

ORIGINAL PAPER

Outcomes in occupational asthma caused by reactive dye after long-term avoidance

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Summary

Background Reactive dye (RD) is known to be a causative agent of occupational asthma (OA). However, to date, no report has been issued concerning the long-term outcomes of RD-induced OA.

Objectives We sought to evaluate the long-term outcomes in cases of OA caused by RD.

Methods A total of 11 OA patients confirmed by RD bronchial challenge were enrolled in this study. First and second follow-up examinations were conducted at 4.3 ± 2.3 and 13.7 ± 2.3 years (means $\pm$ SD) after the initial examinations, respectively. Skin prick test with RD and 11 common inhalant allergens, pulmonary function test, methacholine bronchial provocation testing, symptom and medication scores were determined at each visit. In addition, inflammatory cells in induced sputum were measured at the second follow-up examinations.

Results Reduced lung function at initial examinations did not recover at the first and second examinations despite cessation of exposure and proper pharmacological treatment. In addition, asthma severity (as determined by symptom and medication scores) and non-specific airway hyper-responsiveness to methacholine also did not improve. However, skin reactivity to RD almost disappeared at the second examinations. Interestingly, four of the six patients who showed negative skin responses to all 11 common inhalant allergens at initial examinations were found to be atopic at the second examinations. Moreover, in terms of airway inflammation, seven of the 11 patients showed eosinophilia in induced sputum ($\geq 3\%$) at the second examinations despite having been on high-dose inhaled corticosteroid medication.

Conclusion The present study demonstrates that reduced lung function and asthmatic symptoms persist in RD-induced OA even after long-term exposure avoidance.

Keywords asthma, follow-up, occupational disease, outcome, reactive dyes

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Introduction

About 9–15% of adult asthma is associated with occupational factors [1, 2], which makes occupational asthma (OA) one of the most common occupational lung diseases in industrialized countries [3, 4]. Several low-molecular-weight reactive chemicals are known to cause OA [5], and the fibre-reactive dyes are one of these chemicals [6]. These dyes contain a chromogen linked to a reactive group that enables the covalent attachment of the dye to hydroxyl groups on cellulose fibres, which act as haptens and can stimulate an immune response. Since the first report of reactive dye (RD)-induced OA was issued in

1978 [7], many cases of occupational respiratory diseases resulting from such dyes, including lethal asthma, have been described [8–10]. Correct OA management must be based on a good knowledge of the natural history of the disease concerned and of its prognostic factors. In the case of OA caused by toluene di-isocyanate (TDI; a low-molecular-weight agent similar to RD), long-term consequences have been well characterized by many investigators [11–14]. However, no report has been issued concerning the long-term outcomes of RD-induced OA. Here, we present our results of a long-term follow-up examination conducted on 11 patients with RD-induced OA.

Methods

All subjects enrolled in this study provided written informed consent, and the study protocol was approved by the Institutional Review Board of Seoul National University Hospital.

Initial examinations

The 11 enrolled subjects were confirmed to have RD-induced OA by a bronchial challenge test with causative dyes at initial examination. All underwent skin prick test (SPT) with 11 common inhalant allergens (Bencard, Brentford, UK) and four reactive dyes (Ehwa Co., Seoul, Korea): Remazol black GR (colour index reactive black 5), Remazole black B (colour index reactive black 5), Remazole orange 3R (colour index reactive orange 16) and Procion yellow HE4R (colour index reactive yellow 84). The common inhalant allergens included house dust mites (*Dermatophagoides pteronyssinus*, *D. farinae*), dog fur, cat fur, moulds (*Aspergillus*, *Alternaria*), pollens (tree pollen mixture, grass pollen mixture, mugwort, ragweed) and cockroach. Non-specific airway hyper-responsiveness (AHR) to inhaled methacholine was evaluated and the provocative dose of methacholine causing a decline of 20% (PC20) in forced expiratory volume in 1 s (FEV₁) was calculated for all 11 patients. A scoring system based on symptoms [4-point scale; 0 (no symptoms), 1 (symptoms $\leq$ 1/week), 2 (symptoms $\geq$ 2/week or nocturnal symptoms), 3 (daily or persistent symptoms)] and medication status [5-point scale; 0 (no medications), 1 (prn. β_2 -agonist), 2 (low-dose inhaled steroid, budesonide $<$ 800 μ g/day), 3 (high-dose inhaled steroid, budesonide $\geq$ 800 μ g/day) and 4 (systemic steroid)] was applied to quantify the severity of asthma. The characteristics of the study patients at initial examination are described in Tables 1 and 2.

