Direct-Writing Recorder of the Dose-Response Curves of the Airway to Methacholine*

Clinical Application

T. Takishima, M.D., F.C.C.P.; W. Hida, M.D.; H. Sasaki, M.D.; S. Suzuki, M.D.; and T. Sasaki, M.D.

We report a new device for examining the bronchial hyperresponsiveness by directly writing the dose-response curve of respiratory resistance (Rrs) during the continuous inhalation of the methacholine in stepwise incremental concentrations. Respiratory resistance was measured by the forced oscillation method. We found that the Rrs began to increase at a certain threshold concentration of methacholine (bronchial sensitivity) and that it has a curvilinear slope (bronchial reactivity). Subsequent inhalation of the bronchodilator drug returned the Rrs to the control level. Thus, we were able to examine bronchohyperresponsiveness in the patterns of the cumulative dose-response curves of methacholine. All normal subjects were nonresponders; while all of the asthmatic subjects, 63 percent (10) of the 16 patients with chronic bronchitis and 50 percent (7) of the 14 patients with acute bronchitis were responders. The dose-response curves were reproducible. Our device may be clinically applicable for examinations of bronchial hyperresponsiveness and for screening tests.

Bronchial responsiveness to various stimuli is used as one of the characterizing criteria for bronchial asthma.1 Orehek et al2 measured bronchial hyperresponsiveness by constructing cumulative dose-response curves to an inhaled bronchoconstrictor drug and reported that the dose of bronchoconstrictor needed to cause a bronchoconstriction (bronchial sensitivity) and the slope of the curves (bronchial reactivity) were not correlated. These investigators3 suggested that bronchial sensitivity and reactivity might be determined by different factors. Since the bronchial response to bronchoconstrictor agents could not validly be assessed by the determination of a single dose-response relationship, it would be necessary to examine the degree of constriction of the airways after the frequent inhalation challenge of incremental concentrations. So far, the bronchial provocation test for estimating the hyperresponsiveness of the airways has been generally examined by spirometric measurement4 or by the maximum expiratory flow-volume curve5 after the inhalation of a bronchoconstrictor agent. In this procedure, subjects expire periodically with maximum efforts after each inhalation of increasing doses of a bronchoconstrictor agent, in order to obtain the dose-response relationship; however, forced expiration itself might introduce bronchoconstriction.6 Furthermore, this test would be time-consuming because of the intermittent procedures involved. Even though the measurement of airway resistance with a body plethysmograph7,8 has been used for the evaluation of bronchial responsiveness, the time-consuming problem might not be solved. Therefore, we are looking for a new technique which avoids these problems and which would be clinically applicable for examinations of bronchial hyperresponsiveness, as well as for screening tests.

In the present study, we report a new device for examining the bronchial hyperresponsiveness by directly-writing the dose-response curve of respiratory resistance (Rrs) measured by the forced oscillation method9 during the continuous inhalation of methacholine in stepwise incremental concentrations. By using this device, we examined the bronchial hyperresponsiveness in normal subjects and in patients with acute bronchitis, chronic bronchitis, and bronchial asthma.

Materials and Methods

We studied ten normal subjects (all men, nine nonsmokers and one smoker; mean age, 30 years) without cardiopulmonary disease and without any allergy history or family history of allergy; 14 patients with acute bronchitis (eight men and six women; mean age, 44 years); 16 patients with chronic bronchitis (eight men and eight women; mean age, 48 years); and 60 patients with extrinsic asthma (36 men and 24 women; mean age, 42 years). Chronic bronchitis was diagnosed on the basis of the clinical history of cough and sputum for at least three months of the year over a minimum period of two years.1

*From the First Department of Internal Medicine, Tohoku University School of Medicine, Sendai, Japan.
Reprint requests: Dr. Takishima, First Department of Internal Medicine, Tohoku University School of Medicine, Seiryo-machi, Sendai, Japan 990
Subjects with chronic bronchitis had no allergic rhinitis or bronchial asthma. Bronchial asthma was diagnosed on the basis of clinical history and evidence that the disease was markedly reversible through the use of bronchodilator drugs. All subjects with bronchial asthma were asymptomatic without audible wheezing upon examination. Patients with acute bronchitis had a history of symptoms of sputum and cough for a short period only and had no chronic bronchitis, allergic rhinitis, or bronchial asthma prior to their acute illness. All were outpatients who avoided all medication for at least ten hours before the examination.

