Determinants of the bronchial response to high molecular weight occupational agents in a dry aerosol form

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Determinants of the bronchial response to high molecular weight occupational agents in a dry aerosol form. B. Nguyen, K. Weytjens, Y. Cloutier, H. Ghezzi, J-L. Malo. ©ERS Journals Ltd 1998.

ABSTRACT: In occupational challenge tests with isocyanate vapours, bronchial responsiveness is determined by the total dose rather than the concentration or duration of exposure. Whether the same applies for high molecular weight (HMW) agents in powder form is unknown. The aim of this study was to determine whether the total dose of HMW agents in powder form is responsible for the immediate reaction documented in specific challenge tests.

Included in the study were nine subjects (seven males and two females) with a diagnosis of occupational asthma proved by specific challenge tests carried out on a preliminary visit. Two challenge tests (using a closed-circuit exposure chamber) were performed at an interval of 2 weeks; the concentrations administered in a random order on these two visits were half and double the one that had caused a 20% fall in forced expiratory volume in one second (FEV₁) on a preliminary visit. The duration of exposure was adjusted until a significant fall in FEV₁ (target of 20%) occurred.

The two concentrations obtained were significantly different, by 2.07 ± 0.36 -fold (SD). The observed durations of exposure leading to a 20% fall in FEV₁ on the two visits also differed significantly by 0.46 ± 0.32 -fold. Consequently, the cumulative efficient doses were not significantly different between the two visits: 12 ± 5.4 and 9 ± 5 mg·mL⁻¹·min⁻¹, respectively. The corresponding cumulative dose ratio was 0.96 ± 0.61 . The expected duration of exposure (10.8 ± 24 min) was not significantly different from the observed duration (5.4 ± 9 min). The mean and 95% confidence interval for the difference in concentration between the two visits was 1.83-fold (1.48–2.21).

In conclusion, the total dose rather than the concentration or duration of exposure *per se* determines bronchial responsiveness to high molecular weight agents in powder form.

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Occupational asthma (OA) is the most frequently diagnosed condition among respiratory diseases caused by the workplace environment [1]. Specific inhalation challenges (SIC) are an important tool for diagnosing OA [2]. For asthma induced by common inhalants, determinants of the bronchial response include the dose of the exposed allergen, the degree of immunological sensitization and the level of nonspecific bronchial responsiveness [3]. VANDENPLAS *et al.* [4] demonstrated that it is the total dose rather than the concentration or duration of exposure that determines the degree of bronchial responsiveness in occupational challenge tests with isocyanate vapours. Whether this is also the case for occupational high molecular weight (HMW) agents is unknown. As it has been shown that bronchial reactions are affected by the chemical and/or physical properties of the agents, the same hypothesis was tested for occupational HMW agents existing in powder form. Whereas COCKCROFT *et al.* [3] assessed the determinants of specific responsiveness among different subjects, the present study examined the same determinants by comparing each subject to themselves (within-subject variability).

Materials and methods

Subjects

Nine subjects with OA to HMW agents in powder form, confirmed by SIC on a preliminary visit, gave their informed consent to participate in the study. All subjects had experienced an immediate reaction, defined as a $\geq 20\%$ fall in forced expiratory volume in one second (FEV₁) during the first hour after the end of exposure. These subjects all had well-controlled asthma. Baseline FEV₁ had to be >2 L. The study was approved by the ethics committee.

Study protocol

Two challenge tests were performed at an interval of 2 weeks. Challenges were performed only when the pre-challenge FEV₁ was within $\pm 10\%$ and the provocative concentration of methacholine causing a 20% fall in FEV₁ (PC₂₀) was within 3.2-fold of the baseline value, as determined on a control day [5]. Half and double the concentration that had caused a 20% fall in FEV₁ on the preliminary

visit was administered in a random order on the two visits to balance for the potential enhancement of the allergic reaction by the first exposure. The duration of exposure was modified until a significant fall in FEV₁ (≥20%) occurred. The chronological order of exposure was randomized, *i.e.* on the first visit, subject numbers 1, 3, 7 and 9 were exposed to the lower concentration whereas the rest of subjects were exposed to the higher concentration.

