Slope of the dose-response curve: usefulness in assessing bronchial responses to inhaled histamine

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ABSTRACT The value of determining the slope of the histamine dose-response curve, in addition to the histamine provocation concentration producing a 20% reduction in FEV₁ (PC_{20-FEV₁}), was assessed by analysis of histamine dose-response curves in 40 patients selected as having a wide range of increased non-specific bronchial responsiveness to inhaled histamine. The histamine dose-response curves were found to fit the linear curve (dose ν response, mean $r^2 = 0.97$) better than the logarithmic curve (log dose ν response, mean $r^2 = 0.93$), the difference being significant ($p < 0.001$). There was a strong negative correlation between the PC_{20-FEV₁} and the slope ($r = -0.98$, $p \leq 0.001$) and a weak negative correlation between the PC_{20-FEV₁} and the log-dose-response slope ($r = -0.38$, $p < 0.05$). Sixteen normal subjects and 16 asthmatic patients were compared on the basis of histamine dose-response curves measuring fall in sGaw. In this study there was no difference between r^2 for the linear determination and for the logarithmic determination ($0.91 \nu 0.90$, $p > 0.05$). The PC_{35-sGaw} showed a strong negative correlation with the dose-response slope ($r = -0.95$, $p \leq 0.01$) and no correlation with the log-dose-response slope ($r = 0.09$, $p > 0.05$). In the two studies there appeared to be little information gained from the determination of either the dose-response slope or the log-dose-response slope. The slope and the PC_{20-FEV₁} were equally reproducible, duplicate determinations showing less than a two-fold difference in 14 of 15 paired PC₂₀ measurements and in 13 of 15 paired slope measurements. In summary, the slope of the histamine dose-response curve appears to fit the linear model better than the logarithmic model. It is feasible to calculate it from the results of a standardised histamine inhalation test; determination of either the slope or the log-dose-response slope, however, appears to add little useful information. It is recommended that bronchial provocation test results should be expressed in terms of a threshold concentration such as the PC_{20-FEV₁} or the PC_{35-sGaw}.

Measurements of non-specific bronchial responsiveness to inhaled histamine or inhaled cholinergic agonists such as methacholine has become an important diagnostic and research aid.^{1,2} Inhalation tests are performed with increasing doses of the provoking agents, and the results are generally expressed either as the first dose or concentration which produces more than a predetermined response or as the interpolated dose producing a given response. For example, the histamine provocation concentration producing a 20% decline in the one-second forced expired volume (FEV₁) is referred to as the histamine PC_{20-FEV₁}³ and that producing a 35% decrease in specific airway conductance (sGaw) as

the histamine PC_{35-sGaw}.⁴ Recently, however, Orehek *et al* suggested that a more detailed examination of bronchial provocation dose-response curves was valuable.⁵ These authors made a distinction between the slope of the inhaled carbachol dose-response curve, referred to as "reactivity," and the dose causing a 25% reduction in sGaw (PC_{25-sGaw}), referred to as "sensitivity." It was suggested that different mechanisms may determine "reactivity" and "sensitivity," that "hyper-reactivity" was more important to the asthmatic state than was "hypersensitivity," and that both should be determined when bronchial provocation tests are being interpreted.

We have developed a standardised, reproducible histamine inhalation test which has proved useful in extensive clinical research.^{3,6-9} Test results have

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been expressed as the histamine PC_{20-FEV_1} or the histamine $PC_{35-sGaw}$. In the current study we have used this histamine inhalation test and determined the slope of the histamine dose-response curves. We have evaluated both the feasibility and the usefulness of measuring "reactivity" by this method. We have also assessed whether the histamine dose-response slope fits the linear or the logarithmic curve better.