The apparatus for examination is diagrammed in Figure 1. The system for delivering serially increasing doses of methacholine consisted of 12 identical nebulizers. The nebulizers (Vaponephrin; USV Pharmaceutical Corp.) were driven with a constant air flow of 5 L/min by an air compressor (Nissho). Each nebulizer was connected to the main tube between the mouthpiece and the flowmeter. The air compressor could be switched in turn from one nebulizer to the next one at constant intervals of time. In this way, 12 kinds of aerosol could be sequentially delivered. Each nebulizer delivered approximately 0.15 ml of solution per minute. The sizes of the particles produced from the nebulizers were measured under conditions in the study by the application of the laser radar technique. According to the manufacturer’s specification the sizes of the particles were 2.5 μ in mean diameter; however, they were 9 μ in median diameter and 11 μ ± 6 μ in mean diameter (± SD) in the present system for delivery.

Bronchial responsiveness was displayed with the Rrs measured by the oscillation technique. The constant-amplitude pressure generator was connected to the mouthpiece and produced a constant-amplitude pressure of sine wave 3 Hz at the mouth with a loudspeaker box system. A constant bias flow of 0.4 L/sec was introduced between the nebulizer and the flowmeter to minimize the dead space. The airflow at the mouth (V) was measured with a pneumotachometer (Fleisch) and a differential pressure transducer (Validyne model MP45-1). The mouth pressure (Pao) relative to ambient pressure was measured with the differential pressure transducer (Validyne model MP45-1). In order to write the dose-response curves directly, the calculation of Rrs was performed by an analog computer according to Hyatt et al. In order to extract the component of only the 3-Hz wave of flow and pressure, V and Pao were passed through a 3-Hz bandpass filter with the width of 0.4 Hz and the time constant of 0.3 second. The flow was differentiated, and the zero point of differentiated flow was identified by a zero comparator circuit. Moreover, the maximal and minimal flow, corresponding to the zero point of the differentiated flow, was sampled and held. From the maximal and minimal flow, AV was derived by another subtraction circuit. Furthermore, two samples of pressure corresponding to maximal and minimal flow were subtracted to generate ΔPao. The Rrs was computed by a division circuit as ΔPao/V. In order to avoid rapid fluctuations in Rrs during continuous measurement, the Rrs was filtered by a 0.2-Hz low pass filter, the time constant of which was 4.0 seconds. The response time for 90 percent change of Rrs with this apparatus was 10 seconds. The Rrs was continuously displayed on the time scale of an X-Y recorder (Hewlett-Packard 7045A), so that the dose-response curves to methacholine were directly observed.

As the bronchial agonist, we used a solution of methacholine hydrochloride in isotonic saline. The following ten increments of dilution were used: 49μg/ml, 98μg/ml, 195μg/ml, 390μg/ml, 781μg/ml, 1,563μg/ml, 3,125μg/ml, 6,250μg/ml, 12,500μg/ml, and 25,000μg/ml. The concentrations of methacholine were approximately of the same order of magnitude as recommended by Chai et al. The first nebulizer contained the 2-ml saline solution, while a 2-ml solution of methacholine of each concentration was contained from the second to 11th nebulizers, respectively. Each concentration of the methacholine solution was inhaled for a period of one minute. The 12th nebulizer con-

\[\text{Figure 1. Block diagram of apparatus. Respiratory resistance is measured with oscillation method. Twelve nebulizers are connected to main tube near mouthpiece.}\]
tained metaproterenol as a bronchodilator agent. When Rs reached twice the initial Rs, metaproterenol was inhaled for a period of two minutes; or when the inhalation of methacholine was performed to its maximum concentration; or when subjects indicated a sign of dyspnea. All subjects were examined during quiet breathing in a sitting position. The breathing patterns of the subjects were not controlled, since under patterns of controlled breathing, fluctuations of Rs were increased, probably due to the unstable glottic aperture. Both sides of the cheeks were pressed by two balloons containing air, in order to minimize the oscillation of the cheeks.

