

ORIGINAL ARTICLE Asthma and Rhinitis

Distinct temporal patterns of immediate asthmatic reactions due to high- and low-molecular-weight agents

J.-L. Malo, H. Ghezzo and J. L'Archevêque

Université de Montréal and the Department of Chest Medicine, Hôpital du Sacré-Coeur de Montréal, Montreal, Canada

Clinical & Experimental Allergy

Summary

Background Exposure to occupational agents can cause immediate asthmatic reactions.**Objective** It can be hypothesized that the pattern of immediate reactions is different for high (HMW)- and low-molecular-weight (LMW) agents. To test this, we studied the temporal features of reactions in workers who underwent specific inhalation challenges for possible occupational asthma.**Methods** We examined 467 immediate reactions due to HMW ($n = 248$, 53%) and LMW ($n = 219$, 47%) agents in regards to timing of the maximum reaction and recovery.**Results** The median duration of exposure to elicit significant immediate reactions was comparable for HMW and LMW agents (15 min). The median maximum fall in FEV₁ occurred after 20 min for LMW by comparison with 10 min for HMW agents ($P < 0.001$). The median timing of recovery of FEV₁ to 10% baseline was shorter for HMW (60 min) than for LMW (90 min) agents ($P < 0.01$), and significantly more subjects recovered to 10% baseline (89.5%) for HMW than for LMW agents (72.6%) ($P < 0.001$). Confounding variables such as age, atopy, baseline airway calibre and the maximum fall in FEV₁ at the time of the immediate reaction did not alter the significant effect of the nature of the agent *per se*. Immediate reactions were followed by a late asthmatic reaction more often in the case of LMW (37.3%) than HMW (26.2%) agents ($P < 0.05$). Significant changes in non-specific bronchial responsiveness were significantly ($P = 0.02$) more frequent after reactions to LMW (31.9%) than to HMW (21.4%) agents. We found similar trends by comparing reactions to flour ($n = 113$), the principal cause of reactions to HMW agents, and diisocyanates ($n = 111$), the principal LMW agent.**Conclusions and Clinical Relevance** This study shows distinct patterns for immediate reactions due to occupational agents. These results can provide useful guidelines for performing specific inhalation challenges and improve the safety of the procedure.**Keywords** asthmatic reactions, bronchial hyperresponsiveness, occupational asthma, specific inhalation challenges

Submitted 01 November 2011; revised 13 January 2012; accepted 16 January 2012

Correspondence:

Jean-Luc Malo MD, Université de Montréal and the Department of Chest Medicine, Hôpital du Sacré-Coeur de Montréal, 5400 West Gouin Blvd, Montreal, Canada H4J 1C5.
E-mail: jean-luc.malo@umontreal.ca
Cite this as: J.-L. Malo, H. Ghezzo and J. L'Archevêque, *Clinical & Experimental Allergy*, 2012 (42) 1021–1027.

Introduction

Exposure to an inhaled allergen can cause asthmatic reactions in sensitized subjects. Three main temporal patterns of reactions have been identified: (1) the immediate reaction that 'starts rapidly, is maximal in 10–30 min, resolves in about 1.5–2 h' [1]; (2) late reactions, initially described by Herxheimer [2], which have been associated with asthmatic inflammation and remodelling; (3) dual reactions that are a combination of immediate and late reactions. Asthmatic reactions to specific agents can be induced by general ubiquitous

inhaled allergens or occupational agents. General environmental allergens were often used to confirm the aetiology of asthma in the beginning of the 20th century, as reviewed [3], whereas challenges with occupational agents were initiated in the 1970s [4] and are considered as the gold standard for confirming the aetiology of occupational asthma (OA) [5]. Immunologically induced OA can be caused by high- and low-molecular-weight agents. High-molecular-weight (HMW) agents are proteinaceous products that induce reactions by an IgE-mediated mechanism, whereas the mechanism of asthmatic reactions induced by

low-molecular-weight (LMW) (chemical) agents is unknown for most chemicals and is apparently not IgE-mediated. Asthmatic IgE-mediated reactions result in a cascade of immunological events that lead to eosinophilic inflammation [6]. Neutrophilic inflammation is also present as a result of asthmatic reactions, especially in non-IgE mediated reactions such as those that occur after exposure to LMW agents [7], especially diisocyanates [8].

