

Nasal response in subjects undergoing challenges by inhaling occupational agents causing asthma through the nose and mouth

Desrosiers M, Nguyen B, Ghezzo H, Leblanc C, Malo J-L. Nasal response in subjects undergoing challenges by inhaling occupational agents causing asthma through the nose and mouth.

Allergy 1998; 53: 840–848. © Munksgaard 1998.

Background Subjects with occupational asthma (OA) often report nasal symptoms, but nasal reactions to inhalation challenges with occupational agents have not been well characterized.

Methods Fifteen subjects with OA (eight due to high-molecular-weight agents – flour and guar gum – and seven due to isocyanates) underwent inhalational challenges using closed-circuit devices (dry particles for high-molecular-weight agents and gas generator for isocyanates) on two occasions, 2–4 weeks apart in a random fashion. On one occasion, they inhaled through the nose and, on another, through the mouth. The FEV₁ was monitored for up to 8 h afterward, and symptoms were documented with a standardized questionnaire on nasal symptomatology, assessment of nasal resistance by rhinomanometry, and nasal lavage for the examination of cells and mediators.

Results Inhaling through the mouth and through the nose:

- 1) yielded similar asthmatic responses ($25 \pm 8\%$ and $22 \pm 10\%$ maximum changes in FEV₁)
- 2) more than doubled the peak nasal symptoms and nasal resistance when the maximum daily response was compared with prechallenge results.

This increase occurred on the days of inhalational challenges through the mouth and through the nose. There were some significant responses assessed by nasal lavage in terms of cells and mediators, again with no differences between the days of challenges through the mouth and through the nose.

Conclusions Inhaling occupational agents of high or low molecular weight, including isocyanates, whether through the mouth or nose:

- 1) results in a similar asthmatic response
- 2) causes a significant nasal response in terms of symptoms and an increase in nasal resistance
- 3) causes some significant changes in inflammatory cells and mediators.

Agents causing occupational asthma (OA) often induce nasal and conjunctival symptoms (1). A recent retrospective study showed that high-molecular-weight (HMW) agents, which generally exert their mechanism through an IgE-mediated mechanism, caused more rhinoconjunctivitis symptoms than did low-molecular-weight (LMW) agents (2). Moreover, rhinoconjunctivitis symptoms precede the onset of asthmatic symptoms more often for HMW than for LMW agents.

Although nasal challenges have been developed for common aeroallergens, these tests are not

routinely carried out with occupational agents. In the investigation of OA, specific inhalation tests are carried out by having the subject inhale through the mouth with the nose clipped. Therefore, nasal reactions in subjects undergoing bronchial challenges with occupational agents have not been studied by exposing the subject to nonirritant concentrations of these agents. It can be assumed that inhaling the occupational agent through the nose would result in a lesser bronchial response in terms of functional changes, due to the “filtering” process in the upper airways; this would be partic-

**M. Desrosiers¹, B. Nguyen²,
H. Ghezzo², C. Leblanc², J.-L. Malo²**

¹Department of Otorhinolaryngology, Centre hospitalier universitaire de Montréal (CHUM), Montreal; ²Department of Chest Medicine, Sacré-Coeur Hospital, Montreal, Canada

Key words: occupational asthma; rhinitis; toluene diisocyanate.

Jean-Luc Malo, MD
Department of Chest Medicine
Sacré-Coeur Hospital
5400 West Gouin
Montreal
Canada H4J 1C5

Accepted for publication 1 April 1998

Table 1. Baseline anthropometric, clinical, and functional results

Patient no.	Sex	Age (years)	Atopy	Interval since end of exposure (months)	Medication (total daily dosage [μ g])	Agent	FEV ₁ (% pred)	PC ₂₀ (mg/ml)
HMW (<i>n</i>=8)								
1	M	45	+	49	nil	Flour	99	10.8
3	F	49	+	17	B2	Flour	85	0.3
4	M	40	—	3	nil	Flour	102	0.5
10	M	60	+	29	B2	Flour	104	3.7
11	M	62	+	27	B2; be 1000	Flour	80	0.7
12	M	37	+	4	nil	Guar gum	82	128
13	M	38	+	41	nil	Guar gum	84	18
15	F	26	—	15	B2; bu 800	Flour	76	0.03
LMW (<i>n</i>=7)								
2	F	58	—	37	B2; be 2000	TDI	90	0.25
5	M	47	+	6	B2	TDI	121	128
6	M	45	—	23	nil	TDI	105	13.3
8	M	50	+	42	B2; bu 800	HDI	89	1.0
9	M	41	+	5	B2	MDI	118	23
14	M	52	—	49	B2; be 1000	TDI	81	0.25
16	M	34	+	3	B2; flu 500	TDI	92	0.5
Mean		46		23			94	2.5
SD		10		17			14	—

Atopy was defined as at least one immediate skin reaction to 15 common inhalants by prick method.

B2: inhaled β_2 -adrenergic agent; be: beclomethasone; bu: budesonide; flu: fluticasone; TDI: toluene diisocyanate; HDI: hexamethylene diisocyanate; MDI: diphenylmethane diisocyanate.

ularly true for agents in dry aerosol form (powder, particles). It can also be assumed that inhaling the agent through the nose would result in a nasal reaction in terms of symptoms, resistance, and inflammatory influx, whereas such a reaction would be of lesser magnitude, when the agent was inhaled through the mouth.