We examined the bronchial hyperresponsiveness using the direct-writing recorder of the dose-response curves to methacholine in ten normal, 60 asthmatic, and 30 bronchitic subjects. The reproducibilities of the dose-response curves to methacholine were studied among the subjects who had bronchial responsiveness to methacholine. We examined the dose-response curves on two separate days within seven days after the first challenge in six asthmatic subjects and in two subjects with acute bronchitis. Furthermore, we examined the dose-response curves on three to four separate days within a month in two asthmatic subjects and in one subject with acute bronchitis. All data were statistically analyzed using a paired or unpaired t-test.

RESULTS

Figure 2 shows the dose-response curve in a patient with bronchial asthma. In bronchial asthma after the Rs remained at an almost constant value for a short period, it increased curvilinearly after a threshold concentration of methacholine and decreased rapidly after the inhalation of metaproterenol. In general, the patterns in the cases of bronchial asthma were similar to isosceles tri-

angles. On the other hand, in the normal subjects, the Rs did not increase up to the maximum concentration of methacholine hydrochloride of 25,000μg/ml. In acute or chronic bronchitis, in general, the Rs increased slowly from a concentration of methacholine hydrochloride of 3,125μg/ml to 25,000μg/ml and decreased after the inhalation of metaproterenol. Thus, we might be able to distinguish different patterns of the dose-response curves among those of the normal subject, patients with bronchitis, and those with bronchial asthma.

To evaluate the dose-response curve, we calculated the three parameters. Since the calculated respiratory conductance (Gr) from Rs showed approximately linear slope, the best fitting straight line was drawn by eye. A linear regression equation was derived to describe the slope of Grs in some curves, but there were little differences between the lines drawn with the linear regression equation and those drawn by eye.

The first calculated parameter was the control value of the conductance (Grcont), ie, the reciprocal of Rs during the inhalation of the saline aerosol.

The second calculated parameter was the minimum dose of methacholine (Dmin), that is, the amount of the cumulative dose at the inflection point where the reciprocal of Rs (Grs) decreases linearly. When the inflection point where Grs started to decrease was not clear, we chose the point of intersection of the control value (Grcont) and the linear slope of Grs. We defined the Dmin as the
indicator for bronchial sensitivity. We calculated the cumulative dose of methacholine by the unit in a similar way to the standardization of inhalation challenge technique recommended by Chai et al. In that is, one unit equals one minute of inhalation of aerosol solution at 1.0 mg/ml during quiet tidal breathing. Therefore, the total cumulative dose of methacholine became 50 units from the beginning to the end of the inhalation challenge. In Figure 2 the cumulative dose of methacholine is also shown by the unit.

The third parameter calculated was the linear slope of the Grs decreased (SGrs), that is SGr = ΔGrs/Δt (in L/sec/cm H2O/min), which was defined as the bronchial reactivity.

Figure 3 shows the Grs-cont, Dmin, and SGr in ten normal, 60 asthmatic, and 30 bronchitic subjects. All data were from the dose-response curves obtained on the first day. In asthmatic patients, Grs-cont (0.23 ± 0.09; mean ± SD) was significantly lower than Grs-cont in normal subjects (0.35 ± 0.09) and those with acute bronchitis (0.32 ± 0.15; P < 0.01), but there was no significant difference of Grs-cont in normal subjects and those with acute and chronic bronchitis (0.28 ± 0.15).