Tests

Spirometry was performed on a vitalograph apparatus (Vitalograph, Buckingham, UK) or a Collins spirometer (W.E. Collins, Braintree, MA, USA) according to the standards of the American Thoracic Society [6]. Predicted values for FEV₁ and forced vital capacity (FVC) were taken from KNUDSON *et al.* [7].

Nonspecific bronchial responsiveness to methacholine was assessed with a Wright nebulizer (Baltimore, MD, USA; output = 0.14 mL·min⁻¹) at tidal breathing for 2 min using the procedure outlined by COCKCROFT *et al.* [8].

SIC were carried out according to a standardized procedure [2] using a closed-circuit apparatus that keeps the concentration of particles relatively constant and below the irritant level throughout exposure (threshold limit value-short-term exposure level (TLV-STEL) [9, 10]; for dry particles, the TLV-STEL is determined at 10 mg·m⁻³ [11]. Exposure to HMW agents was progressive on the same day (one breath, 15 s, 40 s, 2 min, 4 min, *etc.*) until a 20% fall in FEV₁ occurred. FEV₁ was monitored every 10 min for the first hour, every 30 min for the second hour, then hourly for 8 h. Inhaled β₂-adrenergic agents were withheld before the challenge (short-acting at least 8 h, long-acting 72 h); the total daily dose of inhaled steroids was administered at the end of the day, at least 10 h before the challenge.

Analysis of results

The analysis was designed to show that the mean concentration (graphic record by a photometer) and the observed duration of exposure to obtain a comparable asthmatic reaction (≥20% fall in FEV₁) on two visits were significantly different, while the calculated total dose (mean concentration × duration of exposure) was not.

The mean concentration, the observed duration of exposure (*i.e.* the summation of the progressively increasing periods of exposure on each study day) and the calculated dose on the two visits were compared using a two-sided paired t-test. On the assumption that the total dose on the two visits was not significantly different, the expected duration of exposure was calculated using the following formula: lower concentration × duration of exposure / higher concentration; this result was compared with the observed duration of exposure to the higher concentration, using a paired t-test.

The mean±SD of the ratios of concentrations, durations of exposure and doses were computed in the following way: high concentration day (day H)/low concentration day (day L).

A satisfactory reproducibility of the dose causing the immediate reaction was defined as a variation not exceeding a factor from single to double or from single to half, a criterion that has also been used in a recent study [12]. For this purpose, the mean±SD of the ratios of doses was calculated in the following way: higher dose/lower dose (regardless of the concentration of the day). To obtain information concerning the reproducibility of the dose-response curve of SIC, a 95% confidence interval (CI) for the absolute difference between the doses on the two visits was calculated.

The level of statistical significance was set at a p-value ≤0.05.

Results

Some clinical and functional features of the subjects who took part in the study are presented in table 1. Baseline FEV₁ varied from 2.2–4 L, corresponding to 74–109% of predicted. Baseline PC₂₀ varied from 0.05–22 mg·mL⁻¹. Seven subjects were exposed to flour, one to psyllium and one to garlic. At the time of the study, all but three subjects used short-acting β₂-adrenergic medication as needed; five subjects also took inhaled steroids, associated in two subjects with long-acting β₂-adrenergic bronchodilators. All but one of the patients were atopic, as defined by an immediate skin-prick reaction to at least one of 15 common inhalants.