Therefore, to test these hypotheses, we performed inhalation challenges with occupational agents through both the mouth and the nose, by a method that allows quantification of the dose (3, 4), in 15 subjects with OA due to HMW agents and a LMW agent (isocyanate). Therefore, this study aimed to determine whether:

- 1) the magnitude of the asthmatic reaction would be comparable in inhalation of occupational agents through the mouth or through the nose
- 2) a nasal response could be documented and, if so, whether its magnitude would be greater in inhalation through the nose.

Material and methods

Subjects

We studied 15 subjects with OA due to either isocyanates (*n*=7; toluene diisocyanate [TDI] in 5/7 instances) or HMW (*n*=8; flour in 6/8 instances) agents (Table 1). All of these subjects had previously undergone specific inhalation challenges and

had shown a fall in FEV₁ of 20% or more after exposure. These subjects were selected only on the criterion that previous challenges were positive, and not because of developing nasal symptoms on the occasion of these challenges or in relation to their work. As shown in Table 1, 10 subjects were atopic. Six subjects were on inhaled steroids at the time of the study, which was kept at a constant dosage during the study period, although they did not inhale this preparation on the days of challenge. None took nasal inhaled steroids. All subjects gave informed written consent, and the project was approved by the local ethics committee.

Testing

Specific inhalation challenges were carried out by the closed-circuit method developed for particles (3, 5, 6) and isocyanates (4), which allows subjects to be exposed to low, nonirritant levels of these agents. Exposure by inhaling through the nose or the mouth was randomized. Subjects inhaled through a buccal mouthpiece in the case of challenges through the mouth. Subjects inhaled through a nasobuccal face-mask on the occasion of challenge through the nose. They were then asked to inhale through the nose with the mouth closed. A period of 2–4 weeks was observed between each challenge (nasal and oral) provided that baseline FEV₁ and the degree of responsiveness to metha-

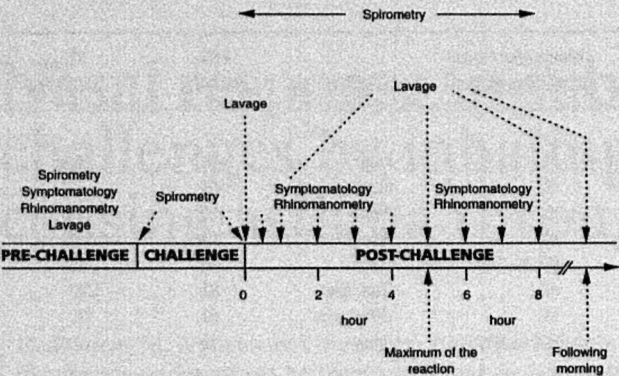


Fig. 1. Protocol in event of immediate reaction. Assessments of spirometry were done every 10 min for first hour, every 30 min for second hour, and every hour for total of 8 h.

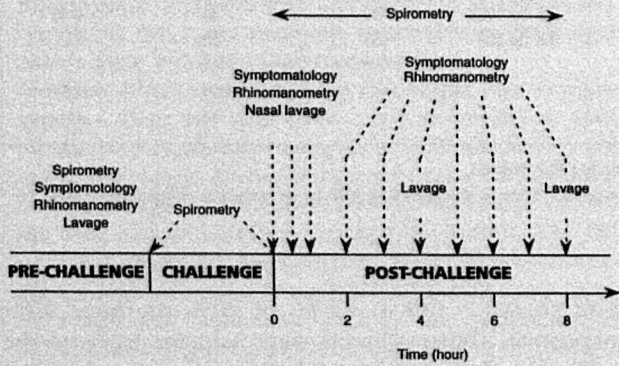


Fig. 2. Protocol in event of late-phase reaction. Spirometry was monitored every 10 min for first hour, every 30 min for second hour, and every hour for total of 8 h. Maximum reaction was arbitrarily placed at 5 h after challenge; it could occur at any time.

choline were back to the prechallenge assessment (no change in the provocative concentration of methacholine causing a change of 20% in FEV₁ – PC₂₀ – beyond 3.2-fold from one visit to the next (7).

The study design is illustrated in Figs. 1 and 2. On the first study day, a fall in FEV₁ of ≥15% was required after a maximum total exposure of 2 h split into progressive intervals (one breath, 15 s, 45 s, 2 min, etc.) to continue with the study. Subjects were exposed at a similar concentration but by the alternative method (through the mouth or through the nose) for a maximum of 2 h on the other occasion. The intervals at which the questionnaire, rhinomanometry, and nasal lavages were performed after the exposure are shown in Figs. 1 and 2.

Subjects were asked to fill out a subjective assessment of symptoms sheet on a visual analog scale (8). Each 100-mm line represented scales for sensation of rhinorrhea, nasal congestion, and itchy nose or throat. Sneezes were also counted (8).

