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Occupational Asthma and IgE Antibodies to Reactive Dyes

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Reactive dyes have been widely used in recent years. This paper reports nine cases of immediate type occupational asthma to reactive dyes in one dye industry. All patients had had asthmatic symptoms, four had had rhinitis and they had worked for 6 to 25 months. Skin prick tests with reactive dyes were positive and bronchoprovocation tests also produced immeidate or dual types of bronchoconstriction. We used the radioallergosorbent test (RAST) technique with nitrocellulose filter paper as a solid phase to detect specific IgE to four reactive dye- human serum albumin conjugates. High specific IgE binding was found in eight asthmatic workers compared with 13 negative controls. The RAST inhibition test revealed that there was no immunological cross-reactivity between 4 reactive dyes. These results suggested that the mechanism of their asthmatic symptoms was immunological, mostly an IgE-mediate reaction.

Key Words: Occupational asthma, reactive dyes, specific IgE

Since the first reactive dye for cotton was marketed in 1956 by Imperial Chemical Industries Ltd (ICI, USA), reactive dyes have been increasingly used to bind covalently to textiles as coloring agents in the textile industry.

A few studies have been performed about a type of occupational asthma caused by reactive dyes. Recently, Luczynska and Topping (1986) observed a specific IgE to reactive dye using the radioallergosorbent test (RAST) with reactive dye conjugate discs. Docker *et al.* (1987) reported 21 cases of occupational asthma associated with dye exposure among over 400 workers handling reactive dyes.

In this study, we report the immediate type of sensitization to four different reactive dyes in 9 asthmatic patients working at one dye industry and the cross-reacting allergenicity between the four reactive dyes is discussed.

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MATERIALS AND METHODS

Patients

Nine patients, all men aged between 32 to 46 years, were dye process workers in the same industry located in Incheon, Korea. None had a previous history of medical or atopic diseases. They all complained of asthmatic symptoms following various lengths of exposure to reactive dyes.

Sera

Sera from the 9 patients were collected and stored

Table 1. The specific IgE binding on nitrocellulose filter paper discs (bound %) according to various concentrations of Black GR

	Black GR Concentration (mg/ml)		
	0.01	0.1	1.0
Patient CH	4.04	13.38	12.97
Negative control I	0.79	0.78	1.77
Negative control II	0.89	0.78	1.28

Negative control: Individuals who had never been exposed to reactive dyes

at −20°C. Sera from individuals who showed a negative response on skin prick tests with 50 common inhalant allergens and who had never been exposed to reactive dyes were pooled and used as negative controls.

Preparation of dye solution for skin prick test

Four kinds of reactive dyes were presented by the dye industry, Their trade names were Black GR (color index: BK 5), Red BBN, Orange GR and Yellow GR. Three of them are not listed in the color index and the structures of these three dyes are shown in Fig. 1. Ten mg of four reactive dyes were dissolved in one ml of modified Coca solution (NaCl 9 gm, phenol crystal 4 gm, NaHCO3, 2.9 gm in 1000 ml of distilled water; Phillips 1967) containing 50% sterile glycerine and used for skin prick tests.

Preparation of dye-human serum albumin (HSA) conjugate discs

A solution of adequate amounts of reactive dyes in 100 mM Na2C03 (pH 11.0) was prepared. Twently mg of HSA (Sigma Chemical Co., St Louis, MO, USA) was added to 10 ml of each dye solution. This was incubated with stirring at 4°C for 18 hours and dialysed against 100 mM Na2C03, with four changes, at 4°C for 48 hours. A similar technique was used to prepare a solution of HSA alone without reactive dyes. This was termed sham HSA. The discs of nitrocellulose filter paper (NFP; Millipore, Cat. No. HAHY 08250, 0.45μm, Millipore Co., Beford, Massachusetts, USA) were immersed into this dye-HSA conjugate solution for 72 hours at 4°C. Then the discs were dried at room temperature and used as a solid phase in the subsequent RAST assay.

