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Variability of the plateau response to methacholine in subjects without respiratory symptoms

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Abstract

***Background*—Interpretation of measurements of limited maximal airway narrowing, or plateau response, requires knowledge of its variability within subjects and between methods.**

***Methods*—The repeatability of the plateau response to inhaled methacholine with a dosimeter (D) method (maximal dose 210 μmol) and a tidal breathing (T) method (730 μmol), and the agreement of the two methods, were measured in 16 subjects with mild or no asthma. Two tests by each method (D1,D2,T1,T2) were performed in random order over four consecutive days, with a third dosimeter (D3) test one week later. The dose producing a decrease in forced expiratory volume in one second (FEV_1) of 10% (PD_{10}) and the plateau were calculated from each dose-response curve.**

***Results*—A plateau was reached in all five tests in 12 subjects and in all tests except D3 in 14 subjects. PD_{10} was inversely related to the plateau ($r = -0{\cdot}95$ for D, $r = -0{\cdot}77$ for T). The 95% ranges for differences between two determinations of the plateau in a subject were ± 11·9% (change in FEV_1), ± 19·2%, and ± 20·3%, estimated from D1-2 and 1-3, and T1-2 tests, respectively. From the same tests the 95% ranges for the difference of a single determination from an individual's true mean value were ± 8·3%, ± 13·6%, and ± 14·3%. The limits of agreement between methods indicated that 95% of the measurements of the plateau by tidal breathing ranged from 15·2% below to 13·3% above those obtained by dosimeter. There was no significant bias between methods. Tachyphylaxis over 24 hours occurred with PD_{10} but not with the plateau response.**

***Conclusions*—The plateau response is a subject characteristic which is independent of the method of inhalation challenge testing. Repeatability of the plateau is low in this group of subjects with low airway responsiveness.**

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Bronchial responsiveness is often assessed by measuring the changes in lung function induced by inhaling increasing doses of bronchoconstricting agents such as methacholine. The two most commonly used methods of inhalation challenge testing are the tidal breathing method[1 2] and the dosimeter method.[3 4] The position[5] and shape[6] of the dose-response curves obtained differ in patients with asthma from those in normal subjects. In patients with asthma the curve is shifted to the left, reflecting increased airway sensitivity.[5] This is commonly expressed as the provocative concentration (PC_{20})[1] or dose (PD_{20})[3] causing a 20% fall in forced expiratory volume in one second (FEV_1).

It has been noted that the dose-response curves are sigmoid in shape and reach a maximum response (plateau) in individuals with normal or mildly increased airway sensitivity.[6 7] Individuals with moderate or severe asthma fail to reach a plateau despite a fall in FEV_1 of up to 60%.[6] It has been proposed that the presence of a plateau response indicates a limit to the degree to which airways can narrow, while moderate to severe asthma is characterised by the absence of such a limitation to maximal airway narrowing.[6 7]

The possibility that the level of the plateau response is related to the method of inhalation challenge has not been fully investigated. If the plateau is a stable characteristic of an individual, it should be similar with different methods of testing. The aims of this study were to assess the repeatability of the plateau response in subjects with no asthma or with mild asthma by a dosimeter method and a tidal breathing method, and to assess the agreement between the two methods.

Methods

SUBJECTS

Sixteen adult volunteers from the hospital and laboratory staff took part in the study (table 1). Subject no. 6 had had intermittent wheeze and chest tightness within the previous month and was taking salbutamol as required and regular beclomethasone but did not use salbutamol within six hours of testing on any of the test days. Two subjects had a previous history of asthma but were free of symptoms at the time of the study and were not taking medication. Seven subjects had a history of hay fever although none had any respiratory symptoms during the period of the study. Atopic state was not tested and no subject had an upper respiratory infection. All

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Table 1 Subject characteristics

Subject no.	Sex	Age (y)	Height (cm)	History of: Asthma	Wheeze	Hay fever	Initial FEV_1 (l)	(%pred)
1	M	38	165	N	N	Y	3·67	102
2	M	34	180	N	N	Y	3·35	81
3	F	28	167	N	Y	N	2·75	85
4	F	25	160	N	N	N	3·02	96
5	M	27	185	N	N	Y	4·12	90
6	M	30	178	Y	Y	Y	3·60	86
7	F	28	165	Y	Y	Y	2·83	87
8	F	33	160	N	N	N	2·74	94
9	M	45	183	N	N	N	4·40	108
10	M	45	165	Y	N	Y	2·89	86
11	F	21	160	N	N	N	2·46	78
12	F	28	167	N	N	N	2·54	78
13	F	27	178	N	N	N	4·05	112
14	M	22	180	N	N	N	3·51	80
15	M	38	184	N	N	Y	3·68	85
16	M	27	168	N	N	N	4·01	100
Mean		31	172					91
Range		21–45	160–184					78–112

FEV_1—forced expiratory volume in one second.

subjects were either lifelong non-smokers (n = 11) or ex-smokers (n = 5) and had an initial FEV_1 greater than 78% of the predicted value[8] at the time of the study.