Table 2. Results of specific inhalation challenges

Subject no.	Temporal type of reaction		Maximum fall in FEV ₁		Dose*	
	Nasal	Buccal	Nasal	Mouth	Nasal	Mouth
Dry particles						
1	immed	immed	28	32	28	25
3	immed	immed	21	21	28	62
4	immed	immed	21	36	11	4
10	immed	immed	20	23	131	22
11	immed	Dual	20	29	5	6
12	immed	immed	18	15	144	243
13	immed	immed	20	34	2	3
15	immed	immed	17	25	257	68
Mean	–	–	20.6	26.9	28	21
SD	–	–	3.1	6.7	–	–
Isocyanates						
2	Late	Late	55	25	138	130
5	Late	Late	19	28	50	77
6	immed	immed	22	21	432	272
8	Late	Late	15	11	1836	1824
9	Late	immed	20	27	20	8
14	Late	Late	17	14	441	333
16	Late	Late	19	30	54	68
Mean	–	–	23.9	22.3	164	134
SD	–	–	12.9	6.8	–	–
Mean	–	–	22.1	24.7	–	–
SD	–	–	9.2	7.1	–	–

* Expressed in mg/min for HMW agents and in PPB/min for isocyanates (i.e., concentration × duration of exposure). Geometric mean values are given for dose. Temporal type of reaction – immed: immediate (maximum in first hour after exposure); late (maximum at first hour after exposure). No significant differences in maximum changes in FEV₁ or in dose needed to provoke these changes.

Nasal resistance was measured from each nostril by anterior rhinomanometry (MultiSPIRO Inc., Irvine, CA, USA). The nostril selected for assessment of resistance was the one with the most reliable results at baseline, and this was the one monitored thereafter. Results are expressed in resistance to flow at –150 kPa inspiratory pressure, according to the recommendations of the International Committee on Rhinomanometry (9).

The nasal lavages were done by the technique described by Naclerio et al. (10). It consisted of the injection of 5 ml of normal saline in each nostril while the subjects were in a sitting position with their necks extended at about 30° from the horizontal. The liquid was kept for 10 s; then, as the subjects flexed their necks, the lavage fluids were recovered, transferred into a sterile reservoir, and maintained on ice until processing for cells. Cell processings from the nasal lavages were conducted within 4 h after sampling. Cell counts were performed with a hemocytometer. Each lavage was

Table 3. Baseline values for clinical and functional parameters

Agent	Rhinorrhea (left)		Rhinorrhea (right)		Congestion (left)		Congestion (right)		Itching		Sneezing		Total score	
	Nasal	Buccal	Nasal	Buccal	Nasal	Buccal	Nasal	Buccal	Nasal	Buccal	Nasal	Buccal	Nasal	Buccal
Questionnaire														
HMW (<i>n</i> =8)	0 (0)	0.06 (0.2)	0 (0)	0 (0)	1.5 (2.9)	1.05 (2.2)	0.06 (0.2)	0 (0)	0.15 (0.4)	0.2 (0.7)	0 (0)	0 (0)	1.8 (3.5)	1.4 (2.9)
LMW (<i>n</i> =7)	0.83 (1.8)	1.2 (3.2)	0.81 (1.8)	1.7 (3.3)	1.3 (1.5)	0.16 (0.4)	0.49 (0.9)	0.97 (2.6)	0.13 (0.3)	1.1 (2.9)	0 (0)	0.4 (1.1)	3.6 (5.4)	5.6 (13.2)
Total (<i>n</i> =15)	0.38 (1.2)	0.59 (2.2)	0.38 (1.2)	0.81 (2.3)	1.4 (2.3)	0.63 (1.7)	0.26 (0.6)	0.5 (1.8)	0.14 (0.4)	0.7 (2.0)	0 (0)	0.2 (0.8)	2.6 (4.4)	3.3 (9.1)
Cellular characteristics of nasal lavage														
Agent	Volume (ml)		No. of cells		No. of cells/ml		Macrophages		Lymphocytes		Neutrophils		Eosinophils	
	Nasal	Buccal	Nasal	Buccal	Nasal	Buccal	Nasal	Buccal	Nasal	Buccal	Nasal	Buccal	Nasal	Buccal
HMW (<i>n</i> =8)	6.2 (1.5)	5.9 (1.3)	0.2 (0.3)	0.7 (1.9)	0.05 (0.1)	0.14 (0.3)	33 (19)	33 (33)	0.5 (0.8)	0.3 (0.5)	51 (36)	49 (38)	2 (2.4)	0.6 (0.9)
LMW (<i>n</i> =7)	6.6 (0.6)	6.3 (1.0)	0.1 (0.2)	0.1 (0.1)	0.02 (0.02)	0.02 (0.02)	47 (32)	33 (28)	0.5 (0.5)	2 (4)	39 (34)	46 (34)	0.2 (0.4)	0.5 (0.8)
Total (<i>n</i> =15)	6.3 (1.2)	7.0 (0.8)	0.1 (0.3)	0.3 (0.8)	0.033 (0.04)	0.03 (0.04)	39 (25)	33 (30)	0.5 (0.7)	1.0 (2.5)	46 (34)	48 (35)	1.1 (1.9)	0.6 (0.9)
Mediator characteristics of nasal lavage														
Agent	Histamine		IL-1		IL-2		IL-5		GM-CSF		TNF- α			
	Nasal	Buccal	Nasal	Buccal	Nasal	Buccal	Nasal	Buccal	Nasal	Buccal	Nasal	Buccal	Nasal	Buccal
HMW (<i>n</i> =8)	17 (21)	21 (25)	75 (137)	67 (78)	38 (89)	15 (29)	28 (52)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
LMW (<i>n</i> =7)	13 (9)	18 (20)	14 (13)	18 (27)	11 (12)	0 (0)	16 (38)	6 (16)	0.3 (0.8)	0 (0)	0 (0)	3.1 (8.3)	0 (0)	4 (15)
Total (<i>n</i> =15)	16 (17)	18 (22)	46 (102)	43 (63)	26 (65)	8 (22)	22 (45)	4 (9)	0.1 (0.5)	0 (0)	0 (0)	4 (15)	0 (0)	4 (15)