It is generally accepted that 'both common allergens and chemical agents can elicit similar...reactions' [1]. However, thorough characterization of the time-course of asthmatic reactions has not been carried out in a sufficient number of subjects and is based on case series with small numbers of participants. In particular, it is not known if the temporal pattern of immediate asthmatic reactions due to LMW agents is similar to what occurs after exposure to HMW agents. Since the mechanism of reactions is IgE-mediated in the latter whereas it generally remains unknown in the former, this strongly suggests the possibility that different mechanisms of reactions can result in different temporal patterns.

We therefore examined a large sample of immediate asthmatic reactions due to occupational agents to better characterize these reactions and test the hypothesis that they show different temporal patterns in the case of LMW vs. HMW agents.

Subjects and methods

All ($n = 467$) positive ($\geq 20\%$ fall in forced expiratory volume in 1 s, FEV₁) immediate specific inhalation challenges that were documented in subjects investigated for possible OA from 1980 onwards at our centre were examined. These tests are routinely performed in our unit to diagnose OA provided that the suspected agent is known and the baseline FEV₁ is 2 L or more. The methodology of these tests has been extensively described [9, 10]. Briefly, after exposure to a control agent on the previous day, subjects are exposed to the suspected occupational agent in a progressive manner up to a maximum total period of 2 h. For HMW agents, exposure is carried out on the same day for progressively increasing periods (one breath, 10 s, 20 s, 30 s, 2 min, 5 min, 30 min, etc., for a total of 2 h). For LMW agents, exposure is done on different days: one breath, 10 s, 20 s, 30 s (total of one min) on the first day; 1, 2 and 2 min (total of 5 min) on the second day; 5, 10 and 15 min (total of 30 min) on the third day; 15, 15, 30, 30, 30 min (total of 2 h) on the last day [10]. Exposure is performed in a realistic way in small cubicles [4] (this was the case in 333/467 (71%) instances) or using apparatuses that can generate dry particles such as flour dust or vapour and aerosols such as diisocyanates and allow for better control of exposure, reducing the risk

of major immediate reactions (this was possible in 134 (29%) instances) [11, 12]. Spirometry is assessed before exposure, immediately after stopping exposure, every 10 mins for 1 h, every half hour for 2 h and hourly for 7 or 8 h. On the control day and after exposure, 7–8 h after ending exposure, airway responsiveness to methacholine is assessed with the provocative concentration causing a fall of 20% (PC20) in FEV₁ [13, 14]. On the day of exposure to the causal agent, in case of a significant late reaction, if the FEV₁ is not back to $\pm 10\%$ of the baseline FEV₁ 7–8 h after ending exposure, PC20 is assessed on the following morning instead. Since 1995, induced sputum has also been obtained to assess airway inflammation on the control and active exposure days using a standardized methodology [15, 16]. Long-acting bronchodilators are stopped 3 days before the start of testing whereas inhaled steroids are unchanged but the total daily dose is given only once in the evening preceding the testing day but not on the morning preceding exposure.

The following information was obtained: (1) Anthropometric (gender, age); (2) Clinical data: atopic status (atopy present if at least one immediate reaction to 15 common aeroallergens), inhaled medication, type of occupational agent (HMW or LMW); (3) Functional data: FEV₁, FEV₁/forced vital capacity (FVC); serial changes in FEV₁; PC20 and features of induced sputum, including percentages and total counts of eosinophils. A significant change in PC20 was considered to occur when there was a ≥ 3.2 -fold decrease [17] on the control day and after exposure to the causal agent.

Data analysis

Descriptive and associative analysis of data was carried out using parametric (chi square, unpaired *t*-test) or non-parametric (Mann–Whitney) statistical tests according to the distribution of data. Median regression was used to examine the possibility of a confounding effect of variables found to be significantly different in the univariate comparison of HMW and LMW agents.

The protocol was accepted by the ethics committee of Hôpital du Sacré-Coeur de Montréal.