Methods

Subjects

Normal subjects with no history of asthma, rhinitis, or other respiratory disorder; subjects with asthma¹⁰; and subjects with rhinitis were selected from the respiratory diseases clinic at the University Hospital in Saskatoon and from volunteers within the laboratory. Those who were taking medications were asked to refrain from using antihistamines for 48 hours and bronchodilators for eight hours; corticosteroids were continued in the same dose. The subjects were non-smokers. This investigation was approved by the ethics committee of the University of Saskatchewan and signed informed consent was obtained.

Histamine inhalation test

The standardised histamine inhalation test was performed as previously outlined.^{3,7} After a control inhalation of normal saline, doubling concentrations of histamine acid phosphate from 0.03 to 8.0 mg/ml were inhaled for two minutes at five-minute intervals. Inhalations were made via a face mask from a Wright nebuliser operated with an airflow rate of 7 l/min and with 5 ml of solution in the vial. The output of the nebuliser was 0.130 ml/min and the particle size mass median diameter 1 μ m.

The histamine PC_{20-FEV_1} was determined by means of FEV_1 measurements from a Godart 9-litre water spirometer. The FEV_1 was measured in triplicate before the inhalations and again 30 and 90 seconds after each inhalation. If the 90-second FEV_1 was less than the 30-second FEV_1 , the measurement was repeated three and if necessary five minutes after the inhalation. The test was continued until FEV_1 had fallen by 20% or more, or until the top concentration had been inhaled. The percentage reduction in FEV_1 was calculated from the lowest post-saline FEV_1 to the lowest post-histamine FEV_1 for each histamine concentration. The histamine PC_{20-FEV_1} was then calculated from the dose-response curve by linear interpolation between the last two points, as previously described.³

The histamine $PC_{35-sGaw}$ was determined (in sepa-

rate tests) by means of measurements of sGaw performed in a constant-volume variable-pressure body plethysmograph (Cardio-Pulmonary Instruments, Model 2000). The mean of four measurements of sGaw was calculated before the inhalations, and again 30-90 seconds after each inhalation. The percentage reduction in sGaw was calculated from the post-saline mean sGaw to the post-histamine mean sGaw at each histamine concentration. The test was continued until there had been a 35% or greater reduction in sGaw, or until the top histamine concentration had been administered. The histamine $PC_{35-sGaw}$ was determined from the last two points of the histamine dose-response curve in a manner similar to the histamine PC_{20-FEV_1} .

The slope of each histamine dose-response curve was determined by means of the method of least-squares linear regression.¹¹ The first point showing a measurable reduction in either FEV_1 or sGaw and all subsequent points were used in the regression. Both the slope of the linear curve (percentage reduction versus dose) and the slope of the logarithmic curve (percentage reduction versus log dose) were calculated. The coefficient of determination (r^2) was determined for each regression.

STUDY NO 1: PC_{20-FEV_1}

Forty subjects were selected to participate in the study. All were known to have increased bronchial responsiveness to inhaled histamine with a histamine PC_{20-FEV_1} of less than 8.0 mg/ml. They included 30 with bronchial asthma, six with rhinitis, and four normal individuals with mildly increased responses to histamine. The subjects were selected to represent a wide range of bronchial responsiveness; their PC_{20s} ranged from 0.06 to 8.0 mg/ml. The histamine PC_{20-FEV_1} and both the dose-response slope and the log dose-response slope were calculated from each of the 40 curves. Determination of the slopes of the dose-response curves was based on two points in nine subjects, three points in 26 subjects and four points in five subjects.

Logarithmic transformation of PC_{20} and of slope of the dose-response curve, but not the slope of the log dose-response curve, was required for the analysis. Firstly, the coefficients of determination (r^2) of the slope of the linear dose-response curve and of the slopes of the log dose-response curve (from the 31 curves with more than two points) were compared by means of the paired t test.¹² Secondly, correlation between the PC_{20-FEV_1} and the slopes of both the linear and the log dose-response curves was sought by the method of least-squares linear regression.¹¹ Finally, the 20 subjects with the highest and the 20 with the lowest log-dose-response slopes were compared with a view to finding differences in

clinical features; Student's *t* test¹³ and the χ^2 test¹⁴ were used.