For questionnaire, maximum values reached correspond to cm of 10-cm-long analog scale. Total score is maximum sum ever recorded of all listed symptoms. Zero value means that questionnaire was negative or that mediators were not detectable. Cell types are expressed in %. HMW: high-molecular-weight agents; LMW: low-molecular-weight agents.

centrifuged at 1500 rpm at 4°C for 15 min. The lavage supernatant liquid was divided into three parts and kept at -70°C for further assays of histamine and cytokines. The cell pellet was resuspended in PBS (phosphate-buffered saline). The quantity of PBS added depended on the cell count for each lavage specimen. Finally, Cytospin slides of the cell suspension were prepared in a Cytospin 3 centrifuge (Shandon, Pittsburgh, PA, USA) rotating at 600 rpm for 6 min. The slides were stained with Diff-Quik (Baxter Healthcare Corporation, Miami, FL, USA), a modified Wright stain for cell differentials (11).

Histamine assay was done with a radioenzymatic assay sensitive to 250 pg/ml (12). Interleukin (IL)-1 and IL-2 were assayed by a previously described method (13). IL-5, GM-CSF, and tumor necrosis factor- α (TNF- α) were assessed with commercial kits (Quantikine®, Research and Diagnostic Systems, Minneapolis, MN, USA). Given that the

precise mechanism of LMW agent-induced inflammation is currently undetermined, measurement of cytokines was performed in order to determine whether different cytokine generation profiles are involved for both HMW and LMW agents. All markers are reported in terms of total quantity measured in the eluate because it represents the amount of mediator generated in a fixed time interval. Total quantity is obtained by multiplying the concentration by the volume of secretions by the volume of eluate fluid.

Spirometry was monitored by the usual scheme during specific inhalation challenge tests: every 10 min for the first hour, every 30 min for the second hour, and then hourly for up to a total of 8 h (14). Assessments of FEV₁ values fulfilled the standards of the American Thoracic Society (15) and were done with a Vitalograph instrument (Vitalograph Ltd, Buckingham, UK) while the subjects were standing. At the end of each day, bronchial respon-

Table 4. Symptoms according to type of challenge

Agent	Rhinoirrhoea (left)		Rhinoirrhoea (right)		Congestion (left)		Congestion (right)		Itching		Sneezing		Total score	
	Nasal	Buccal	Nasal	Buccal	Nasal	Buccal	Nasal	Buccal	Nasal	Buccal	Nasal	Buccal	Nasal	Buccal
Mean $\pm$ SD differences between maximum and baseline values														
HMW (<i>n</i> =8)	0.64*	0.17	0.85*	0.15	0.26	0.23	0.76	1.5	3.0*	2.5*	0.5	0.13	4.6**	4.1*
	(0.7)	(0.3)	(1.1)	(0.3)	(1.0)	(0.5)	(2.0)	(2.7)	(3.0)	(3.3)	(1.1)	(0.4)	(4.2)	(4.0)
LMW (<i>n</i> =7)	0.36	0.67	0.46	0.16	1.3	1.7*	0.55	2.7*	1.7	0.47	0.43	0.29	3.4	3.6*
	(1.1)	(1.3)	(1.0)	(2.1)	(3.6)	(1.5)	(2.5)	(3.7)	(2.2)	(1.7)	(1.1)	(0.8)	(8.5)	(3.5)
Total (<i>n</i> =15)	0.51*	0.40	0.67*	0.16	0.74	0.93**	0.66	2.1*	2.4**	1.6*	0.47	0.2	4.0*	3.9**
	(0.9)	(0.9)	(1.0)	(1.4)	(2.5)	(1.3)	(2.2)	(3.1)	(2.6)	(2.8)	(1.1)	(0.6)	(6.4)	(3.7)
	Rhinoirrhoea (left)		Rhinoirrhoea (right)		Congestion (left)		Congestion (right)		Itching		Sneezing		Total score	
	Nasal	Buccal	Nasal	Buccal	Nasal	Buccal	Nasal	Buccal	Nasal	Buccal	Nasal	Buccal	Nasal	Buccal
Mean $\pm$ SD (maximum nasal/maximum mouth) ratios														
HMW (<i>n</i> =8)	1.4		2.6		1.4		0.8		1.0		1.0		1.1	
	(1.6)		(3.4)		(1.3)		(0.4)		(0.5)		(1)		(0.9)	
LMW (<i>n</i> =7)	1.1		1.1		1.8		0.6		1.8		0.9		1.1	
	(1)		(1.1)		(1.8)		(0.4)		(2.5)		(0.2)		(1.3)	
Total (<i>n</i> =15)	1.3		1.9		1.6		0.7*		1.4		1.0		1.1	
	(1.3)		(2.6)		(1.5)		(0.4)		(1.7)		(0.1)		(1.1)	
Mean $\pm$ SD (nasal/mouth) ratios at time of maximum changes in FEV ₁														
HMW (<i>n</i> =8)	0.8		2.0		1.6		0.6		1.0		1.0		1.1	
	(0.4)		(3.1)		(1.5)		(0.5)		(0.5)		(0)		(0.9)	
LMW (<i>n</i> =7)	1.0		1.4		1.6		1.6		1.8		0.9		1.5	
	(1.1)		(1.6)		(1.6)		(2.4)		(2.5)		(0.4)		(1.9)	
Total (<i>n</i> =15)	0.9		1.7		1.6		1.1		1.4		0.9		1.3	
	(0.8)		(2.5)		(1.5)		(1.7)		(1.8)		(0.3)		(1.4)	