Results

The total number of subjects in our data bank was 2313. Of these, 1462 had negative challenges and 384, asthmatic reactions other than immediate. Table 1 shows the baseline characteristics of the 467 subjects with immediate asthmatic reactions. Slightly more subjects (248/467, 53%) had reactions caused by HMW agents. Among the HMW agents, wheat flour was the main causative agent (113/248, 45.6%), followed by

Table 1. Baseline characteristics of subjects

	All	Low-molecular-weight agents	High-molecular-weight agents
Number	467	219	248
Females/Males	117 (25.1%)/350 (74.9%)	32 (14.6%)/187 (85.4%)	85 (34.3%)/163 (65.7%)
Age (years)	40.1 ± 11.5	42.7 ± 11.2	37.8 ± 11.2***
Atopy	323/442 (73.1%)	118 (57.3%)	205 (86.9%)*
Duration of exposure (years)	15.3 ± 17.7	17.1 ± 20.6	13.8 ± 14.6*
Duration of symptoms (years)	4.6 ± 6.7	4.6 ± 8.8	4.6 ± 4.1
Smoking habits(S/ES/NS)	111 (23.8%)/169 (36.2%)/187 (40.0%)	47 (21.5%)/91 (41.6%)/81 (37.0%)	64 (25.8%)/78 (31.5%)/106 (42.7%)
Number on inhaled corticosteroids	228/462 (49.4%)	107 (49.8%)	121 (49.0%)
FEV ₁ (% pred)	91.9% ± 18.9%	90.2 ± 19.2%	93.4 ± 18.5%
Number with values <LLN	66 (14.1%)	33 (15.1%)	33 (13.3%)
FEV ₁ /FVC (%)	75.5% ± 9.8%	73.9 ± 9.6%	76.8 ± 9.5%**
Number with values <LLN	105 (22.5%)	57 (26.0%)	48 (19.4%)
PC20 ≤ 16 mg/mL	387/441 (87.8%)	179/202 (88.6%)	208/239 (87.0%)
Sputum eosinophils			
>1%	55/99 (55.6%)	15 (44.1%)	40 (61.5%)
>2%	43/99 (43.4%)	14 (41.2%)	29 (44.6%)
>3%	35/99 (35.4%)	12 (35.3%)	23 (35.4%)

Atopy: at least one immediate reaction to 15 common aeroallergens; S: smokers; ES: ex-smokers; NS: non-smokers; LLN: lower limit of normal; mean ± SD values are presented as well as% in parentheses;

* $P \leq 0.05$;

** $P \leq 0.01$;

*** $P \leq 0.001$.

various cereals ($n = 22$, 8.9%), latex ($n = 20$, 8.1%) seafoods ($n = 16$, 6.5%) and laboratory animals ($n = 15$, 6.0%). As regards LMW agents, the principal causal agent was diisocyanates (111/219, 50.1%) followed by wood dusts ($n = 39$, 17.8%), drugs ($n = 31$, 14.2%), welding fumes ($n = 17$, 7.8%), and resins and glues ($n = 17$, 7.8%). Subjects exposed to LMW agents were slightly older and were less atopic than those with OA due to HMW agents. They also tended to have slightly lower airway calibre with a significantly lower FEV₁/FVC ratio. The frequency of airway hyperresponsiveness and eosinophilic inflammation were not significantly different in the two groups. Subjects with negative challenges in our data bank ($n = 1846$) had some baseline features that were similar to the studied group: 70% males, age of 41.1 ± 11.7 years, 75.9% with atopy, smoking habits (28% smokers, 35% ex-smokers and 37% non-smokers), FEV₁ of $88\% \pm 26.2\%$ pred, FEV₁/FVC of $76.5 \pm 9.5\%$; but, they had relatively lower duration of exposure at work: 10.9 ± 10.5 years and duration of symptoms: 3.2 ± 4.3 years; only 62.2% of subjects in this group had a PC20 ≤ 16 mg/mL, and smaller proportions of subjects had levels of sputum eosinophils respectively ≥ 1% (46.1%), ≥ 2% (26%) and ≥ 3% (16.4%).