To observe the optimal concentration of dye-HSA conjugate on NFP, various concentrations of Black GR-HSA conjugate solution were prepared as in Table 1. The highest specific IgE bindings with minimal non-specific binding were noted at 0.1mg Black GR-2mg/ml HSA conjugate. We determined that 0.1mg Black GR-2mg/ml HSA conjugate was the optimal concentration and the other three reactive dye-HSA conjugate discs were also prepared similarly to above.

RAST assay

The dried discs were blocked with 10% newborn calf serum for 1 hour and incubated with 50 μl of patient sera for 6 hours at room temperature. The discs were washed three times with 2.5 ml of 0.9% NaCl containing RAST washing solution additive (Pharmacia, Uppsala, Sweden). Fifty μl of 125 I-labelled anti-human IgE (Pharmacia, Uppsala, Sweden) were added and left for 18 hours at room temperature. The discs were washed again with washing solution and the bound 125-I was measured using a gamma counter (Packard). All assays were performed in duplicate. The results were expressed as RAST percent binding, defined as percentage of added counts per minute (cpm) bound to the reactive dye-HSA conjugate disc.

Measurement of total IgE

Total IgE was measured using a Phadebas paper radioiommunosorbent test (PRIST) kit (Pharmacia) ac-

Table 2. Patient characteristics

Patients	Sex/Age	Cigarette smoker	Exposure duration (M)	PC_{20} methacholine (mg/ml)	Atopy	Total IgE (IU/ml)
CH	M/32	Current	16	1.0	−	23.9
PH	M/27	Former	16	1.45	+	388.7
SE	M/36	Former	6	3.3	−	6625.3
PK	M/32	Former	24	1.0	+	605.0
UC	M/46	Current	25	3.7	+	206.4
PS	M/34	Former	25	0.49	−	153.8
KI	M/35	Current	19	2.3	−	107.3
KS	M/44	Former	20	0.53	−	8022.6
KA	M/34	Current	12	5.3	−	1374.9

PC_{20} : Provocation concentration of methacholine required to reduce FEV_1 20% below baseline
Atopy : Patient who showed greater than 2+ response to more than one antigens on skin prick test with 9 common inhalant allergens
M : Months

cording to the manufacturer's directions. One anti-IgE disc was added to the bottom of each tube and incubated with 100 μl of diluted serum at room temperature for 3 hours. The discs were washed three times with 2.5ml of 0.9% NaCl containing PRIST washing additives. One hundred μl of anti-IgE-I 125 PRIST tracer was then incubated for 18 hours at room temperature and rinsed three times. The binding radioactivities were determined using a gamma counter, and absolute amounts of total IgE were determined using a standard curve.

RAST inhibition with reactive dyes

Competitive RAST inhibition was used to investigate the relationship among the 4 reactive dyes. Sera of patients who showed high IgE binding (>10% bound radioactivity) on Black GR-conjugate discs in the previous experiments were pooled. Fifty μl of this pooled sera was incubated with various concentrations (0.0025-0.1 mg/ml) of 4 reactive dye-HSA conjugates for 1 hour at room temperature. The inhibited sera pool was incubated with Black GR-conjugate discs for 6 hours at room temperature. After washing three times with 2.5 ml of 0.9% NaCl containing RAST washing additives, 50 μl of 125I-labelled anti-human IgE (Pharmacia) was incubated with the discs for 18 hours at room temperature. After the repeated washing step, the bound radioactivities were determined using a gamma counter (Packard). The inhibition of the specific IgE binding to allergen discs was calculated by comparing to control samples in which an equal volume of phosphate buffered saline (PBS, pH 7.5) was preincubated instead of inhibitors, and expressed as percent inhibition as follows:

$$100 - \frac{\text{cpm bound to dye-HSA disc with inhibitor}}{\text{counts per minute (cpm) bound to dye-HSA disc without inhibitors}} \times 100$$