Maximum values reached correspond to cm of 10-cm-long analog scale. Total score is maximum sum ever recorded of all listed symptoms. Significant changes from baseline at $P \leq 0.05^*$ and 0.01^{**} levels. HMW: high-molecular-weight agents; LMW: low-molecular-weight agents.

siveness to inhaled methacholine was assessed. A Wright nebulizer was used, and the subjects inhaled methacholine at tidal breathing volume for 2 min. The concentration causing a 20% fall in FEV₁ was obtained by interpolating the individual dose-response curves drawn on a semilogarithmic scale (16).

Analysis of results

The following indices were compared by Student's paired *t*-test:

- 1) baseline values on the days of challenges through the nose and through the mouth
- 2) maximum differences (maximum value – baseline) in symptom scores and nasal resistance and absolute (for cellular features and mediators) values obtained separately on the days of challenges through the nose and through the mouth
- 3) maximum (nasal/mouth) ratios of maximum values or of differences in maximum values reached (in the case of mediators) any time after challenge

- 4) maximum (nasal/mouth) ratios or differences in maximum values reached (in the case of mediators) at the time of maximum changes in FEV₁.

An individual *P* value of ≤ 0.05 was considered significant. As the questionnaire and rhinomanometry were carried out at several intervals after the end of challenge, the area under the curve relating the time interval on the abscissa to the values on the ordinate was also analyzed. As this analysis yielded similar results as with the maximum values (see above), these results are not presented.

Results

Table 2 gives the results of specific inhalation challenges. The temporal pattern of reaction after inhalation through the nose and through the mouth was similar in all subjects except for subjects 11 and 9. The maximum changes in FEV₁ were not significantly different on the two study days. The dose of HMW agents and of isocyanates was not significantly different either ($t=0.7$ and 0.8 , respectively), although the mean absolute

Table 5. Cellular characteristics of nasal lavage

Agent	Volume (ml)		No. of cells		No. of cells/ml		Macrophages		Lymphocytes		Neutrophils		Eosinophils	
	Nasal	Buccal	Nasal	Buccal	Nasal	Buccal	Nasal	Buccal	Nasal	Buccal	Nasal	Buccal	Nasal	Buccal
Mean $\pm$ SD differences between maximum and baseline values														
HMW (<i>n</i> =8)	0.8*	1.2**	0.2*	-0.2	0.03	-0.1	21*	14**	1	4	17**	26**	1	2*
	(1.0)	(1.1)	(0.3)	(2.1)	(0.1)	(0.3)	(21)	(11)	(1)	(8)	(14)	(25)	(2)	(4)
LMW (<i>n</i> =7)	0.5*	0.5*	0.5	-0.1	0.07	-0.1	23	26*	12	5**	24	16	11	1**
	(0.5)	(0.5)	(1.4)	(0.1)	(0.2)	(0.02)	(50)	(25)	(30)	(10)	(39)	(33)	(27)	(2)
Total (<i>n</i> =15)	0.7**	0.9**	0.4	-0.1	0.05	-0.1	21*	19**	6	4*	20**	21**	5	2*
	(0.8)	(0.9)	(0.9)	(0.2)	(0.1)	(0.2)	(36)	(20)	(21)	(8)	(28)	(28)	(19)	(3)
Mean $\pm$ SD (maximum nasal/maximum mouth) ratios														
HMW (<i>n</i> =8)	1.0		2.5		2.2		2.4		1.8		11.0		10.0	
	(0.1)		(2.2)		(2.0)		(2.2)		(2.6)		(28.2)		(22.3)	
LMW (<i>n</i> =7)	1.1		5.1		5.0		5.6		6.7		6.8		53	
	(0.1)		(10.0)		(10.1)		(9.5)		(9.7)		(14.0)		(91.2)	
Total (<i>n</i> =15)	1.0		3.7		3.5		3.8		4.3		9.3		24.4	
	(0.1)		(6.9)		(6.9)		(6.3)		(7.2)		(22.6)		(53.4)	
Mean $\pm$ SD (nasal/mouth) ratios at time of maximum changes in FEV ₁														
HMW (<i>n</i> =8)	1.0		12.6		15.4		0.3		0.1		0.5		(0.1)	
	(0.2)		(30.6)		(38.7)		(0.5)		(0.4)		(0.8)		(0.4)	
LMW (<i>n</i> =7)	1.0		2.1		2.1		0		0		0		0.1	
	(0.1)		(3.1)		(2.9)		(0)		(0)		(0)		(0.4)	
Total (<i>n</i> =15)	1.0		7.7		9.2		0.1		0.06		0.2		0.1	
	(0.1)		(22.4)		(28.3)		(0.3)		(0.3)		(0.6)		(0.3)	

Macrophages, lymphocytes, neutrophils, and eosinophils are expressed in % counts. * $P \leq 0.05$; ** $P \leq 0.01$. HMW: high-molecular-weight agents; LMW: low-molecular-weight agents.

dose necessary to cause a significant fall in FEV₁ was marginally larger in the case of inhalation through the nose.