The duration of exposure to elicit a significant immediate reaction was not significantly different for HMW (median duration: 15 min, interquartile interval: 29 min) and LMW (median duration: 15 min, interquar-

tile interval: 56 min) agents. The main features of immediate reactions are shown in Table 2. Nearly two-thirds of subjects experienced an isolated immediate reaction. The mean percentage fall in FEV₁ was slightly greater in subjects exposed to LMW (30.5%) vs. HMW

Table 2. Features of immediate reactions

	Low-molecular-weight agents	High-molecular-weight agents
Number	219	248
Reactions		
Isolated immediate	137 (62.7%)	183 (73.8%)
Dual	82 (37.3%)	65 (26.2%)*
% fall in FEV ₁		
Isolated immediate		
Mean±SD	30.5 ± 10.3	25.4 ± 7.0**
Number 20–30%	137 (62.6%)	203 (81.9%)*
Number ≥ 40%	36 (16.4%)	8 (3.2%)*
Number ≥ 50%	9 (4.1%)	3 (1.2%)
Dual	27.4 ± 9.8% (immed)/ 25.0 ± 10.3% (late)	25.4 ± 7.0% (immed)/ 22.9 ± 9.2% (late)
For the late-phase:		
Number 10–15%	14 (17.1%)	14 (21.5%)
Number ≥ 20%	50 (61%)	36 (55.4%)

Presence of a dual phase reaction in the presence of an immediate reaction followed by at least a 10% fall in FEV₁ after the first hour following end of exposure:

* $P \leq 0.05$;

** $P \leq 0.001$.

(25.4%) agents and there were significantly more subjects exposed to HMW agents who had a maximum fall in FEV₁ between 20% and 30%, that is what was aimed at, more subjects (16.4%) exposed to LMW agents having a fall in FEV₁ $\geq$ 40%. The magnitude of the late phase of the reaction in subjects with a dual reaction was similar after exposure to HMW and LMW agents. Features related to the timing and recovery of reactions are listed in Table 3. The median maximum fall in FEV₁ was 20 min vs. 10 min after stopping exposure in subjects exposed to LMW and HMW, with significantly more subjects (41% vs. 28.8%) having a maximum fall in FEV₁ 10 min after stopping exposure in the case of HMW agents. The timing of the late-phase of the reaction in subjects with dual reactions was not different. The timing of recovery from the immediate reactions to 10% of baseline was more delayed in subjects exposed to LMW vs. HMW agents (median values of 90 min vs. 60 min respectively). There were also significantly fewer subjects (72.6% vs. 89.5%) who recovered to 10% of baseline value in the case of LMW agents. Whereas there were no significant differences in the frequency of significant changes in PC20 ($\geq$ 3.2-fold decrease)

by comparing subjects who experienced an isolated immediate reaction vs. those with a dual reaction, 53/166 (31.9%) subjects exposed to LMW agents vs. 43/201 (21.4%) subjects exposed to HMW agents ($P = 0.02$) showed such significant changes. There were no significant differences in the frequency of significant changes in PC20 (3.2-fold diminution or more) in subjects who experienced peak immediate reactions 10 min after cessation of exposure (25.3%) vs. 20 min or more after (27.1%). There were no significant differences in the percentages of eosinophils nor the total number of eosinophils in induced sputum on the control and active exposure days by comparing subjects exposed to HMW and LMW agents. Finally, we found no significant associations between the baseline sputum% eosinophils and the timing of the peak immediate reactions (10 min vs. 20 min or more): 33/58 subjects (56.1%) with peak reactions at 10 min vs. 22/41 subjects (53.7%) with peak reactions at 20 min or more had $\geq$ 1% eosinophils in induced sputum; corresponding figures for a threshold of $\geq$ 2% eosinophils were 25/58 (43.1%) and 18/41 (43.9%), and, for $\geq$ 3% eosinophils, 20/58 (34.4%) and 15/41 (36.6%).

To investigate the possibility that variables significantly associated to the molecular weight of the agents (as shown in Table 1) could exert a confounding effect on the significance of the molecular weight *per se*, median regressions were carried out by incorporating variables that were found significantly different for HMW and LMW agents in the univariate analysis. First, differences in the timing of peak effects of immediate reactions showed statistically significant coefficients for the molecular weight of the agent by incorporating separately in the model the following variables: age (coefficient = - 10; upper and lower bounds of the 95% CI = - 1.21, - 10.0) and FEV₁/FVC (coefficient = - 10; upper and lower bounds of the 95% CI = - 5.02, - 10.0), but not atopy (coefficient = - 10.0; upper and lower bounds of the 95% CI = 15.8, - 10.0). Moreover, incorporating in the median regression the maximum fall in FEV₁ to the molecular weight of the agent resulted in a significant coefficient for the molecular weight of the agent, of - 5.5 (upper and lower bounds of the 95% CI = - 1.1, - 6.5). Second, differences in the timing to recovery to 10% baseline showed statistically significant coefficients for the molecular weight of the agent by incorporating separately the following variables: age (coefficient = - 13.5, upper and lower bounds of the 95% CI = - 3.3, - 28.2) and FEV₁/FVC (coefficient = - 30.0, upper and lower bounds of the 95% CI = - 6.0, - 30.0) but not atopy (coefficient = - 30.0, upper and lower bounds of the 95% CI = 6.5, - 30.0). Finally, incorporating in the median regression the maximum fall in FEV₁ to the molecular weight of the agent resulted in a persistently significant