Table 2 gives information on the PC₂₀ level and the SIC on the two visits. The PC₂₀ level was confirmed to be within a 3.2-fold difference from the baseline value before each exposure; a methacholine challenge was not repeated

Table 1. – Baseline anthropometric, clinical and functional data

Subject No.	Sex	Age yrs	Atopy	Smoking	Medication	Agent	Interval since last exposure months	FEV ₁ %pred
1	M	26	+	Yes	Be300	Flour	1	94
2	M	59	+	Yes	Be400	Flour	30	87
3	F	51	+	No	Be1600	Psyllium	22	86
4	M	46	+	Yes	None	Flour	56	88
5	M	48	-	No	BDT <i>p.r.n.</i>	Flour	4	78
6	F	26	+	No	None	Flour	58	109
7	M	33	+	Exsmoker	Be500	Flour	8	86
8	M	34	+	No	Be1000	Garlic	14	90
9	M	25	+	Exsmoker	None	Flour	50	74

FEV₁: forced expiratory volume in one second; M: male; F: female; Be: inhaled beclomethasone with daily dose (in µg); BDT: bronchodilator. Atopy was present if there was at least one immediate skin reaction to 15 common inhalants by the prick method.

Table 2. – Features of the bronchial reaction

Subject No.	Concentration mg·m ⁻³		Duration of exposure min		Dose mg·m ⁻³ ·min ⁻¹		Maximum change in FEV ₁ %		PC ₂₀ mg·mL ⁻¹			
	Day L Day H		Day L Day H		Day L Day H		Day L Day H		Day L		Day H	
									pre-	post-	pre-	post-
1	3.0	6.7	3.0	3.1	8.9	20.8	21	18	3.4	0.8	3.6	2
2	1.9	3.7	11	3.0	20.4	11.1	24/17*	28	3.4	1.0	-	1.2
3	2.4	5.0	8.5	3.0	20.1	15	22	20	0.05	0.06	-	0.04
4	2.0	3.9	8.0	4.5	16	17.5	26	22	22	38	-	8
5	1.9	3.5	3.0	1.0	5.6	3.5	19	23	7.4	16	-	16
6	1.1	2.9	15	2	16.5	5.8	25/34*	23	0.9	1.8	-	0.7
7	3.0	4.9	120	30	360	147	19	24/21*	3.4	0.2	1.5	1.1
8	1.9	4.8	0.3	0.1	0.6	0.4	23	22	2.9	2.0	-	4.0
9	2.1	3.5	2.2	2.0	4.6	7.0	24	19	2.0	1.2	-	2.1

FEV₁: forced expiratory volume in one second; PC₂₀: provocative concentration of methacholine causing a 20% fall in FEV₁; Day H: visit for which the higher concentration was used; Day L: visit for which the lower concentration was used. The order of presentation of data does not correspond to the chronological order of exposure that was randomized, *i.e.* on the first visit, subjects numbers 1, 3, 7 and 9 were exposed to the lower concentration whereas the rest of subjects were exposed to the higher concentration. *: Changes for the immediate and late reactions; PC₂₀ was not reassessed before day H if no change had been documented after the challenge on day L.

before the second SIC if there had not been a significant change in nonspecific bronchial responsiveness after the first exposure. The magnitude of the asthmatic reactions in terms of changes in FEV₁ was comparable on the two visits ($p=0.7$). The concentration of the agent administered on the two visits differed significantly by 2.07 ± 0.4 fold. The observed duration of exposure was also significantly different, by 0.46 ± 0.32 fold. As expected, the total dose (concentration $\times$ duration of exposure) did not significantly differ between the two visits: 12 ± 5.4 and 9 ± 5 mg·m⁻³·min⁻¹, respectively ($p>0.2$). The corresponding cumulative dose ratio was 0.96 ± 0.61 .

When the higher of the two concentrations was used, less time was required to elicit a significant bronchial response, in all but two individuals (numbers 1 and 9), for whom there was no difference in duration of exposure on the two visits. The expected duration of exposure obtained from the formula detailed in Materials and Methods (10.8 ± 24 min) was not significantly different from the observed duration (5.4 ± 9 min) ($p=0.3$). Moreover, the difference in concentration between the two visits (1.83 ± 0.58 fold, *i.e.* the mean ratio of the higher dose/lower concentration), with a 95% CI of 1.48–2.21, was within the limits of reproducibility defined previously, *i.e.* within two-fold [12].