There were no significant differences in prechallenge baseline values in the case of challenges through the nose and the mouth (Table 3). Table 4 shows the mean maximum differences (maximum value - prechallenge baseline), mean maximum (nasal/mouth) ratios at any time after challenge, and mean maximum (nasal/mouth) ratios at the time of maximum changes in FEV₁ for symptoms. These reveal significant differences in symptom scores whatever the type of challenge (through the nose and through the mouth). Although the mean (maximum nasal/maximum mouth) ratios any time after challenge and the mean (maximum nasal/maximum mouth) ratios at the time of the maximum changes in FEV₁ tended more often to be greater than 1 (indicating more symptoms in inhalation through the nose), this did not reach significant differences.

Baseline values for nasal resistance obtained by rhinomanometry were 0.388 ± 0.202 kPa·l⁻¹·s on the day of challenge through the nose and 0.310 ± 0.240 kPa·l⁻¹·s on the day of challenge through the mouth. The mean maximum absolute increase in nasal resistance was significant ($+0.511$ kPa·l⁻¹·s by inhalation through the nose and $+0.439$ kPa·l⁻¹·s by

inhalation through the mouth). This increase was not superior in comparison of challenges through the mouth and through the nose.

Although there were no significant differences in the number of cells nor in the number of cells/ml in the nasal lavage on the 2 days of challenges, the maximum increases reached for macrophages, lymphocytes, and neutrophils were significant in most instances for inhalation through the nose and through the mouth (Table 5). Again, the ratios of nasal/mouth were more often greater than 1, but this was not significant. Table 6 shows that the increase in levels of IL-2 and IL-5 reached on the day of challenge through the nose with LMW agents was significant. This was the case for IL-1 on the day of challenge with LMW agents through the mouth and for histamine for challenges with LMW agents through the mouth and the nose. Higher increases were obtained for IL-1, IL-2, and IL-5 in inhalation through the mouth, but this was not significant.

Discussion

This study shows that:

- 1) the dose of occupational agents in dry-particle or vapor forms required to cause a significant

Table 6. Mediator characteristics of nasal lavage

	Histamine		IL-1		IL-2		IL-5		GM-CFS		TNF- α	
Agent	Nasal	Buccal	Nasal	Buccal	Nasal	Buccal	Nasal	Buccal	Nasal	Buccal	Nasal	Buccal
Mean $\pm$ SD differences between maximum and baseline values												
HMW	76 (153)	12.2** (13)	169 (281)	77 (154)	58 (143)	38 (102)	51 (90)	7 (12)	2.6 (7.1)	3.9 (11)	7.0 (19)	0 (0)
LMW	16.2** (11)	6.6** (3.8)	35 (46)	16** (10)	10.5** (8.2)	4.1 (9.3)	33* (44)	0 (0)	3.0 (5.4)	2.2 (5.9)	0 (0)	3.1 (8.3)
Total	48 (113)	9.8** (10.0)	111 (219)	48 (113)	37 (108)	22 (75)	43* (71)	4 (9)	2.8 (6.2)	3.1 (8.7)	3.7 (14.5)	1.5 (5.7)
Mean $\pm$ SD (maximum nasal – maximum mouth) differences												
	Histamine		IL-1		IL-2		IL-5		GM-CFS		TNF- α	
HMW (<i>n</i> =8)	-92 (179)		92 (178)		21 (42)		44 (94)		-1.2 (3.9)		7.0 (20)	
LMW (<i>n</i> =7)	-14 (47)		17 (51)		5 (15)		33 (44)		0.7 (8.9)		-3.1 (8.3)	
Total (<i>n</i> =15)	-55 (136)		60 (140)		13 (32)		38 (73)		-0.3 (6.5)		2.3 (16)	
Mean $\pm$ SD (nasal – mouth) differences at time of maximum changes in FEV ₁												
HMW (<i>n</i> =8)	-98 (174)		28 (197)		-15 (53)		12 (39)		2.7 (7.1)		7 (19)	
LMW (<i>n</i> =7)	-4 (50)		8 (49)		0 (12)		13 (26)		0.1 (0.4)		0 (0)	
Total (<i>n</i> =15)	-54 (136)		19 (143)		-8 (39)		12 (32)		1.5 (5)		4 (15)	

Histamine in ng/ml; other mediators in pg/ml; HMW: high-molecular-weight agents; LMW: low-molecular-weight agents.

and equivalent reduction in airway caliber is not significantly different whatever the mode of inhalation; i.e., through the mouth or through the nose

2) exposure to low levels of agents causing OA can also elicit clinical, functional, and pathologic nasal responses.

These changes can be documented by inhaling through the mouth or through the nose, the differences being not significant for either HMW or LMW agents.

Although much attention has been given to OA (17), less has been given to occupational rhinoconjunctivitis. It is well known that concomitant nasal and conjunctival symptoms are common in subjects with OA (1). The frequency is higher for HMW than for LMW agents and precedes the appearance of chest symptoms in the case of HMW agents (2). Moreover, the nose can be used as a model for exploring the clinical, functional, and physiopathologic manifestations of asthma caused by aeroallergens. The ability to carry out specific inhalation challenges with occupational agents, either in dry-particle form (3) or with isocyanates in vapor form (18), with instruments that allow quantification of the dose inhaled makes it possible to test inhalation

of the occupational agent through the mouth or through the nose.