Table 3. Timing and recovery of reactions

	Low-molecular-weight agents	High-molecular-weight agents
Number	219	248
Timing of the maximum fall in FEV ₁ for the immediate reactions (min)		
Median (first, third quartile values)	20 (10, 30)	10 (10, 20)**
0min (immediately after exposure)	30 (13.7%)	40 (16.1%)
10min post-exposure	63 (28.8%)	102 (41.1%)*
20min post-exposure	55 (25.1%)	66 (26.6%)
30min post-exposure	27 (12.3%)	13 (5.2%)*
Timing of the maximum fall in FEV ₁ for the late-phase of the reactions (min)		
Median (first, third quartile values)	360 (300, 420)	360 (300, 420)
Timing of recovery (min)		
(Median, and first, third quartile values in parentheses)		
to 5% of baseline	105 (60, 180)	90 (60, 180)
to 10% of baseline	90 (50, 120)	60 (40, 90)*
Number of subjects with recovery of the immediate reaction to :		
5% or less of baseline value	114 (52.1%)	170 (68.5%)**
10% or less of baseline value	159 (72.6%)	222 (89.5%)**

Presence of a dual phase reaction in the presence of an immediate reaction followed by at least a 10% fall in FEV₁ after the first hour following end of exposure:

* $P \leq 0.01$;

** $P \leq 0.001$.

coefficient for the molecular weight of the agent, of -14.3 (upper and lower bounds of the 95% CI = -4.2 , -28.3). By incorporating all these variables together in a single model, the nature of the agent (HMW and LMW) remained significantly significant in the case of the timing to recovery to 10% (coefficient = -11.5 , upper and lower bounds of the 95% CI = -4.9 , -11.5) but not to the timing of the peak effect.

We also compared subjects with immediate reactions to flour ($n = 113$) and diisocyanates ($n = 111$). Subjects exposed to flour were more often atopic (93.5% vs. 56.1%) ($P < 0.001$) and had higher spirometric values (FEV₁ of $94.3 \pm 17.7\%$ pred vs. $89.4 \pm 17.1\%$ pred) ($P = 0.03$). The magnitude of the immediate reactions was slightly but significantly ($P < 0.001$) greater in the case of diisocyanates ($31.1 \pm 10.6\%$) vs. flour ($25.1 \pm 7.3\%$). The median maximum fall in FEV₁ was 10 min (interquartile interval: 15 min) for flour agents and 20 min (interquartile interval: 20 min) for diisocyanates ($P < 0.001$). The timing of recovery of FEV₁ to 10% of baseline was also longer, though not significantly so for diisocyanates (90 min, interquartile range: 70 min) than flour (50 min, interquartile range: 50 min). The frequency of significant changes in PC20 after exposure to diisocyanates (34/94, 36.2%) was significantly higher ($P = 0.02$) than for flour (20/95, 21.1%).

The features of reactions were not significantly different in subjects taking ($n = 228$) or not taking ($n = 234$) inhaled steroids in regards to magnitude of immediate reactions (27.5% for both groups), median time of maximum reaction (10 min for both groups), as well as timing for recovery (median values of recovery to 5% baseline in 90 min for both groups).

Discussion

This study is the first to show that the temporal patterns of immediate asthmatic reactions differ in the case of HMW and LMW occupational agents in two important aspects, maximum effect and recovery that were found to be slightly delayed for LMW agents by comparison to HMW. Furthermore, reactions to LMW agents are more often accompanied by changes in non-specific bronchial responsiveness (BR).