Follow-up examinations

All the 11 study patients had given up smoking after diagnosis and had been treated on their asthma severity basis. For all subjects, eventually, exposure to RD stopped. First follow-up examinations were performed at 4.3 ± 2.3 (mean $\pm$ SD) years after initial examinations (the average duration of freedom from RD exposure was 2.4 ± 1.8 years). At this stage, patients underwent a pulmonary function test and a methacholine bronchial provocation test (MBPT). Symptom and medication scores were measured using the same criteria as those used at initial examinations.

Second follow-up examinations were performed at 13.7 ± 2.3 years after initial assessments, and subjects underwent a pulmonary function test, MBPT and SPT with 11 common inhalant allergens and four RDs. In addition, symptom and medication scores were determined and sputum induction was performed. Sputum induction was performed as follows: after measurement of basal FEV₁, all subjects were pretreated with 200 μ g albuterol (VentolinTM, GlaxoSmithKline, Brentford, UK). Sterile hypertonic saline solution (4.5%) was then administered using an ultrasonic nebulizer, with output set at 4.5 mL/min (Omron Co., Tokyo, Japan), repeated up to four times at an interval of 5 min. FEV₁ was measured in order to detect bronchoconstriction immediately after each nebulization. The subjects were asked to rinse their mouths and encouraged to spit the sputum into a petri dish. The more dense and opaque portions of the expectorate were collected and treated with 4% solution of the mucolytic agent dithiothreitol. Then, total cell and differential cell counts were determined by counting $>$ 300 non-squamous cells. Statistical analyses were performed using Mann-Whitney non-parametric testing, and *P* values of ≤ 0.05 were considered to be significant. As the distribution of PC20

Table 1. Characteristics of enrolled patients at initial evaluations

Patient	Sex/age	Smoking (PY*)	Exposure to Sx. [†]	Sx. to Dx. [‡]	Atopy	PC20 [§]	FEV ₁ % [¶]
1	M/35	Current (15)	4.9	1.1	—	3.18	100
2	M/52	Current (30)	18.6	2.0	+	12.52	108
3	M/35	Current (5)	4.2	1.4	—	3.07	91
4	M/43	Current (3)	0.6	7.4	+	1.25	66
5	M/44	Current (5)	15.0	5.7	—	9.50	79
6	M/38	Former (15)	11.3	7.2	+	8.05	79
7	M/47	Current (30)	19.8	4.0	—	1.01	61
8	M/47	Current (15)	0.4	4.9	+	1.57	68
9	M/33	Former (5)	11.2	4.6	—	0.15	73
10	M/51	Current (20)	0.6	7.3	—	14.80	65
11	M/53	Current (10)	21.8	5.0	+	0.27	56

*Pack years.

[†]Duration from the onset of exposure to the development of symptoms (years).

[‡]Duration from the development of symptoms to the diagnosis (years).

[§]Methacholine-PC20 (mg/mL).

[¶]FEV₁ % predicted value.