STUDY DESIGN

Subjects were questioned about smoking history, history of wheeze, hay fever or doctor diagnosed asthma, and current medications. Each subject performed two dosimeter and two tidal breathing inhalation challenges, in random order, over four consecutive days. To test the repeatability of the dosimeter method over a longer interval a third test was performed about one week later. All five tests were performed at the same time of day.

Dosimeter method

Solutions were delivered from two DeVilbiss #646 nebulisers driven by an air compressor (Dynavac, Sydney, Australia). Nebulisation time was 0·6 seconds. The mean (SD) output of the nebulisers was determined by weighing them before and after 10 nebulisations on 10 occasions, without a subject. The mean (SD) outputs were 0·014 (0·001) ml and 0·016 (0·002) ml per nebulisation of 0·6 seconds. The same nebuliser was used for all solutions in a given test. Using a noseclip and with the nebuliser held 1–2 fingerbreadths from the open mouth, subjects were instructed to inhale slowly from functional residual capacity towards total lung capacity over 2–3 seconds. The technician triggered the dosimeter at the onset of each inhalation. After holding the breath for 1–2 seconds the subject exhaled normally. Five inhalations constituted one dose of test solution. FEV_1 was measured 30 and 90 seconds after each dose with a wedge spirometer (Vitalograph, Buckingham, UK). Five doses of normal saline were delivered, followed by increasing doses of methacholine (table 2) until the FEV_1 decreased by 50% of the post saline values, or until the total cumulative nebulised dose of 210 μmol had been delivered to the mouth.

Tidal breathing method

Solutions were delivered from a Wright nebuliser, containing 3 ml of solution, driven by continuous air flow at 7·5 l/min. The mean (SD) outputs of two nebulisers were determined by weighing each nebuliser before and after two minute nebulisations without a subject on 10 occasions and were 0·145 (0·005) and 0·140 (0·010) ml/min. The same nebuliser was used for all solutions in a given test. Using a noseclip and with a mask held loosely over the nose and mouth, subjects inhaled aerosol by tidal breathing for two minutes per dose. FEV_1 was measured 30, 90, and 180 seconds after each dose. Three doses of normal saline, followed by doubling concentrations of methacholine (0·03–256 mg/ml) were delivered until the FEV_1 decreased by 50% of the baseline value, or until the total cumulative dose of 730 μmol had been nebulised.

Analysis of dose-response curves

"Baseline FEV_1" was calculated as the mean FEV_1 of all the post saline values (10 values for the dosimeter method; nine values for the tidal breathing method). Dose-response curves were generated by plotting the percentage change in FEV_1 (lowest value after each dose) from the baseline FEV_1 against the

Table 2 Dosimeter method: methacholine dose schedule

Concentration (mg/ml)	No. puffs*	Dose (µmol)	Cumulative dose (µmol)
0·05	1	0·04	0·04
0·05	1	0·04	0·08
0·05	2	0·08	0·16
0·05	5	0·20	0·36
2·0	5	0·70	1·06
5·0	5	1·8	2·9
10·0	5	3·6	6·5
25·0	5	9·0	15·5
50·0	5	17·9	33·4
100·0	5	35·5	69·0
100·0	5	71·0	140·0
100·0	5	71·0	210·0

*Nebuliser output = 0·014 ml/puff.

$\log_{10}$ cumulative, nebulised dose of methacholine. A plateau response was considered present if there was less than a 5% change in FEV_1 over two or more of the final doses. The plateau was calculated as the mean of all values from the first of these dose steps and was expressed as the percentage change in FEV_1 from the baseline FEV_1. In 22 dose-response curves the change in FEV_1 was never more than 5% for each dose. For these curves the plateau was calculated as the mean of all values from the first dose at which the change in FEV_1 was zero or decreased. The provocative dose causing a decrease in FEV_1 of 10% (PD_{10}) from the baseline was calculated by linear interpolation of two adjacent points on the cumulative log dose-response curves.