Immediate asthmatic reactions were initially elicited after experimental exposure to ubiquitous allergens made of proteinaceous HMW material, as reviewed [3]. Similar reactions have been described after exposure to occupational allergens [4], although the pattern of reaction has not been discriminated in the case of LMW agents, a frequent cause of OA, by comparison with HMW agents derived from proteinaceous material, as for ubiquitous allergens. Previously, the only difference in the pattern of asthmatic reactions that has been

found for HMW vs. LMW agents is the occurrence of isolated late reactions (not preceded by immediate reactions) in the case of LMW but not for HMW agents [4].

The fact that LMW agents can cause different patterns of immediate asthmatic reactions and isolated late asthmatic reactions (LAR) might well be related to a physiopathological mechanism that is different from the mechanism identified for HMW agents. In the case of HMW agents, the mechanism is IgE-mediated as for ubiquitous sensitizing inhalants [6], whereas it is not generally IgE-mediated and remains unknown for most LMW agents, although for the latter, various mechanisms have been proposed [18, 19]. In regards to diisocyanates, the most common cause of OA due to LMW agents, mechanisms based on the presence of specific IgG [20] and monocyte chemoattractant protein-1 (MCP-1) synthesis [21, 22] have been proposed. Increased specific IgG and MCP-1 levels are more sensitive but less specific than specific IgE levels in the diagnosis of OA due to diisocyanates confirmed by specific inhalation challenges [20, 23, 24]. Specific IgG antibodies also seem to persist for a longer duration than specific IgE after removal from exposure in subjects with OA due to diisocyanates [25, 26]. Moreover, specific IgG antibodies are involved in the case of another LMW agent, reactive dyes [27]. It is therefore probable that these different mechanisms can also influence the genesis of and recovery from immediate asthmatic reactions. There might also be other mechanistic explanations for the difference in the pattern of reactions. These, that were not explored in our work but would worth being examined, are among others: (1) the deposition of dry particles in the airways, that represent the way high-molecular-weight agents were generated, can differ from wet aerosols and vapours, this being how low-molecular-weight agents were inhaled; (2) the small molecular weight agent has to bind to specific proteins to exert its allergenicity which is not the case for high-molecular-weight agents; (3) the activation of the bronchospastic cascade, from activation of cells to release of mediators, can be more rapid in the case of high-molecular-weight agents.

We do not think that the fact that exposure to high-molecular-weight agent was carried out on a single day in the case of high-molecular-weight agent and on different days for low-molecular-weight agents could have affected the patterns of reactions. As described in the method section, the total duration of exposure is broken into shorter periods for low-molecular-weight agents on all days of exposure in the same way as this is done for high-molecular-weight agents. Moreover baseline FEV₁ on subsequent exposure days is similar. Although PC20 is not assessed at the end of each day of exposure to low-molecular-weight agents, it can be suspected that exposure on the day that preceded the day where

significant changes in FEV₁ occurred can have enhanced non-specific BR [9]. This, contrary to what we found i.e. the peak reaction was delayed to 20 min in the case of low-molecular-weight agent, would rather promote brisker reactions to the specific agent.

Also, it can be argued that the percentage fall in FEV₁ was larger after exposure to low-vs. high-molecular-weight agent. However, the mean difference that was found (5%) is at the limit of a physiological significance. Moreover, median regressions were carried out by incorporating factors that were found to be significantly different for LMW and HMW agents in the univariate analysis (atopy, age, FEV₁/FVC) as well as the maximum fall in FEV₁, all variables that might have been considered as confounding factors. This analysis showed that the molecular weight of the agent still exerted an effect *per se* as shown by incorporating each variable separately in the analysis and, in the case of the timing to recovery, by even incorporating all variables in the model.

As a secondary outcome, we found that the occurrence of dual reactions (a late reaction following the immediate) was slightly but significantly more frequent after exposure to LMW (37.3%) vs. HMW (26.2%) agents. This higher frequency of dual reactions after exposure to LMW agents has also been suggested by others, although precise figures on the frequency of this phenomenon have not been published [4]. Another ancillary outcome of our study is that reactions to LMW as opposed to HMW agents were more often accompanied by a significant increase in BR. The same finding applies to diisocyanates, the most frequent cause of OA due to LMW agents, as compared to flour, the most common cause of OA due to HMW agents. Interestingly, in our study, the type of reaction (isolated immediate vs. dual reactions) was not associated with a different frequency of significant changes in BR. The LAR can be accompanied by changes in non-specific BR [28], although such changes were found to be only marginally commoner than after isolated immediate reactions [29]. Our study suggests that it is not the type of reaction (immediate vs. late) that affects the likelihood of changes in non-specific BR, but rather the nature of the causal agent.