We found no significant differences in the dose required to cause a significant reduction in airway caliber whatever the mode of inhalation. We expected some filtering process in the upper airways, especially in the case of agents in dry-particle forms. We propose the following explanation of this finding. Our exposure apparatuses allow the generation of agents at a concentration below the TLV-STEL (threshold limit value–short-term exposure level) (19) threshold; that is, a concentration at which nonspecific irritant reactions are unlikely to occur. One may suspect that the filtering process which occurs in the upper airways is particularly efficient in cases where higher concentrations than those used in our study occur.

In commonly performed and widely reported nasal challenge studies, a supraphysiologic stimulus is employed in order to produce a strong response. Nasal secretions are collected, and mediators generated during this response can be assayed. In these studies, allergen challenge has been shown to produce increases in cells and mediators such as histamine (20), prostaglandins, leukotrienes (21), and cytokines (22). However, the mediator levels

produced may not necessarily correspond to the degree of symptomatology (23). It has been argued that these supraphysiologic challenges induce massive changes not necessarily representative of clinical disease, as they do not reflect the pattern of natural exposure. Indeed, in these experiments, an entire season's worth of pollen can be insufflated into the nose at one sitting. Challenges more closely resembling natural exposure are less apt to show these changes. In nasal lavage studies of allergic rhinitis performed during natural seasonal exposure, obvious clinical disease, manifested by symptoms of sneezing, ocular and nasal pruritus, rhinorrhea, and nasal congestion, occurs. However, this is rarely accompanied by increases in mediators and cells recovered from lavage fluid. Histamine levels remain relatively constant throughout the season despite obvious symptomatology (24). The number of cells does not increase, but the percentage of eosinophils does (25).

It is somewhat surprising that both types of challenges, through the mouth and through the nose, produced equivalent responses. The magnitude of the effect appeared similar for both routes of administration. We suspect that this was due to a significant amount of postnasal exposure occurring through reflux from the nasopharynx. This reflux mechanism or passage of air from the buccal cavity is well known and is believed to be principally responsible for the conduction of odor molecules and scents to the olfactory region. The nasal lavage technique samples the entire nasal cavity and the nasopharynx indiscriminately; therefore, changes occurring in the posterior regions of the nasal cavity are reflected in the lavage fluids. More specific lavage collection methods, such as the filter paper disk model (26) or biopsy techniques (27), would allow selective sampling exclusively of the anterior regions. One could obviate nasal contamination from inhalation through the mouth by blocking the posterior part of the nasopharynx. To test the hypothesis that a reflux mechanism from the mouth to the nose occurs in inhalation of the occupational agents through the mouth, one might perform challenge studies through the mouth while completely isolating one of the nasal passages from any potential exposure by means of an inflatable plug in the anterior nares and choana, and then comparing the degree of response from both the challenged and control nostrils. In such a design, it would be possible to assess the magnitude of the nasal response by comparison with a total nonexposure procedure. However, this would represent an artificial setup, much different from the methodology used for inhalational challenge with occupational agents, in which the subject breathes through an orofacial mask. We were interested to know whether a nasal

reaction could be documented – and we did this – and whether it would be, in these circumstances, of greater magnitude as a result of inhalation through the nose. To our surprise, it was not, although it could reasonably be expected that the dose deposited in the nasal passages would be more potent with inhalation through the nose.

Exposure to HMW agents caused significant nasal symptoms, whereas this was not the case for isocyanates, thereby confirming the results of a retrospective questionnaire administered to subjects assessed for OA due to HMW agents and isocyanates (2). The influx of cells was also significant in the case of HMW agents, but not isocyanates, this being due to a wide dispersion of results in the case of isocyanates, some subjects showing a significant influx of cells, and others not. The reason for the significant presence of neutrophils, but not of eosinophils, observed in the case of HMW agents is unclear. However, we believe this was due primarily to the difference in stimulus strength previously alluded to. Significant levels of mediators (histamine, IL-1, IL-2, and IL-5) were reached after the challenges, these levels being higher, though not significantly so, for nasal challenges. Higher levels of mediators might have been obtained by using the filter disk method initially described by Sim (22), in which filter paper is left in contact with the nasal mucosa for a more prolonged period (15 min); however, given the low volume of secretions produced (on average, 7 ml, including 5 ml of the instilled solution), this would probably not have been practical in our study. Finally, examination of the inflammatory reaction in nasal biopsy specimens, instead of in lavage, may better characterize the pathophysiologic mechanism. Studies of simultaneously performed lavage and biopsies after allergen challenge have also highlighted the differences in nasal secretions and the mucosal compartments. In a study by Lim et al., the predominant cell recovered in nasal lavage was the eosinophil, while the predominant cell in the epithelium was a mononuclear cell presumed to be a lymphocyte (28).

This study has practical implications. Indeed, when performing challenges with occupational agents in order to document bronchial responses, it would be interesting to monitor possible nasal responses as well, particularly for HMW agents. A diagnosis of occupational rhinitis, associated or not with OA, would then be possible, even if the subject is exposed by inhalation through the mouth.