Table 2. Changes in skin reactivity to reactive dyes

Patient	Skin prick test (A/H ratio*)				Second examinations				Bronchoprovocation test initial examinations	
	Initial examinations (diagnosis)									
	Yellow HE 4R	Orange 3R	Black B	Black SF-GR	Yellow HE 4R	Orange 3R	Black B	Black SF-GR	Allergen	Reaction
1	ND	1.67	2.22	1.00	0.00	0.00	0.00	1.00	Black B	Dual
2	ND	2.21	1.00	0.63	0.00	0.00	0.80	0.80	Orange 3R	Early
3	0.00	1.44	1.67	1.66	0.00	0.00	0.00	0.00	Black SF-GR	Early
4	0.91	1.32	2.36	2.64	0.00	0.00	0.00	0.00	Black SF-GR	Early
5	0.00	0.00	0.00	1.33	0.00	0.00	0.00	1.00	Black SF-GR	Early
6	0.00	0.00	1.31	1.00	0.00	0.00	0.00	0.00	Black B	Dual
7	0.00	0.00	0.00	1.55	0.00	0.00	0.00	0.00	Black SF-GR	Dual
8	0.00	0.00	0.67	1.38	0.00	0.00	0.00	0.00	Black SF-GR	Early
9	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	Black B	Dual
10	0.00	0.00	0.00	1.71	0.00	0.00	0.00	0.00	Black SF-GR	Dual
11	0.00	0.83	0.69	1.00	0.00	0.71	0.00	0.00	Black SF-GR	Dual

ND, Not done.

*Wheal of allergen/wheal of histamine ratio.

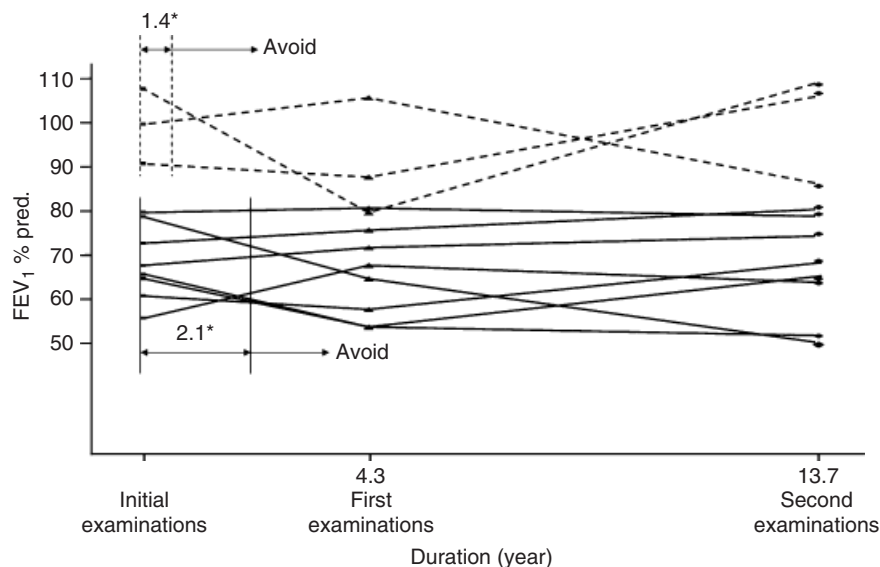


Fig. 1. Changes in forced expiratory volume in 1 s (FEV_1) % predicted values. *Mean duration from the diagnosis to complete removal from exposure (years). Dotted line: Patients with normal FEV_1 % predicted values ($\geq 80\%$) at initial examinations (Group 1). Solid line: patients with reduced FEV_1 % predicted values ($< 80\%$) at initial examinations (Group 2). Mean of FEV_1 % predicted values was not significantly different between initial examinations and the first follow-up examinations or between the first and second follow-up examinations for patients in Groups 1 and 2.

was highly skewed, the findings were log10 transformed for statistical analysis.

Results

Three patients had a normal FEV_1 % predicted value ($\geq 80\%$; Group 1) and their disease durations from symptom onset to diagnosis were significantly shorter than those of patients with a reduced FEV_1 % predicted value ($< 80\%$; Group 2; 1.5 ± 0.5 vs. 5.7 ± 1.4 years; $P = 0.012$). However, durations from exposure to symptom onset did

not significantly differ for these two groups (9.3 ± 8.1 vs. 10.0 ± 8.8 years; $P > 0.05$).