STATISTICAL ANALYSIS

The effect of the order of testing was examined in each subject. Since plateau responses did not always occur in all subjects (see below), the results of order analysis are given for the 12 subjects in whom a plateau response was measurable on all occasions. For baseline FEV_1 and plateau the results of both methods (tidal breathing and dosimeter) were grouped and differences between days 1, 2, 3, and 4 were examined with the general linear models procedure of the SAS statistical package.[9] Examination of each method separately did not affect the result statistically. For PD_{10} the tidal breathing and dosimeter methods were examined separately in the same 12 subjects, since dose was calculated as the nebulised dose and is not directly comparable between methods. In subjects in whom there was less than a 10% change in baseline FEV_1, the maximal cumulative doses of 210 μmol or 730 μmol were used in testing for the effect of order. This would tend to underestimate differences between days. Paired t tests were used to test differences between the initial and subsequent tests under two circumstances: (1) when the initial test (with a given method) was on day 1; and (2) when the initial test was on days 2 or 3. For the dosimeter method paired t tests were also used to examine differences between day 1 and day 7, and between days 2, 3, or 4 and day 7.

The within subject repeatability of the level of the plateau measured by each method and the agreement between the two methods were analysed by the method described by Bland and Altman.[10 11] All analyses of PD_{10} were performed on logarithmically transformed data (base 10). The repeatability of each method was assessed from the mean (x) and standard deviation (SD) of individual differences between replicates, and the within subject standard deviation (σr). Where $(x_1 - x_2)$ was the difference between replicates by a given method, the formulae were:

$$SD = \sqrt{(\Sigma_n (x_1 - x_2)^2/n)}$$

$$\sigma r = \sqrt{(\Sigma_n (x_1 - x_2)^2/2n)} = SD/\sqrt{2}$$

These two values provide 95% ranges which have different implications. The 95% range based on ± 2 SD values represents the range for change from one test to the next. The 95% range based on ± 2 σr represents the interval within which 95% of the differences between a single determination and that individual's true mean measurement will lie. The 95% ranges were corrected for sample size by the t distribution.[12] Where SD (or σr) was on a log scale, the 95% ranges were calculated by antilog transformation.

Independence of repeatability from the size of the measurements was assessed by plotting the absolute value of individual differences between replicates against the individual means of the replicates, and comparing the correlation coefficient (r) with the null hypothesis of $r = 0$.

Agreement between methods was assessed by analysing the individual differences between the means of replicates for each method. The mean (x) of the differences between each method was the bias. A paired t test examined the hypothesis of zero bias. Since the SD of the differences is underestimated when replicates are averaged, the corrected SD (SD_c) was: $\sqrt{(SD^2 + 1/4S_D^2 + 1/4S_T^2)}$, where S_D and S_T are the SD values of differences between replicates for the dosimeter and tidal breathing methods respectively. Limits of agreement were estimated as $x \pm 2SD_c$. Independence of agreement between methods from the size of the measurements was assessed by plotting the individual differences between the means of replicates for each method against the individual averages of the means of the two methods, and comparing r with the null hypothesis of $r = 0$.

Results

A plateau was reached in all five tests in 12 subjects, and in all but the third dosimeter test in an additional two subjects (table 3). Replicates of the plateau response were available in all subjects for the first two dosimeter tests, and in 14 subjects for the tidal breathing tests. One subject (no. 8) stopped one test because of dyspnoea before a plateau was reached. In three subjects the FEV_1 continued to change by more than 5% per dose step without reaching a plateau by the final dose of methacholine. Subject no. 6, with current asthma, had a fall in FEV_1 greater than 50% on one occasion. A PD_{10} could be measured at least once for all subjects. There was a close inverse relationship between log PD_{10} and the plateau response, $r = -0{\cdot}95$, $p < 0{\cdot}001$ for the first dosimeter test and $r = -0{\cdot}77$, $p < 0{\cdot}01$ for the first tidal breathing test (fig 1).

REPEATABILITY

The baseline FEV_1 did not change significantly from day to day (fig 2). The order in which the tests were performed affected the PD_{10} and plateau response differently (fig 2). For each subject the plateau responses were not significantly different on any test day with either method. PD_{10} was, however, signifi-