STUDY NO 2: PC_{35-sGaw}

The second study was carried out to compare asthmatic with normal subjects. Sixteen patients with well-documented asthma¹⁰ and 16 normal subjects participated. All had a 30% or greater reduction in sGaw after inhalation of the top concentration (8 mg/ml) of histamine—that is, the PC_{35-sGaw} was less than 10 mg/ml. We had to test 36 normal subjects to find 16 with a 30% reduction in sGaw after 8 mg/ml of histamine. Histamine dose-response curves were obtained and the PC_{35-sGaw} was determined as well as the slopes of the dose-response curve and the log-dose-response curve. Determination of the slopes was based on two points in two subjects, three points in 19 subjects, four points in 10 subjects, and five points in one subject.

The analysis was performed in the same way as in the first study. The coefficients of determination for the linear and log slopes were compared for the 30 curves where more than two points were used in the regression. The PC_{35-sGaw} was regressed with the slopes of both the linear and the log-dose-response curves. In addition, the asthmatic were compared with the normal subjects with regard to anthropometric data, baseline sGaw, PC_{35-sGaw}, slope of dose-response curve, and slope of log-dose-response curve by means of Student's *t* test.¹³

STUDY NO 3: REPRODUCIBILITY

The reproducibility of measurements of PC_{20-FEV₁}, and the slope of the curve was assessed by duplicate histamine inhalation tests. The tests were performed at the same time of day within one week; baseline FEV₁ varied by less than 10% and there was no history of recent respiratory infection or allergen

exposure. Fifteen asthmatic subjects whose PC₂₀ was less than 8 mg/ml participated in this study. From the two tests the percentage difference between the two determinations of PC₂₀ and of the slope of the curve was calculated by the formula

$$\frac{\text{difference in 2 tests' results}}{\text{mean value of 2 tests' results}} \times 100.$$

The results were compared by means of the paired *t* test.¹²

Results

STUDY NO 1: PC_{20-FEV₁}

Details of the 40 subjects in this study are given in table 1. The slope of the histamine dose-response curve fitted the linear model better than the logarithmic model; the mean *r*² for the percentage fall versus dose regressions was 0.97 ± 0.03 and for the percentage fall versus log-dose-regressions 0.93 ± 0.06 (difference significant, $p < 0.001$). The histamine PC₂₀ is compared with the slope of the dose-response curve in figure 1. There was a more than 100-fold range in PC₂₀ values, from 0.06 to 8.0 mg/ml; and there was a similar range in slopes, values ranging from 3 to 460 (% fall/mg/ml). There was a strong negative correlation between the PC₂₀ and the slope ($r = -0.98$, $p \ll 0.001$), the slope of the curve being larger in subjects with a low PC₂₀. Only one subject fell outside the 95% confidence of limits of this regression (fig 1). The histamine PC₂₀ was then compared with the slope of the log-dose-response curve (fig 2). The range of the values for this slope was smaller, from 12 to 90 (% fall/log mg/ml). There was a weak negative correlation between the PC₂₀ and the log-dose-response slope ($r = -0.38$, $p < 0.05$). In an effort to determine whether there was any significance in the differences

Table 1 Comparison of subjects with high and low log-dose-response curves

	High slope: 60 ± 15 (% FEV ₁ fall/log mg/ml)	Low slope: 27 ± 6	Significance
Number of subjects	20	20	
Number with asthma	17	13	NS
Number with rhinitis	2	4	NS
Number of normal subjects	1	3	NS
Age (y) (mean $\pm$ SD)	38 ± 18	35 ± 17	NS
Sex (male: female)	7:13	11:9	NS
Number with atopy	16	7	$p < 0.05$
Duration of symptoms (y)	11 ± 11	7.7 ± 9.5	NS
FEV ₁ (l)	2.61 ± 0.81	2.98 ± 0.84	NS
Histamine PC _{20-FEV₁}	0.79*	1.97*	$p < 0.05$
Asthma treatment: number having			
none	9	11	NS
adrenergic bronchodilators	10	9	NS
theophylline	4	2	NS
steroids	8	5	NS