Eventually, all patients were removed from exposure to RD. The mean duration from diagnosis to complete removal from exposure was 2.1 ± 3.0 years, and this was shorter in Group 1 but not significantly so (1.4 ± 2.3 vs. 2.1 ± 3.0 years; $P > 0.05$). The serial changes in FEV_1 % predicted values are shown in Fig. 1. No significant improvement was observed, despite the fact that patients were completely removed from exposure to RD over a 10-year period. Moreover, patients in Group 2 persistently

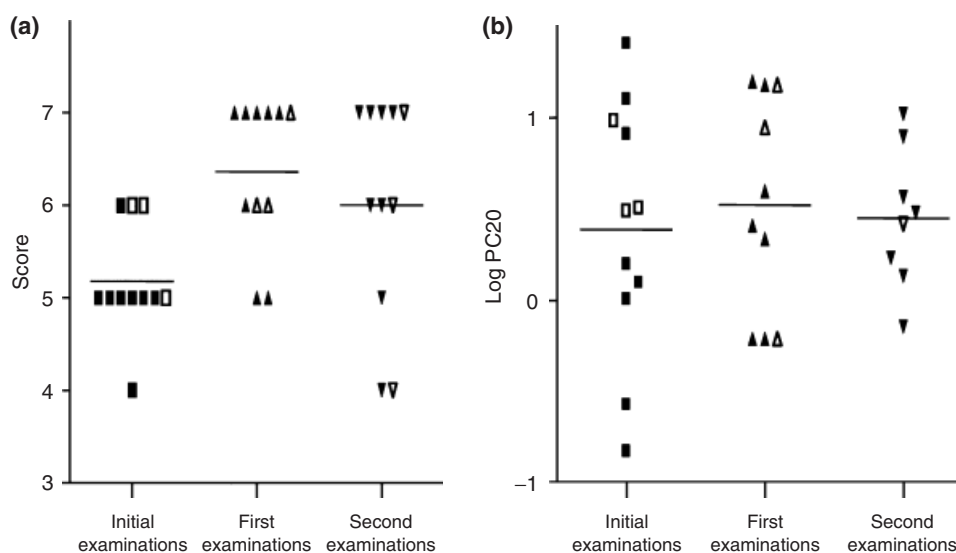


Fig. 2. Changes in sum of symptoms and medication scores and methacholine-PC20. (a) Sum of symptoms and medication scores. (b) Methacholine-PC20. Open symbols: patients with normal forced expiratory volume in 1 s (FEV₁) % predicted values ($\geq 80\%$) at initial examinations. Closed symbols: patients with reduced FEV₁ % predicted values ($< 80\%$) at initial examinations. Horizontal bars: mean values (geometric mean values for PC20). No significant changes were found between initial examinations and the first follow-up examinations or between the first and second follow-up examinations.

Table 3. Inflammatory cells in induced sputum and medications at second follow up examinations

Patient	Sputum eosinophil (%)	Sputum neutrophil (%)	Current medication
1	7.33	21.67	High-dose ICS*, theophylline, LTRA [†]
2	4.33	22.67	High-dose ICS, oral steroid, LTRA
3	1.33	16.33	High-dose ICS, oral steroid, theophylline, LTRA
4	1.33	11.00	High-dose ICS, oral steroid, theophylline, LTRA
5	2.00	27.00	High-dose ICS, theophylline
6	3.00	21.33	High-dose ICS, oral steroid, theophylline, LTRA
7	3.67	17.67	High-dose ICS, oral steroid, theophylline
8	3.67	9.00	High-dose ICS, theophylline, LTRA
9	5.67	16.33	High-dose ICS
10	3.33	35.00	High-dose ICS, oral steroid, theophylline, LTRA
11	1.00	9.00	High-dose ICS, oral steroid, theophylline, LTRA

*Inhaled corticosteroid (budesonide ≥ 800 $\mu\text{g/day}$).

[†]Leukotriene receptor antagonist.

showed lower FEV₁ % predicted values, and significant differences in FEV₁ % predicted value between Groups 1 and 2 persisted at the second follow-up examination. No significant changes in geometric mean of PC20-methacholine were observed during the study period, although negative conversions of PC20-methacholine (> 16 mg/mL) were found in two patients in Group 1 and one in

Group 2 at second examinations (Fig. 2). Contrary to our expectations, the sum of symptoms and medication scores did not decrease despite exposure avoidance (Fig. 2). Skin reactivity to RD had become almost undetectable in all patients at the second examinations (Table 2). Interestingly, four of the six patients who were non-atopic (negative skin response to any of the 11 common inhalant allergens) at initial examinations were found to be atopic at the second examinations. In terms of airway inflammation, seven of the 11 patients showed eosinophilia in induced sputum ($\geq 3\%$) at the second examinations, despite having been on high-dose inhaled corticosteroid since diagnosis (Table 3).