Table 3 Individual methacholine test results

Subject no.	Plateau response (% change in FEV_1)					PD_{10} (μmol)				
	D1	D2	D3	T1	T2	D1	D2	D3	T1	T2
1	17	14	14	9	8	17	67·8	103	>730	470
2	11	6	20	12	12	67·8	>210	78	76	147
3	40	43	50	44	30	1·8	3·4	<1·8	3·6	<2·9
4	19	13	18	29	28*	50	84·5	52	126	216
5	36	38	38	26	32	3·8	2·8	3·6	27	50
6	46	35	54*	37	26	0·7	2·5	0·8	<0·1	28·7
7	14	17	33	17	31	71·5	46·2	14·4	118	29·5
8	19	26	41**	47	27	25	14·4	7·3	10·8	48
9	12	7	6	9	8	139	210	>210	730	>730
10	36	37	43	40	36	1·8	3·8	4·2	5	10·6
11	16	10	21	17	7	103	86	32·3	24	280
12	34	32	32	28	25	10·2	14·4	9·4	48	126
13	6	7	10	34*	9	>210	>210	103	100	>730
14	17	26	31	39	24	40·2	32·3	15·4	<2·9	24
15	9	4	2	9	4	139	>210	>210	38	>730
16	10	6	13	7	11	92	>210	140	>730	180

D1, D2, D3—first, second and third dosimeter tests, respectively; T1, T2—first and second tidal breathing tests, respectively.
*Values are the maximal change in FEV_1 which occurred in the absence of a plateau.
**Subject stopped before a plateau was reached.

cantly greater at any subsequent test (day 2, 3, or 4) compared with a test on day 1, with both methods. PD_{10} was not significantly different between tests on days 2 and 3, days 2 and 4, or days 3 and 4 and, for the dosimeter method, when day 1 was compared with day 7. The repeatability of PD_{10} was therefore examined only for the dosimeter method, comparing day 1 with day 7. The within subject standard deviations of the plateau responses between the first and second dosimeter tests, the first and third dosimeter tests, and the first and second tidal breathing tests, and for PD_{10} between the first and third dosimeter tests, are shown in table 4. The 95% ranges for change from one test to the next, based upon 2 SD, were ± 11·9% (change in FEV_1), ± 19·2%, ± 20·3%, and a 7·9 fold change, respectively. The 95% ranges for a single determination in relation to an individual's mean value, based on 2 σr, were ± 8·3% (change in FEV_1), ± 13·6%, ± 14·3%, and a 5·0 fold change, respectively. Repeatability of the plateau response and the log PD_{10} were independent of the size of the measurements for all comparisons.

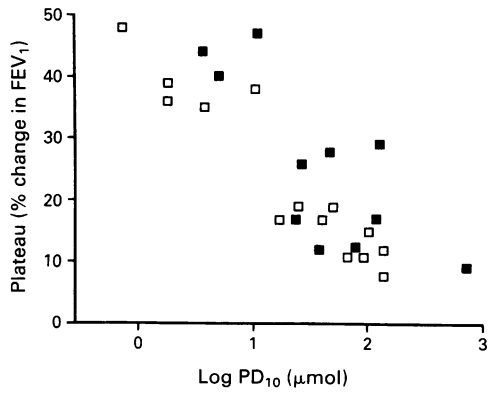

Figure 1 Relationship between log PD_{10} and the plateau response at the first dosimeter test in 14 subjects (open squares, $r = -0·95$, $p < 0·001$) and at the first tidal breathing test in 11 subjects (closed squares, $r = -0·77$, $p < 0·01$).

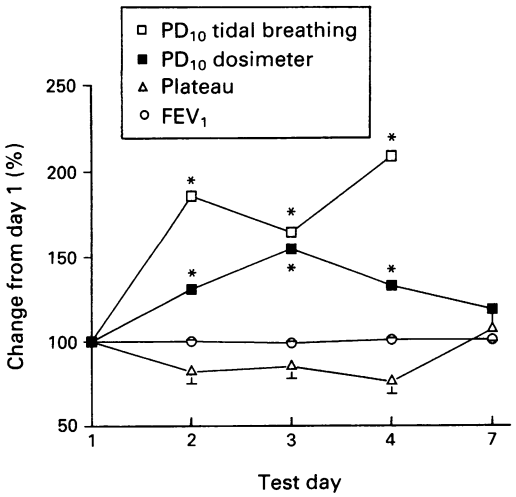

*Figure 2 Effect of order of testing on baseline FEV_1 and dose-response curve characteristics. FEV_1 and plateau response did not change significantly after day 1, whereas PD_{10} was significantly greater at 24 hour intervals after the first test with both methods of delivery. After a further three days without testing (day 7) there was no significant difference in PD_{10} measured by the dosimeter method compared with day 1. *$p < 0·05$ compared with day 1.*

AGREEMENT

There was no significant bias between methods in assessing the plateau response (mean difference = −1·0%, $p > 0·4$). The limits of agreement for the plateau response indicate that 95% of the measurements by the tidal breathing method ranged from 15·2% below to 13·2% above those obtained by the dosimeter method. Agreement of the plateau response between methods was also independent of the size of the measurements.

