence for each subject. The individual sequence of measurements was randomly defined before the test started. All measurements were completed within 10 min of inhalation; the whole challenge did not last for more than 30 min after inhalation of the first dose of carbachol. Data from FOT were recorded on a floppy disk for subsequent processing.

Expression of results

Changes in sGaw and Grs0 were expressed as the percentage of change from the baseline value. Two dose-response curves were constructed for each subject with either sGaw or Grs0 plotted against cumulative doses of carbachol (fig. 1). Three parameters were calculated from each curve: 1) the threshold dose (TD), defined as the dose of carbachol producing a fall in baseline sGaw or Grs0 of twice their coefficient of variation, and regarded as an indicator of bronchial sensitivity; 2) the slopes (S) of
the regression lines fitted to the dose-response curve assessing bronchial responsiveness; and 3) the provocative dose (PD), defined as the dose which induced a predetermined percentage change from the baseline value. A 50% drop in sGaw was selected [16]. Since sGaw and Grs0 respectively reflect the airway and respiratory systems and are affected differently by lung volume, it was likely that the PD50 would be different with FOT (PD50 FOT) and plethysmography (PD50 PLT). To compare the results of the two methods we attempted to determine a decrease (x) in Grs0 for which PD50 FOT might be the equivalent of PD50 PLT. For each subject, PD50 FOT was computed for all the x values between 25 and 50%, with a 1% step. For each x value, we plotted PD50 FOT versus PD50 PLT, using the data for the 23 subjects, and computed their coefficients of correlation and the slopes of their regression lines. To assess the usefulness of the resonant frequency (Fr) and the Rrs-frequency regression line slope (A), we drew the dose-response curves using these two parameters and compared the slope of the dose-sGaw curve, firstly, with that of the dose-Fr curve and, secondly, with that of the dose-A curve, using the percentage changes in Fr and A.

Statistical method

Correlation coefficients were calculated by the least squares method. We assumed that p < 0.05 was significant.

Results

Baseline values and coefficient of variation (CV) of sGaw and Grs0. Anthropometric and spirometric data of the subjects are indicated in table 1. Baseline values for sGaw and Grs0 were 1.3 ± 0.4 kPa · s⁻¹ (mean ± SD) and 4.2 ± 1.3 l · kPa⁻¹ · s⁻¹ respectively. In ten subjects (five normal and five patients), the mean individual CV for Grs0 and sGaw were 9.2 ± 1.9% and 9.8 ± 3.9% respectively (p > 0.05). The value of 10% was taken for Grs0 but not for sGaw, since the latter was calculated from the mean of

Table 1. Anthropometric and baseline spirometric data of the 23 subjects. Vital capacity (VC) and forced expiratory volume in one second (FEV1) are expressed in ml and in percentage of predicted value (%).

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two determinations and therefore had a CV of 10%. 

An example of a dose-response curve constructed with the $G_{Rs_0}$ values is shown in figure 1.

**Threshold doses (TD).** The regression line was:

$$TD_{FOT} = 0.81 \times TD_{PLT} + 0.24$$

($r = 0.83$, df = 21, $p < 0.001$) (fig. 2a).

**Slopes (S).** The slope of the dose-response curve (S) determined by plethysmography correlated well with that obtained by FOT (fig. 2b); the regression line was:

$$S_{FOT} = 0.69 \times S_{PLT} - 5.7$$

($r = 0.87$, df = 21, $p < 0.001$).

**Provocative doses (PD).** For the decrease $x$ in $G_{Rs_0}$ ranging from 25–45%, the coefficient of correlation between $PD_{50 \times PLT}$ and $PD_{FOT}$ was always above 0.90 ($p < 0.001$), but from 45–50% it decreased. The slope of the regression line increased gradually from 0.46 ($x = 25\%$) to 1.25 ($x = 50\%$). When the provocative dose was computed for a decrease in $G_{Rs_0}$ of 42%, the points were scattered around the line of identity, as follows (fig. 2c): 

$$PD_{42 \times FOT} = 1.005 \times PD_{50 \times PLT} + 0.07$$

($r = 0.90$, df = 21, $p < 0.001$).