*Geometric mean.

observed in the slope of the histamine log-dose-response curve, the 20 subjects with the highest log-dose-response slope (38–90) were compared with the 20 with the lowest values (12–37) (table 1). As was anticipated from the significant regression (fig 2), those with the higher slopes had a slightly lower PC_{20} ($0.79 \nu 1.97$, $p < 0.05$). There were no significant differences in anthropometric data, duration of symptoms, FEV_1 or asthma treatment between the two groups; the only difference was a significantly greater prevalence of atopy ($16 \nu 7$, $p < 0.05$) in the group with the higher slope.

STUDY NO 2: PC_{35} -sGaw

Details of the 32 subjects (16 asthmatic and 16 normal) are given in table 2. The results of the second study are similar to those of the first. The linear model for the regressions was similar to the logarithmic model ($r^2 = 0.91 \pm 0.1 \nu 0.90 \pm 0.1$, $p > 0.05$). The PC_{35} and the dose-response slope were compared (fig 3). There was a similar magnitude of ranges, with PC_{35} ranging from 0.04 to 10 and the slope from 2 to 400. A strong negative correlation between PC_{35} and the dose-response slope ($r = -0.95$, $p \ll 0.001$) is seen in fig 3. There was no significant correlation between the PC_{35} and the log-dose-response slopes between the asthmatic and normal and asthmatic subjects were compared in table 2. The asthmatic subjects were somewhat older ($46 \nu 29$ years) and had more severe airflow obstruction, as shown by lower mean values for both FEV_1 and sGaw. The significant differences between asthmatic and normal subjects in PC_{35} ($0.33 \nu 4.8$, $p < 0.001$) and the dose-response slope ($75 \nu 7.6$, $p < 0.001$) were of the same order of magnitude (about 10-fold). An important point is that the 16 normal subjects included in this study were selected from 36 normal subjects, and they represent those with the lowest PC_{35} values in the group. Consequently, the mean value for normal individuals in this study ($PC_{35} = 4.8$) cannot be taken as representative of our normal subjects. There was no difference in the log-dose-response slopes between the asthmatic and normal subjects.

STUDY NO 3: REPRODUCIBILITY

The histamine PC_{20} - FEV_1 and the dose-response slope were equally reproducible. The PC_{20} was reproducible to within one doubling dilution in 14 of the 15 subjects; this is similar to our previous observation.⁷ There was no significant difference between the percentage differences in the two determinations of PC_{20} (23 ± 23) and the slope (34 ± 26) ($p > 0.05$).

Fig 1 Histamine PC_{20} - FEV_1 versus slope of the histamine dose-response curve. The PC_{20} (mg/ml) is on the horizontal axis and the slope (% FEV_1 fall/mg/ml) on the vertical axis. A marked negative correlation ($r = -0.98$, $p \ll 0.001$) is evident.

Fig 2 Histamine PC_{20} - FEV_1 versus slope of the histamine log-dose-response curve, showing a weak negative correlation ($r = -0.38$, $p < 0.05$).

Table 2 "Reactivity" and "sensitivity" to inhaled histamine in normal and asthmatic subjects

	Normal	Asthmatic	Significance
Number of subjects	16	16	
Age (y) (mean $\pm$ SD)	29 $\pm$ 10	46 $\pm$ 16	$p < 0.05$
Sex (male: female)	6:10	5:11	NS
Height (cm)	169 $\pm$ 11	164 $\pm$ 8	NS
FEV_1 (litres)	3.60 $\pm$ 1.0	2.24 $\pm$ 0.8	$p < 0.001$
sGaw ($l s^{-1} cm H_2O^{-1}$)	0.23 $\pm$ 0.07	0.10 $\pm$ 0.05	$p < 0.001$
Histamine PC_{35} -sGaw (mg/ml)	4.8 $\pm$ 1.7*	0.33 $\pm$ 4.2*	$p < 0.001$
Slope of dose-response curve (% FEV_1 fall/ml)	75 $\pm$ 4.4*	7.6 $\pm$ 2.2*	$p < 0.001$
Slope of log-dose-response curve (% FEV_1 fall/log mg/ml)	61 $\pm$ 32	58 $\pm$ 20	NS

*Geometric mean and geometric standard deviation.