Discussion

The results of this study show that the level of the plateau on the dose-response curve for inhaled methacholine did not change significantly over 48 hours or a week with two different methods of administering methacholine. For individual subjects, however, the repeatability of the plateau response was not high. The plateau was more repeatable by the dosimeter method over a short period (48 hours) with a difference between two estimates of the plateau response in one person of greater than 12% (change in FEV_1) likely to be significant. With the tidal breathing method a difference of greater than 20%

Table 4 Within subject repeatability of the dosimeter and tidal breathing methods

	Difference between two tests					95% ranges for:	
Test pairs	n	Mean	p	SD	σr	Change	Single determination
Plateau response (%)							
D1-D2	16	1·0	> 0·5	5·6	3·9	± 11·9	± 8·3
D1-D3	14	3·6	> 0·1	8·9	6·3	± 19·2	± 13·6
T1-T2	14	4·4	> 0·1	9·4	6·6	± 20·3	± 14·3
Log PD_{10}							
D1-D3	11	0·02	> 0·9	0·42	0·30	7·94*	5·0*

σr—within subject standard deviation; D1–D2, D2–D3, T1–T2—differences between dosimeter (D) or tidal breathing (T) tests.
*Values have been transformed back to original units (that is, antilog values), and represent fold differences.

(change in FEV_1) is likely to be significant, with a similar value for the dosimeter method over one week. The repeatability of PD_{10} over a one week period was low with up to an eight fold change not being significant at the 5% level in this group of subjects. This is similar to the 8·59 fold difference from one test to the next for PD_{10} that can be estimated from the data of Chinn *et al.*[13]

The high values for expected change from one test to the next (low repeatability) are likely to result from studying subjects with very mild airway responsiveness. Flat dose-response curves will exaggerate differences between tests in the same subject, especially since measurements of PD_{10} and plateau were made on the flat portion of the dose-response curve. In subjects with asthma the dose-response curve is steeper and much greater within subject repeatability for PD_{20} has been reported.[14] In addition, the variability of PD_{20} is greater at higher doses of inhaled agonist.[15]

There is no previous report of the repeatability of the plateau response with a dosimeter method. Sterk *et al*[7] reported the plateau response to be highly reproducible by a tidal breathing method, with an intraclass correlation coefficient of 0·88. This statistic depends in part on between subject variance, and there is debate about the best method of assessing repeatability.[16] Reanalysis of their data by the method described by Bland and Altman[10 11] yields a coefficient of repeatability for the maximal plateau response of ± 7·8%—that is, a difference between two estimates of the plateau response of more than 8% change in FEV_1 was likely to be significant. This is similar to the value of 12% we obtained for the dosimeter method but half the value for the tidal breathing method. Possible reasons for this discrepancy include differences in subject characteristics as mentioned above. Furthermore, in their calculations of the maximal response, Sterk *et al*[7] included subjects who did not reach a plateau *per se* and substituted the absolute maximal change, whereas the present study excluded subjects from further analysis if replicates were not available.

In the present study the airway response, assessed by PD_{10}, showed tachyphylaxis at intervals of 24 hours. This effect had disappeared after one week and the plateau response was not subject to tachyphylaxis. The results of this study suggest, however, that, although tachyphylaxis can shift the in vivo dose-response curve to the right, it does not reduce maximal airway narrowing. The factors that normally limit airway narrowing and determine the level of the plateau response include those limiting stimulation of smooth muscle, local neurohumoral influences, and the structural relationships between the airway wall tissues including smooth muscle and the surrounding elastic parenchyma.[17] Many of these factors are unlikely to change over a short period of time.

There was no bias between methods in assessing the level of the plateau response, indicating that the methods were, on average, measuring the same property of the airways and suggesting that the measured plateau response was independent of the method of inhalation challenge testing used. Agreement between the two methods was, however, not high. This was probably partly because of the relatively less repeatable tidal breathing results, as agreement between methods is bound to be poor if either method is not repeatable.

This study confirms the inverse relationship between PD_{10} and the plateau response reported previously by Sterk *et al.*[7] This association is likely to reflect the fact that both parameters are related to some common factor determining bronchial responsiveness. The observation of tachyphylaxis of the PD_{10}, but not of the plateau response, shows that they can behave independently and this is supported by studies undertaken elsewhere.[18–20]

The usefulness of measurements of the maximal (plateau) response to inhaled bronchoconstricting agents remains to be determined. The maximal response may be a more useful predictor of symptom severity[21] and a means of monitoring the efficacy of treatment.[22] To be used in this manner, more information is needed on the stability of the maximal response in larger numbers of normal subjects and patients with mild asthma.

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