Although this study is a retrospective analysis of data, it is based on results obtained using a procedure

that has not changed over the years. For this, subjects are exposed progressively to the suspected occupational agent using a standardized methodology [9, 10]. Moreover, the total duration of exposure that was necessary to cause a significant immediate reaction was comparable for HMW and LMW agents. It is therefore unlikely that this factor could have played a role.

It would be interesting to create international databases that would contain information on results of specific inhalation challenges with occupational agents for larger number of subjects than in our study. This would allow for examining factors affecting separately reactions to HMW and LMW agents as well as to specific agents, particularly those that more rarely cause OA. For the time being, the results of our study may serve to guide the practice of specific inhalation challenges with occupational agents. Indeed, after exposing subjects to LMW agents, a specific recommendation can be made. In the case of a fall in FEV₁ from 10% to 20% as assessed 10 min after the end of exposure, it would be safer, before administering another dose, to assess the spirometry 10 min later, that is 20 min after the end of exposure, because the results of our study show that the maximum reaction is more likely to occur at this time. Over the years, we have worked improving the methodology of such testing by proposing new equipment [12], which has resulted in safer tests, the risk of exaggerated immediate falls in FEV₁ of 30% or more being reduced to 19% [11]. Moreover, all these exaggerated reactions were found to be reversible after administering bronchodilators. Observing a pause of 10 min in the case of immediate falls in FEV₁ between 10% to 20% after exposure to low-molecular-weight agents would further contribute to improved safety. Such a practice may avoid the occurrence of too large falls in FEV₁ and make specific inhalation challenges safer.

Acknowledgements

The authors sincerely thank the MD clinicians who supervised the tests and Kathe Lieber for reviewing this manuscript.

Conflict of interest: The authors have no conflict of interest to declare.

References

- 1 Pepys J. Inhalation challenge tests in asthma. *New Engl J Med* 1975; 293:758–9.
- 2 Herxheimer H. The late bronchial reaction in induced asthma. *Int Arch Allergy Appl Immunol* 1952; 3:323–8.
- 3 Colldahl H. A study of provocation test on patients with bronchial asthma. *Acta Allergol* 1952; 5:133–42.
- 4 Pepys J, Hutchcroft BJ. Bronchial provocation tests in etiologic diagnosis and analysis of asthma. *Am Rev Respir Dis* 1975; 112:829–59.
- 5 Chan-Yeung M, Malo JL. Occupational asthma. *N Engl J Med* 1995; 333: 107–12.
- 6 Chinen J, Fleisher TA, Shearer WT. The immune system: an overview. In: Adkinson NF, Bochner BS, Busse WW, Holgate ST, Lemanske RF, Simons FER