**Usefulness of Fr and A.** Although $Fr$ rose after inhalation of carbachol in all subjects, the slopes of the dose-$Fr$ and dose-$sGaw$ curves did not correlate ($r = 0.55$, df = 21, $p > 0.05$). The $Rrs$-frequency regression line slope (A) increased in some patients and decreased in others and these changes were not related to the changes in $sGaw$ ($r = -0.22$, df = 21, $p > 0.05$).

**Discussion**

This study showed a very close correlation between the threshold dose and the slope of the dose-response curves obtained by FOT and plethysmography in normal and hyperresponsive subjects undergoing bronchial challenge. Furthermore, the same doses of inhaled carbachol induced a 50% drop in $sGaw$ and a 42% drop in $G_{Rs_0}$. FOT and plethysmography therefore provided comparable information.

FOT is a non-invasive, effort-independent technique which is sensitive in detecting airway obstruction even at an early stage involving only small airways [1, 2, 12, 17]. As it only requires quiet breathing, bronchial tone is not affected by FOT as it is by forced expiratory manoeuvres [18]. In addition, the mean intra-subject variability of respiratory resistance has been reported to be 10% or less [6, 19], which is in accordance with our results.

FOT provides resistance values from 2–25 Hz. However, in detecting changes in bronchial tone, $Rrs$ is more sensitive at low frequencies than at high frequencies, as demonstrated by Pimmel et al. [7], and König et al. in asthmatic children [6]. Several studies, using $Rrs$ at low frequencies, have shown that FOT can reliably measure changes in bronchial tone such as bronchoconstriction caused by sulphur dioxide inhalation [20], histamine or methacholine inhalation [3–5, 7] and bronchodilation induced by albuterol in asthmatic children [6]. Takishima et al., who measured the reciprocal of $Rs$ at a frequency of 3 Hz during continuous inhalation of methacholine [5], and Decramer et al., who measured various impedance parameters after isocapnic hyperventilation with cold air [21], were able to differentiate between normal and hyperresponsive subjects. These authors therefore suggested the routine use of FOT for bronchial provocation tests in clinical practice.

Duiverman and co-workers [3] recently compared FOT and maximal and partial expiratory flow-volume curves during bronchial challenge testing in...
asthmatic children: they found that PD_{20,FEV1} and PD_{40,RRs} at 6 Hz were highly correlated and suggested that the TD might be as valuable as the PD. Tjwa et al. [22] found no correlation between decrease in FEV1 and increase in RRs at 3 Hz after inhalation of histamine diphosphate in different groups of adults with acute bronchitis, chronic bronchitis, bronchial asthma and various other respiratory disorders. This may be due to the modification of bronchial tone induced by forced expiratory manoeuvres, but is more likely due to differences in the technique of forced oscillations using only a single frequency of oscillation. We compared dose-response curves to inhaled carbachol obtained by FOT and plethysmography in adults (normal subjects and patients with various types of bronchial hyperresponsiveness), and demonstrated that comparable information was obtained in terms of bronchial sensitivity and responsiveness.

The changes in Grs0 following inhalation of carbachol were smaller than those in sGaw. There are two possible reasons for this discrepancy. FOT and plethysmography do not measure the same resistance parameters. FOT measures the conductance of the respiratory system which includes airways, lung tissues and the chest wall, whereas plethysmography only measures airway conductance. When expressed in percentage of the baseline value, Grs0 is, therefore, less likely to change with increasing bronchoconstriction than is Gaw. By comparison with Grs0 and airway conductance, sGaw reflects the lung volume at which it is measured [8]. We did not measure lung volume for every plethysmographic measurement in order to reduce the duration of the experiments. In five asthmatic patients, Takashima et al. reported a maximum increase in FRC of 0.51 during inhalation challenge with methacholine [5]. Other authors have reported that in asthmatics the thoracic gas volume measured by plethysmography could change by more than one litre following challenge [23]. Thus, as expected, the changes in Grs0 during bronchoconstriction were smaller than those in sGaw although Grs0 is probably affected by changes in lung volume during bronchial challenge. Despite these differences between Grs0 and sGaw, we found a close relationship between the changes in these parameters during carbachol inhalation, indicating that airway narrowing is more important than other factors such as lung volume or extra-bronchial resistance.