Discussion

This long-term follow-up study of OA caused by RD shows that lung function attenuations observed at initial examinations (diagnosis) did not recover despite exposure cessation and proper pharmacological treatment. Moreover, asthma severity and non-specific AHR, as determined by symptoms and medication status and by PC20-methacholine, respectively, did not improve over a period of 10 years, although skin reactivity to RD had nearly disappeared over this time-frame. To the best of our knowledge, this is the first report to present the long-term outcome of RD-induced OA.

So far, conflicting findings have been reported on whether the total duration of removal from exposure [15, 16] and the type of causal agent (high-molecular-weight agent [17, 18] or low-molecular-weight agent [11, 14]) are

prognostic factors for the outcome of OA. On the contrary, airway inflammation and structural changes in the airways are thought to have prognostic implications. A historical study conducted by Chan-Yeung et al. [19] showed higher numbers of inflammatory cells (mainly eosinophils and neutrophils) in bronchoalveolar lavage-fluid of patients with occupational asthma caused by western red cedar after removal from exposure for at least 1 year. In addition, recently, it was reported that airway inflammations measured from induced sputum were not different between patients with OA caused by high- and low-molecular-weight agents [20], which implied that these findings may be a common feature of persisting occupational asthma. Likewise, seven of the 11 patients showed eosinophilia in induced sputum ($\geq 3\%$) despite high-dose inhaled corticosteroid medication over a period of 10 years after diagnosis in the present study. One possible explanation for the persistence of airway inflammation may be that patients with OA become sensitized to a ubiquitous allergen. It was reported that some subjects, especially atopics, could develop new sensitizations after the onset of asthma symptoms, favoured by increased permeability of airway epithelium and by the particular inflammatory milieu [21], which was also observed in patients with occupational asthma [22]. In accordance with this, four of the six patients in the present study who were non-atopic at diagnosis became newly sensitized to common inhalant allergens at the second examinations. However, it should be noted that we did not characterize airway inflammation severity at initial examinations and thus further study is needed to verify the role of airway inflammation in OA. Previously, we observed features of airway remodelling in a bronchoscopic biopsy specimen of RD-induced asthmatics with mean symptom durations of 2.3 ± 0.4 years, such as loss of epithelium, basement membrane thickening, submucosal oedema and increased inflammatory cells [23], which is consistent with findings in TDI-induced asthmatics [12]. Durations from symptom onset to diagnosis of patients with reduced FEV₁ predicted values in the present study were 5.7 ± 1.3 years and thus pathologic changes in bronchial mucosa observed in our previous study [23] might exist at diagnosis. A persistent reduction in lung function, in part, can be attributed to such pre-existing airway remodelling.

The finding in the present study that the duration from symptom onset to diagnosis was the only factor differentiating patients with normal and reduced lung functions at diagnosis suggests that early diagnosis and treatment are essentially required for a good prognosis, as Ameille and Descatha [24] previously described in their review article. In Korea, primarily, occupation-related health problems are screened through the periodic health examinations regulated by the Labor Standard Law and The Industrial Safety and Health Law [25]. However, cases of OA were rarely found. This is mainly attributable to the

fact that the routine health examinations for workers were too simple to make a definite diagnosis of asthma and to disclose the causal relationship between symptoms and occupations [26]. Therefore, workers did not consult with asthma specialists until their symptoms became serious, which, in part, explains the long time to diagnosis observed in this study. To overcome this, the Occupational Asthma Surveillance Center was established in Korea as a non-official agency and steadily encouraged asthma specialists and pulmonary physicians to provide medical care in cooperation with official health managers and to report a new case of OA [26].

In conclusion, in the present study, we found that pulmonary function reductions and severe asthmatic symptoms persisted in RD-induced OA even 10 years after exposure cessation. Despite the small number of subjects enrolled in this study, we are convinced that those observations may add appreciably to our understanding of the natural course of RD-induced OA.

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