Fig 3 Histamine $PC_{35-sGaw}$ versus slope of the histamine dose-response curve, showing a strong negative correlation ($r = -0.95$, $p \leq 0.001$). Solid circles represent the 16 asthmatic and open circles the 16 normal subjects.

Fig 4 Histamine $PC_{35-sGaw}$ versus slope of the histamine log-dose-response curve, with no correlation evident ($r = -0.09$, $p > 0.05$). Solid circles represent the 16 asthmatic and open circles the 16 normal subjects.

Discussion

This investigation was carried out to examine the usefulness of measurements of the slope of the histamine dose-response curve, or the so-called reactivity, when applied to a test procedure which has been well standardised to give reproducible values for histamine PC_{20} , the so-called sensitivity. It must be concluded from this study that on the basis of this testing procedure there appears little to be gained from measuring both "reactivity" and "sensitivity" in assessing the bronchial response to inhaled histamine.

The study was not performed to examine in detail the characteristics of the histamine dose-response curve. From this point of view there are several shortcomings in the current methods. For a detailed analysis of the dose-response curve ideally more data points should have been obtained, certainly more than two, for all regressions. Under ideal con-

ditions the best procedure would be to continue measurements for the dose-response curve until the maximum or "plateau" response had occurred. This is, of course, not possible in man. Consequently, a poor second choice would be to fix the upper limit of response at a set percentage reduction in FEV_1 or sGaw, the theory being that this might approximate a fixed point (that is, a fixed percentage of maximum response) on the total dose-response curve. In the current study a minority of slopes were calculated from only two points—nine of 40 in the FEV_1 study and two of 32 in the sGaw study. Although we might reasonably question the accuracy of the slope calculated from only two points, these slopes did in fact fit as well as those determined from three or four points in the regressions shown in figures 1 and 3. The failure to specify a set upper limit of response in this study is also less than ideal. All subjects in the FEV_1 study had a 20% or greater fall in FEV_1 and the range of maximum response was 20–40% (mean $29 \pm 8\%$), whereas all the subjects in the sGaw study had a greater than 30% reduction in sGaw, with a range of 30–70% (mean $49 \pm 14\%$). Fixing the upper limit of response would be more important if the dose-response relationship was indeed a "curve" or curvilinear. The fact that the relationship fits the linear model well (the mean r^2 , if we exclude those slopes calculated from only two points, was 0.98 in the FEV_1 study) suggests that the setting of a consistent maximum response for all curves is somewhat less important. If the dose-response relationship is indeed linear, the slope should not be greatly affected by variations in the maximum response. Thus with these provisos it appears feasible to calculate with some accuracy the slope of the histamine dose-response curve from data obtained with our inhalation technique. Theoretically, improved accuracy would result if more concentrations of histamine were used—for example, twice as many—and if the maximum response were set within narrow limits—for example, 30–40% FEV_1 reduction or 40–50% sGaw reduction. It may not always be possible, however, either because of histamine-induced symptoms or because of failure to obtain a maximum response in the desired range, to achieve this ideal.