Allergy skin test

Skin prick tests with Bencard's 9 common inhalant allergens (alder, oak, Bermuda grass, Timothy grass, ragweed, wormwood, Aspergillus, Dermatophagoides farinae, cat fur), 4 reactive dye solutions (10mg/ml) and histamine (1mg/ml, Bencard, U.K) were performed on the volar side of both forearms simultaneously. The results were read at 15 minutes after the prick. The wheal size to each antigen (A) and histamine (H) was measured as maximum diameter (Al and H1) and vertical length at the mid-protion of maximal length (A2 and H2). Skin reactivity was expressed as the ratio of wheal size of antigens to histamine $(A1+A2/H1+H2)^2$

Methacholire bronchial challenge test

Nonspecific bronchial hyperreactivity was determined by the previously described standard method (Chai et al. 1975). An aerosol of 0.9% NaCl, followed by doubled concentrations of methacholine (0.075 to 25.0 mg/ml), was inhaled. The forced expiratory volume in one second (FEVI) was measured 5 minutes after each inhalation and continued until FEVI had fallen by 20% (calculated from the post-saline value). The provocative concentration of methacholine required for a 20% decrease in FEVI (PC 20) was obtained from the dose-response curve.

Bronchoprovocation test with reactive dyes

Bronchoprovocation tests were performed according to the modified Chai's method (1975) using three kinds of reactive dye solution which were prepared by dissolving each reactive dye in 0.4% phenolized saline. The antigen reactive dye solution for the bronchoprovocation test was selected for each patient after a personal interview. The FEVI and maximum mid-expiratory flow (MMEF) were measured with a spirometer (HI 298, Japan) before inhalation and 10 minutes after inhalation. The test solutions were delivered by a Vaponefrine nebulizer (Meico Co., Japan) and compressed air source. The patients were asked to breathe the nebulized aerosol 5 times until one's vital capacity was achieved. A 0.4% phenolized saline solution was inhaled for a baseline value and serial increments in antigen concentration (0.01, 0.1, 1.0, 2.5 mg/ml) were given at 10 minute intervals until a 20% or greater decrease in FEVI from the baseline value was recorded. The FEVI and MMEF were mesured freqently during the first hour and then the pulmonary function test was performed every 9 or 10 hours after the challenge.

RESULTS

Patient characteristics

Table 2 illustrates the clinical data of the patients in the study. All workers had a smoking history and in all cases except patient KA, their methacholine PC_{20} was less than 4.66 mg/ml, which is the asthmatic range of methacholine PC_{20} as determined in our previous study (Park *et al.* 1989). The length of exposue was 6 to 25 months. There were three atopic patients who were determined as positive reactors (≥2+) to one or more allergens on skin prick test with 9 common

Table 3. Results of skin prick test and RAST to 4 reactive dyes

Patients	Skin prick test (A/H ratio)				RAST (bound %)				
	Black-GR	Red-BBN	Orange-GR	Yellow-GR	Black-GR	Red-BBN	Orange-GR	Yellow-GR	Sham-HSA
CH	2.3	0	1.8	0	1.0	0.5	0.9	0.5	0.68
PH	0.7	0	0	0	3.0	0.7	1.0	0.7	0.65
SE	1.0	0.6	0.6	0	31.4	14.0	32.1	6.1	0.70
PK	2.6	1.1	0	0	13.5	2.7	4.0	1.7	1.7
UC	1.3	0	0.5	0	23.7	3.2	7.9	1.9	0.56
PS	2.4	0	0	0	2.6	0.6	0.7	0.5	0.79
KL	1.5	0	0	0	2.8	0.5	1.1	0.7	0.65
KS	1.1	0.3	0.7	0	3.9	1.4	3.6	1.1	0.70
KA	1.2	1.6	0.6	0	11.9	2.2	10.8	1.5	0.70
Negative pool	ND	ND	ND	ND	0.58	0.46	1.0	0.44	0.68