All patients with bronchial asthma showed Dmin less than 50 units (1.47 ± 3.29 [mean ± SD]; median, 0.45). In the normal subjects, we could not recognize the increase of Rs at all in the range of the concentrations of methacholine that were used. Since a Dmin of 50 units differentiates the patients with bronchial asthma from the normal subjects, subjects who had a Dmin of more than 50 units were treated as nonresponders and subjects who had a Dmin less than 50 units were treated as responders. In Figure 3, values of Dmin of more than 50 units were plotted at 50 units. Therefore, the mean values of Dmin in normal subjects, acute bronchitis (31.8 ± 20.6) and chronic bronchitis (21.2 ± 23.3) were underestimated. There were responders in 50 percent (7) of the 14 subjects with acute bronchitis and, 63 percent (10) of those 16 subjects with chronic bronchitis. In asthmatic patients the SGr (0.053 ± 0.028 [mean ± SD]; median, 0.040) was significantly higher than that of the other patients (P < 0.001), while there were no significant differences of SGr between patients with acute (0.029 ± 0.037) and chronic bronchitis (0.023 ± 0.023). We examined how the level of the initial state of the narrowing airway (that is, Grs-cont) could modify the dose-response curves. There was a very poor correlation between Grs-cont and Dmin in all responders (r = 0.381). On the other hand, Grs-cont correlated with
SGrs in patients with asthma ($P < 0.01$) or with chronic bronchitis ($P < 0.01$). There was no correlation between the sensitivity and the reactivity in all responders.

All of the dose-response curves studied on the separate days are shown in Figure 4. With some variation, subjects 1 to 8 had reproducible dose-response curves, while subjects 9 and 10 did not. Since in subjects 9 and 10 the control values of $Rrs$ were different, the bronchial responsiveness might also have been different. In subject 11, the clinical symptoms of acute bronchitis were slightly improved when the dose-response curve with the lower bronchial responsiveness was examined. There were highly significant correlations between the first and the second dose-response curves in both sensitivity and reactivity in subjects 1 to 11 ($P < 0.01$).

**DISCUSSION**

**Validity of the Method**

The bronchial provocation test is widely used, not only for examining the bronchial responsiveness in various pulmonary diseases, but also for diagnosing bronchial asthma; however, since the method of examination has not yet been established, each investigator uses his own method. Therefore, the comparison of data among different laboratories has been difficult. Recently Chai et al reported the standardizations of the procedures for bronchial inhalation challenge for antigen, methacholine, and histamine. Following their method, to obtain the dose-response curve, subjects must inhale aerosol from the functional residual capacity (FRC) to the total lung capacity (TLC) and must perform multiple forced expiratory maneuvers or pant many times after every inhalation of the different concentrations of bronchostimulator agents. Forced expiratory maneuvers or deep inflations themselves might modify the degree of bronchoconstriction and furthermore, the method was too time-consuming to be used for a screening test; however, the present technique seems to solve these problems. Using the present method, we are able to measure the continuous change of $Rrs$ during quiet breathing and, therefore, minimum cooperation of the subject. In addition, the test is over a short time (about 10 to 15 minutes for one subject) and is quite safe, because the measurement could be performed monitoring the change in $Rrs$ and finished without inducing an asthmatic
attack. Furthermore, direct-writing dose-response curves showed good reproducibilities (Fig 4). Therefore, our method would be useful for the clinical and screening test of bronchial hyperresponsiveness.

Possible Errors

The bronchoconstriction induced by drugs might be accompanied by laryngeal constriction; however, it has not been shown that even the panting or forced expiratory maneuvers prevent laryngeal narrowing during bronchial provocation. Therefore, further study would be needed in order to know how laryngeal constriction may affect the indirect measurements of bronchoconstriction during bronchial provocation in human beings. The Rrs was variable when the subject coughed, swallowed saliva, or ceased breathing artificially, presumably due to transient narrowing of the glottic aperture. Otherwise, Rrs was quite stable.