A minor finding (for which this study was not designed) was that the histamine dose-response curves appeared to fit the linear model better than logarithmic model. Extrapolation from in vitro muscle studies suggests that dose-response curves for muscle-constricting agents should follow a log dose versus response pattern.¹⁵ Our findings, however, are consistent with those of Orehek *et al*, who found that the dose-response curve for carbachol concentration versus sGaw was linear.⁵ In addition, exami-

nation of the individual dose-response curves for methacholine concentration versus FEV_1 reported by Felarca and Itkin suggests that these fit a linear better than a logarithmic model.¹⁶

We found that the slope of the histamine dose-response curve showed a strong negative correlation both with the PC_{20-FEV_1} and with the $PC_{35-sGaw}$ ($r = -0.98$ and -0.95 respectively). This shows that both increasing dose-response slopes and decreasing PC_{20} or PC_{35} reflect increasing bronchial responsiveness to inhaled histamine. The very close correlation, especially with PC_{20} , suggests that the measurements may be used interchangeably, and that with knowledge of the PC_{20} one could accurately predict the slope of the curve. Thus the slope appears to provide little added information. These results are at variance with those of Orehek *et al*, who showed no correlation between the slope of the carbachol dose-response curve and the $PC_{25-sGaw}$.⁵ That study used a cumulative carbachol dose response curve,⁵ whereas ours used a dose-response curve based on histamine, which has been previously shown to have no cumulative effect.⁷ There are other differences in the design of the studies which might be relevant in explaining the difference. Orehek *et al* studied a smaller number of subjects with a narrower range of bronchial responses, the range for $PC_{25-sGaw}$ being only 20-fold.⁵ A second difference is that they produced a greater fall in sGaw in all subjects, attempting to achieve a 75% reduction in all and reaching at least a 50% reduction in all. Whether the differences between our results and those of Orehek *et al* are due to inherent differences between histamine and cholinergic agonists, to differences between cumulative and non-cumulative dose-response curves, or to other technical factors is not clear.

Although the log-dose-response slope was not as accurate a description of the curve as the linear, it did provide a reasonable fit ($r^2 = 0.93$ and 0.90). The log-dose-response slope showed only a weak negative correlation with PC_{20-FEV_1} and no correlation with $PC_{35-sGaw}$. The relatively narrow range for the values of this log-dose-response slope, in conjunction with the small or absent trend in the regressions when they were plotted against PC_{20} and PC_{35} , shows that the log-dose-response slopes are nearly parallel throughout the entire range of bronchial responsiveness, being only slightly steeper at the more responsive end in the PC_{20-FEV_1} regression (fig 2). This is consistent with the observation of Hargreave *et al*, who showed parallel log-dose-response curves for inhaled histamine.¹⁷ There was no difference in the log-dose-response slope between 16 normal and 16 asthmatic subjects and there was little difference in the clinical features of the

subjects with the high slopes and those with the low slopes in the FEV_1 study. The PC_{20} , as expected from the regression, was slightly lower and there was an unexpected and unexplained higher proportion of atopic subjects in those with the high slopes. Thus the log-dose-response slope also fails to add any obvious useful information when it is determined from the results of histamine inhalation tests by this technique.

Using a different approach, Beaupré and Malo have also examined the clinical usefulness of determining the slope of the histamine dose-response curve.¹⁸ They found that changes in the histamine PC_{20-FEV_1} for individual asthmatic patients correlated with changes in clinical state and that similar information was not available from the slope of the curve.

From this investigation we can draw the following conclusions. Firstly, even though the method fails to fulfil all the theoretical criteria for accurate assessment of the slope of a dose-response curve, it is feasible to obtain a reasonable measurement of the slope of the histamine dose-response curve with our standardised inhalation technique. Secondly, the slope of the dose-response curve, within the range of responses that we measured, appears to fit a linear curve better than a logarithmic curve. Thirdly, the high correlation between the slope and PC_{20} (or PC_{35}) indicates that little information is gained by determining both from the bronchial provocation dose-response curve. Finally, the less significant log-dose-response slope correlated poorly with PC_{20} and PC_{35} , and also appeared to add little information that was not available from the determination of the PC_{20} (or PC_{35}) alone. We suggest that the easiest and most useful measurement of non-specific bronchial responsiveness to inhaled histamine or a cholinergic agonist is a measurement of so-called sensitivity such as the PC_{20-FEV_1} or the $PC_{35-sGaw}$.

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