A/H ratio; The ratio of allergen to histamine reaction

Negative pool; Sera pool from unexposed individuals who visited our allergy clinic

ND: Not done

Table 4. Results of bronchoprovocation test at the workplace and clinic

Patients	Workplace			Clinic	
	Baseline FEV_1 (ml)	Immediate Bronchoconstriction (ml)	Induced symptoms	Antigen	Asthmatic Response
CH	4140	3290	Dyspnea, wheezing sneezing, rhinorrhea	Black-GR	Dual
PH	3250	2540	Dyspnea, cough	Orange-GR	Early
SE	3650	1980	Dyspnea, wheezing rhinorrhea	Red-BBN	Early
PK	3130	1120	Dyspnea, cough wheezing, rhinorrhea	Black-GR	Dual
UC	4440	3490	Dyspnea, sneezing rhinorrhea	Orange-GR	Dual
PS	3250	2260	Dyspnea, abdominal pain	Black-GR	Dual
KL	2930	2100	Dyspnea, cough	Black-GR	Early
KS	ND	ND	ND	Red-BBN	Dual
KA	3450	3450	Cough	Orange-GR	Early

ND: Not done

inhalant allergens. Their total IgE level was variable. Three patients showed a level higher than 1000 IU/ml.

Skin prick test and specific IgE antibodies to reactive dyes.

Table 3 illustrates the results of the skin prick test and specific IgE antibodies to 4 reactive dyes expressed as % binding. Black GR was the most frequent sensitizer on skin prick test. Four workers showed immediate wheal and erythema formation to orange GR and all of them showed concurrent wheal reaction with Black GR. The mean value (%) of Black GR-RAST in 13 negative controls was 0.64 and its standand deviation was 0.20. The bound radioactivities of sham-HSA discs were less than 1% except in one case (Patient CH; 1.7%). When the cut-off value for positive specific IgE antibody for Black GR and other reactive dyes was decided as 2%, eight patients showed positive specific IgE antibodies to Black GR-HSA conjugate, 5

patients to orange GR-HSA conjugate, 4 patients to Red BBN and 1 patient to Yellow GR.

Bronchoprovocation test with reactive dyes

Seven workers developed asthmatic attacks during their usual work, as shown in Table 4. As patient KS was admitted to a hospital due to asthmatic symptoms, the bronchoprovocation test at the workplace could not be performed. All nine workers showed immediate or dual types of bronchoconstriction after the inhalation of reactive dye solution to which they were exposed at the Allergy Clinic as shown in Table 4.

RAST inhibition

Black GR and orange GR RAST inhibition tests were performed with four reactive dye-HSA conjugates as inhibitors. Fig. 2 illustrates the percent inhibition of Black GR RAST with the addition of various concentrations of 4 dye-HSA conjugates and HSA alone. Black GR was the most effective inhibitor and the percent inhibition by three other dye-conjugates was minimal. There was no inhibition by HSA alone.