Other respiratory impedance parameters such as the slope of the Rrs-frequency regression line, which reflects the frequency dependence, were measured with FOT during the provocation test; these parameters were not suitable for differentiation between normal subjects and those with bronchial hyperresponsiveness since the changes in these values were not related to those in sGaw. In two previous studies, frequency dependence of Rrs was observed in hyperresponsive subjects, when marked bronchoconstriction was induced by methacholine inhalation [7] and isocapnic hyperventilation with cold air [21]. An increase in central resistance has been shown to result in an equivalent increase in total resistance at all frequencies, whilst an increase in peripheral resistance induces frequency dependence [15, 21, 24, 25]. It can, therefore, be inferred that low frequency Rrs reflects total resistance whilst high frequency Rrs reflects central resistance [7, 26]. Thus, Rrs was observed to be frequency dependent in patients with small airway obstruction [1, 2, 12]. In this study, after inhalation of carbachol, the presence or absence of a frequency dependence was observed in both normal and hyperresponsive subjects. This observation is in accordance with the data of Sekizawa et al. who found that the sites of airway response to bronchoconstrictor agents differ from one subject to another in both normal and asthmatic populations [27]. Another possibility is the occurrence in some subjects of a central resistance increasing during the bronchial test, at the laryngeal level for instance. Such an obstruction in the presence of a shunt of the upper airway may induce a frequency dependence of resistance [28].

We conclude that FOT is as efficient as plethysmography in providing information about bronchial sensitivity and reactivity during challenge with carbachol inhalation. Furthermore, the data provided by FOT can be adequately analysed using a simple parameter: the extrapolated conductance of the respiratory system at zero frequency. FOT can therefore be recommended for routine use in the examination of bronchial responsiveness.

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RÉSUMÉ: Nous avons comparé les courbes dose-réponse cumula-
tives obtenues au cours de l'inhaleation de carbachol, grâce à des
mesures simultanées de la conductance spécifique des voies
aériennes (sGaw) et de la conductance respiratoire chez 23 sujets
avec ou sans hyperreactivité bronchique. Dans ce but, la
conductance a été mesurée par un plethysmographe corporel,
tandis que la technique d'oscillation forcée avec bruit pseudo-
alaatoire a été utilisée pour déterminer la conductance respiratoire.
La conductance a été comparée à la conductance respiratoire
extrapollée à la fréquence 0 (Grs0). La sensibilité bronchique a été
déterminée par la dose seuil de carbachol (TD) qui entraînait une
diminution de sGaw et de Grs0, atteignant 2 fois le coefficient de
variation de leurs valeurs basales. La réactivité bronchique a été
déterminée par les pentes ($) des courbes individuelles dose-réponse
utilisant les pourcentages de modification de sGaw et de Grs0.
Nous avons également déterminé quelle modification de Grs0 serait
equivalente à une chute de 50% de sGaw en terme de dose
provocation de carbachol. Les valeurs de TD et de S obtenues par
la technique des oscillations forcées et par plethysmographie sont
en étroite corrélation (p<0.001). Les doses de carbachol détermi-
nant une réduction de 50% de sGaw sont équivalentes à celles
provocant une réduction de 42% de Grs0 (r=0.90; p<0.001).
Nous concluons que pendant le test de provocation bronchique, la
technique des oscillations forcées fournirait des informations compar·
able, en terme de sensibilité bronchique et de réactivité, à celles
obtenues par plethysmographie, et qu'elle peut donc être utilisée en
pratique clinique.