The dose-response curves of the airway to methacholine were examined by the display of Rrs but not of Grs. The dose-response curves were curvilinear, and asthmatic subjects who had great reactivity tended to show steeper curves. These subjects were often afraid of inducing too much bronchoconstriction unless we let them inhale metaproterenol after a certain value of Rrs (Fig 2); however, we could not differentiate from the Grs the steep increase of Rrs just before the inhalation of metaproterenol from the initial slow increase of Rrs. In other words, the Grs showed less change than the Rrs when the subjects suffered severe induced bronchoconstriction. Thus, the Rrs seems to be more sensitive than the Grs as an indicator of bronchoconstriction; however, both displays (Rrs and Grs) and the calculation of both sensitivity and reactivity by computer are needed for convenient device.

In the preliminary study, fluctuations in pulmonary volume during the inhalation challenge with methacholine were observed in five asthmatic subjects who were in the volume-type body plethysmograph with a respirometer (Krogh). We observed a maximum of a 0.5-L increase in FRC when Rrs increased twice during the inhalation challenges using the present device. The increase of the FRC by 0.5 L had the effect of increasing airway conductance by approximately 20 percent or less. Therefore, the reactivity would be slightly underestimated.

Comparison to Conventional Method

The present technique is not considerably different from the standardized procedure recommended by Chai et al. They recommended five breaths of a given dilution inhaled from FRC to TLC, while our technique gave one minute of quiet tidal breathing for a given dilution. Thus, the cumulative inhaled volume of methacholine in the standardized procedure would be 12.5 L for a given dilution with an inspiratory capacity of 2.5 L, while it would be about 8 L in the present method. Since the dilutions of methacholine delivered by both techniques are comparable, the inhaled volume at a given dilution in the present study would be about two-thirds that of the standardized method; however, since the nebulizers, the compressors to drive solutions from the nebulizers, and even the connecting system to the mouthpiece are different in the two techniques, precise comparisons of the cumulative doses inhaled could not be made. Bronchial sensitivities in the asthmatic subjects in the present study (Fig 3) are roughly comparable or more sensitive than those obtained in the standardized procedure. The percentages of responders among the subjects with bronchitis (Fig 3) were also roughly comparable to those reported by Parker et al and Empey et al. Rosenthal reported that sensitivity measured by specific airway conductance by the panting method was greater than it was with the spiographic display method. On the other hand, the values obtained from the panting method were approximately equal to those obtained from the oscillation method. Therefore, the different methods of obtaining the values of bronchial sensitivity might be one of the reasons for the variation of values. Different methods of calculation might be another reason for these different values. The standardized procedure defines sensitivity as the cumulative dose at a 20 percent decrease in forced expiratory volume in one second (FEV₁) from the control value. On the other hand, sensitivity in the present study is defined as the cumulative dose from the point where Grs begins to decrease (Fig 2). Therefore, in the case of low reactivity, the standardized procedure would be less sensitive than our method. The values of reactivity for the asthmatic subjects in the present study varied, but they were roughly twice as great as those obtained from the standardized procedure. Again, due to a similar reason as the difference of sensitivity, the methodologic difference of measurement might be one of the reasons for different reactivity. The reactivities are also calculated in different ways. The standardized procedure defines the reactivity as the percentage of decrease of FEV₁ from the control value divided by the dose of agonist, while we define it as the decrease of Grs divided by time. The dose-response curve measured in human subjects is comparable.
to that studied in the isolated smooth muscle of the airway. In the isolated muscle, tension developed is plotted on the log concentration of agonist, and the slopes of the midportion of the dose-response curve are considered as one of the characteristics in assessing the behavior of the muscle. Since in the present method, Rs is displayed on the log concentration of methacholine, the present dose-response curves would be comparable to those studied in the isolated muscle. Therefore, our definition of reactivity would be more comparable to that calculated in the isolated muscle than the standardized definition. The reactivity calculated using the standardized procedure at a high concentration of methacholine would be smaller than that calculated using the present procedure, and vice versa at a low concentration of methacholine; however, average reactivities would not be different due to the different methods of calculations.

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