Discussion

In this study, the influence was assessed of concentration, duration of exposure and total dose (concentration $\times$ duration of exposure) on immediate bronchial responsiveness to occupational HMW agents in powder form causing an immunoglobulin E (IgE)-mediated immediate reaction. VANDENPLAS *et al.* [4] demonstrated that the bronchial response to isocyanate vapours was determined by the total dose rather than the concentration or the duration of exposure *per se*. According to their study, four subjects were challenged using different concentrations and durations, while the total dose of isocyanates (a dose that caused a 20% fall in FEV₁ on a preliminary visit) remained constant. Exposing subjects to a lower total dose but using higher concentrations or longer durations caused a fall in FEV₁ $<20\%$ in all instances. In the present study, the concentration and duration of exposure differed significantly

from one visit to the next, while the total dose administered and the elicited asthmatic reaction were comparable, a fact that extends the results obtained by CHAN-YEUNG *et al.* [1] to other agents, principally flour, causing OA and acting through an IgE-mediated mechanism. HMW agents are a common cause of OA and flour, the main agent tested in our study, is certainly the leading cause in most countries; this outlines the relevance of this choice of agent. The results of this study also apply for immediate and not for late reactions, which was the case in the study by VANDENPLAS *et al.* [4]. Isolated immediate reactions are common after exposure to HMW agents.

The sequence of tests (high *versus* low concentration administered) was performed in a random order to balance for the possible priming of the allergic reaction due to the first exposure. Moreover, to control for the influence of the nonspecific bronchial responsiveness on the bronchial reaction, it was verified that the PC₂₀ level was within a 3.2 fold difference of the baseline level before each exposure [5]. Therefore, it is unlikely that a significant priming effect influenced the results of this study.

In three subjects (numbers 1, 6 and 7), the dose required to elicit a $\geq 20\%$ fall in FEV₁ exceeded the reproducibility criterion defined earlier. In subjects 1 and 6, the administered efficient dose on the second visit was more than double the one given on the first visit 2 weeks earlier. DEHAUT *et al.* [5] determined the reproducibility for histamine PC₂₀ at a ± 3.2 fold difference. The 95% CI for the difference in doses on the two challenges with the occupational challenge was calculated to be ± 2.04 fold. In a study comparing the effect of dose and concentration of bronchial allergen challenges performed with aqueous solutions of common allergens on immediate and late asthmatic reactions, FROLUND *et al.* [13] found that the dose and not the concentration was the key factor influencing the response. These authors showed that administering the solution by 10 breaths of two-fold increasing allergen concentrations resulted in a four-fold greater responsiveness than that provoked by two breaths of two-fold increasing allergen concentrations.

Although the present study showed that the dose, rather than the concentration *per se* or the duration of exposure, had a significant effect on the magnitude of the immediate

reaction. The results are valid only for a situation in which sensitization has already occurred. They cannot be extrapolated to the situation that prevails at the time of acquisition of sensitization. Indeed, it might well be that in such cases it is the concentration, not the dose, that is the relevant factor. Epidemiological surveys have suggested that the concentration may be a relevant factor in those situations [14, 15].

The present findings have practical implications. Since the dose is the main determinant of the reaction and since this variable can be modified by adjusting either the concentration or the duration of exposure, it should be possible to obtain the desired reaction by increasing one or the other of the two factors without preference, depending on the situation. This means that increasing the concentration is unnecessary, provided that exposure is long enough. In order to avoid unduly severe immediate reactions, it would, therefore, be preferable to use low concentrations, since duration can be increased slowly and progressively.

In conclusion, the total dose rather than the concentration or duration of exposure *per se* determines the bronchial response to high molecular weight agents in powder form.

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