Acknowledgments

We thank Anne Kagey-Sobotka, PhD, and Jane MacKenzie-White, MSc, of the Johns Hopkins Asthma and Allergy Center, for her kind assistance in assaying histamine in the nasal

samples, and Dr Jacques Hébert, Québec City, for the assessment of all other mediators. We also thank Lori Schubert for reviewing the manuscript. This study was supported by the Centre québécois d'excellence en santé respiratoire and the International Isocyanate Institute.

References

- Bernstein DI. Clinical assessment and management of occupational asthma. In: Bernstein IL, Chan-Yeung M, Malo JL, Bernstein DI, editors. *Asthma in the workplace*. New York: Marcel Dekker, 1993:103-23.
- Malo JL, Lemièrre C, Desjardins A, Cartier A. Prevalence and intensity of rhinoconjunctivitis in subjects with occupational asthma. *Eur Respir J* 1997;10:1513-15.
- Cloutier Y, Lagier F, Lemieux R, et al. New methodology for specific inhalation challenges with occupational agents in powder form. *Eur Respir J* 1989;2:769-77.
- Vandenplas O, Malo JL, Cartier A, Perreault G, Cloutier Y. Closed-circuit methodology for inhalation challenge test with isocyanates. *Am Rev Respir Dis* 1992;145:582-7.
- Cloutier Y, Malo JL. Update on an exposure system for particles in the diagnosis of occupational asthma. *Eur Respir J* 1992;5:887-90.
- Cloutier Y, Lagier F, Cartier A, Malo JL. Validation of an exposure system to particles for the diagnosis of occupational asthma. *Chest* 1992;102:402-7.
- 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.
- Linder A. Symptom scores as measures of the severity of rhinitis. *Clin Allergy* 1988;18:29-37.
- Clement PAR. Committee report on the standardization of rhinomanometry. *Rhinology* 1984;22:151.
- Naclerio RM, Meier HL, Kagey-Sobotka A, et al. Mediator release after nasal airway challenge with allergen. *Am Rev Respir Dis* 1983;128:597-602.
- Walden SM, Proud D, Bascom R, et al. Experimentally induced nasal allergic responses. *J Allergy Clin Immunol* 1988;81:940-9.
- Baumgarten CR, Togias AG, Naclerio RM, Lichtenstein LM, Norman PS, Proud D. Influx of kininogens into nasal secretions after antigen challenge of allergic individuals. *J Clin Invest* 1985;76:191-7.
- Grassi J, Roberge CJ, Frobert Y, Pradelles P, Poubelle PE. Determination of IL1alpha, IL1beta and IL2 in biologic media using specific enzyme immunometric assays. *Immunol Rev* 1991;119:125-45.
- Cartier A, Malo JL. Occupational challenge tests. In: Bernstein IL, Chan-Yeung M, Malo JL, Bernstein DI, editors. *Asthma in the workplace*. New York: Marcel Dekker, 1993:215-47.
- American Thoracic Society. Standardization of spirometry - 1987 update. *Am Rev Respir Dis* 1987;136:1285-1307.
- Cockcroft DW, Killian DN, Mellon JJA, Hargreave FE. Bronchial reactivity to inhaled histamine: a method and clinical survey. *Clin Allergy* 1977;7:235-43.
- Bernstein IL, Chan-Yeung M, Malo JL, Bernstein D. *Asthma in the workplace*. New York: Marcel Dekker, 1993.
- Vandenplas O, Malo JL, Cartier A, Perreault G, Cloutier Y. Closed-circuit methodology for inhalation challenge tests with isocyanates. *Am Rev Respir Dis* 1991;145:582-7.
- American Conference of Governmental Industrial Hygienists. TLVs and BEIs. Threshold limit values for chemical substances and physical agents. Biological exposure indices. Cincinnati, OH: 1997.
- Naclerio RM, Meier HL, Kagey-Sobotka A, et al. Mediator release after nasal airway challenge with allergen. *Am Rev Respir Dis* 1983;128:597-602.
- Creticos PS, Peters SP, Adkinson NF Jr, et al. Peptide leukotriene release after antigen challenge in patients sensitive to ragweed. *N Engl J Med* 1984;310:1626-30.
- Sim TC. Proinflammatory cytokines in nasal secretions of allergic subjects after antigen challenge. *Am J Respir Crit Care Med* 1994;149:339-44.
- Castells M, Schwartz LB. Tryptase levels in nasal-lavage fluid as an indicator of the immediate allergic response. *J Allergy Clin Immunol* 1988;82:348-56.
- Linder A, Strandberg K, Deuschl H. Variations in histamine concentration in nasal secretions in patients with allergic rhinitis. *Allergy* 1988;43:119-26.
- Pipkorn U, Karlsson G, Enerbäck L. The cellular response of the human allergic mucosa to natural allergen exposure. *J Allergy Clin Immunol* 1988;82:1046-54.
- Flowers BK, Proud D, Kagey-Sobotka A, Lichtenstein LM, Naclerio RM. Localized antigen challenge of the nasal mucosa. *Arch Otolaryngol Head Neck Surg* 1990;116:1407-10.
- Prior AJ, Calderon MA, Lavelle RJ, Davies RJ. Nasal biopsy: indications, techniques and complications. *Respir Med* 1995;89:161-9.
- Lim NC, Taylor RM, Naclerio RM. The histology of allergic rhinitis and its comparison to cellular changes in nasal lavage. *Am J Respir Crit Care Med* 1995;151:136-44.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.