- eds. *Middleton's Allergy*. Philadelphia: Mosby Elsevier, 2009: 3–17.
- 7 Anees W, Huggins V, Pavord ID, Robertson AS, Burge PS. Occupational asthma due to low molecular weight agents: eosinophilic and non-eosinophilic variants. *Thorax* 2002; **57**:231–6.
 - 8 Lemièrè C, Romeo P, Chaboillez S, Tremblay C, Malo JL. Airway inflammation and functional changes after exposure to different concentrations of isocyanates. *J Allergy Clin Immunol* 2002; **110**:641–6.
 - 9 Vandenplas O, Malo JL. Inhalation challenges with agents causing occupational asthma. *Eur Respir J* 1997; **10**:2612–29.
 - 10 Vandenplas O, Cartier A, Malo JL. Occupational Challenge Tests. In: Bernstein IL, Chan-Yeung M, Malo JL, Bernstein DI eds. *Asthma in the Workplace*. New York: Taylor & Francis, 2006:227–52.
 - 11 Malo JL, Cartier A, Lemièrè C *et al.* Exaggerated bronchoconstriction due to inhalation challenges with occupational agents. *Eur Respir J* 2004; **23**:300–3.
 - 12 Caron S, Boileau JC, Malo JL, Leblond S. New methodology for specific inhalation challenges with occupational agents. *Respir Res* 2010; **11**:72.
 - 13 Cockcroft DW, Killian DN, Mellon JJA, Hargreave FE. Bronchial reactivity to inhaled histamine: a method and clinical survey. *Clin Allergy* 1977; **7**:235–43.
 - 14 Malo JL, Pineau L, Cartier A, Martin RR. Reference values of the provocative concentrations of methacholine that cause 6% and 20% changes in forced expiratory volume in one-second in a normal population. *Am Rev Respir Dis* 1983; **128**:8–11.
 - 15 Pizzichini MMM, Popov TA, Efthimiadis A *et al.* Spontaneous and induced sputum to measure indices of airway inflammation in asthma. *Am J Respir Crit Care Med* 1996; **154**:866–9.
 - 16 Lemièrè C, Pizzichini MMM, Balkissoon R *et al.* Diagnosing occupational asthma: use of induced sputum. *Eur Respir J* 1999; **13**:482–8.
 - 17 Dehaut P, Rachiele A, Martin RR, Malo JL. Histamine dose-response curves in asthma: reproducibility and sensitivity of different indices to assess response. *Thorax* 1983; **38**:516–22.
 - 18 Cullinan P. Occupational asthma, IgE and IgG. *Clin Exp Allergy* 1998; **28**:668–70.
 - 19 Lemièrè C, Biagini RE, Zeiss CR. Immunological and inflammatory assessments. In: Bernstein IL, Chan-Yeung M, Malo JL, Bernstein DI eds. *Asthma in the workplace*. New York: Taylor & Francis, 2006:179–97.
 - 20 Cartier A, Grammer L, Malo JL *et al.* Specific serum antibodies against isocyanates: association with occupational asthma. *J Allergy Clin Immunol* 1989; **84**:507–14.
 - 21 Lummus ZL, Alam R, Bernstein JA, Bernstein DI. Diisocyanate antigen-enhanced production of monocyte chemoattractant protein-1, IL-8, and tumor necrosis factor by peripheral mononuclear cells of workers with occupational asthma. *J Allergy Clin Immunol* 1998; **102**:265–74.
 - 22 Bernstein DI, Cartier A, Cote J *et al.* Diisocyanate antigen-stimulated monocyte chemoattractant protein-1 synthesis has greater test efficiency than specific antibodies for identification of diisocyanate asthma. *Am J Respir Crit Care Med* 2002; **166**:445–50.
 - 23 Tee RD, Cullinan P, Welch J, Burge PS, Newman-Taylor AJ. Specific IgE to isocyanates: a useful diagnostic role in occupational asthma. *J Allergy Clin Immunol* 1998; **101**:709–15.
 - 24 Park HS, Kim HY, Nahm DH, Son JW, Kim YY. Specific IgG, but not specific IgE, antibodies to toluene diisocyanate-human serum albumin conjugate are associated with toluene diisocyanate bronchoprovocations test results. *J Allergy Clin Immunol* 1999; **104**:847–51.
 - 25 Park HS, Lee SK, Lee YM, Kim SS, Nahm DH. Longitudinal study of specific antibodies to toluene diisocyanate (TDI)-human serum albumin (HSA) conjugate in patients with TDI-induced asthma. *Korean J Internal Med* 2002; **17**:249–51.
 - 26 Malo JL, L'Archevêque J, Lummus Z, Bernstein D. Changes in specific IgE and IgG and monocyte chemoattractant protein-1 in workers with occupational asthma caused by diisocyanates and removed from exposure. *J Allergy Clin Immunol* 2006; **118**:530–3.
 - 27 Park HS, Hong CS. The significance of specific IgG and IgG4 antibodies to a reactive dye in exposed workers. *Clin Exp Allergy* 1991; **21**:357–62.
 - 28 Cartier A, Thomson NC, Frith PA, Roberts R, Hargreave FE. Allergen-induced increase in bronchial responsiveness to histamine: relationship to the late asthmatic response and change in airway caliber. *J Allergy Clin Immunol* 1982; **70**:170–7.
 - 29 Malo JL, Ghezzi H, L'Archevêque J, Cartier A. Late asthmatic reactions to occupational sensitizing agents: frequency of changes in nonspecific bronchial responsiveness and of response to inhaled beta-2 adrenergic agent. *J Allergy Clin Immunol* 1990; **85**:834–42.