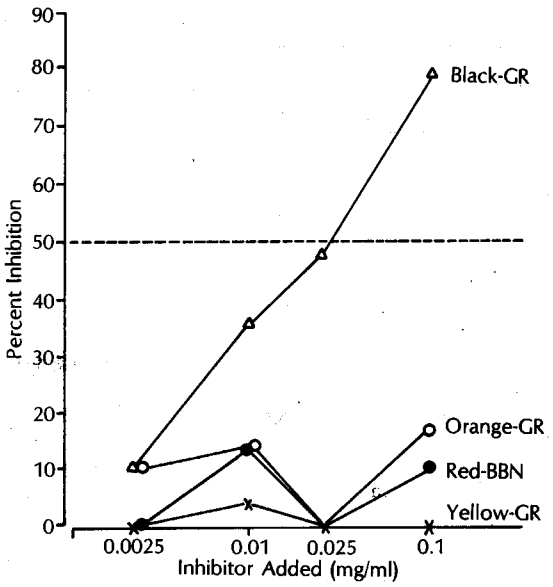
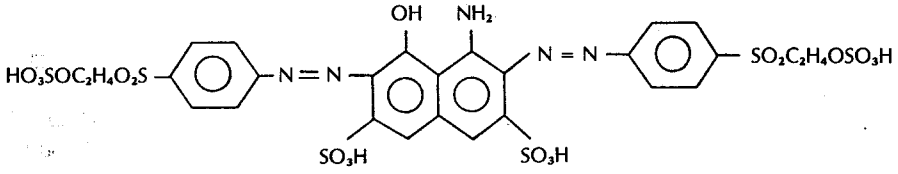
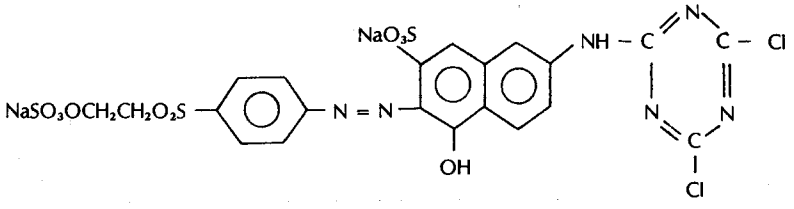


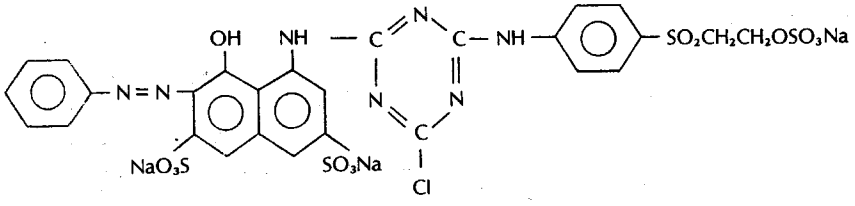
Fig. 2. *Percent inhibition of Black GR RAST with serial addition of 4 reactive dye-HSA conjugates.*



Black-GR



Orange-GR



Red-BBN

Fig. 1. *The structure of reactive dyes in the study.*

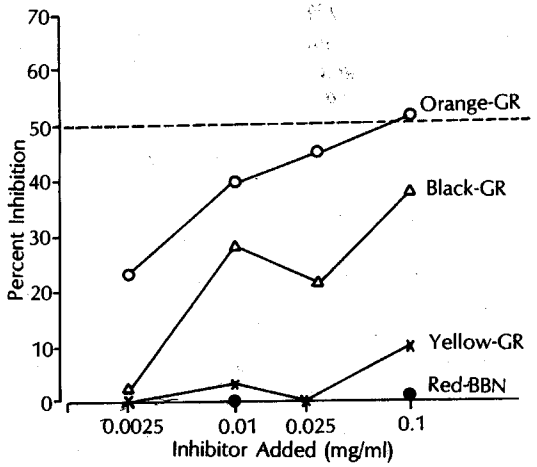


Fig. 3. Percent inhibition of Orange GR RAST with serial addition of 4 reactive dye-HSA conjugates.

Fig. 3 illustrates the percent inhibition of Orange GR RAST with various concentrations of 4 dye-HSA conjugates and HSA alone. Orange GR was the most effective inhibitior. Some inhibition was noted by Black GR, but no inhibitions by Yellow GR, Red-BBN and HSA alone were noted.

DISCUSSION

We have demonstrated significant clinical respiratory and immunologic responses in nine dye workers exposed to reactive dyes.

A few studies have documented immunologic pulmonary diseases resulting from reactive dye exposure. Docker *et al.* (1987) reported that the reactive dyes were major allergenic sources of exposure in the dye house and were implicated as causative agents in 21 employees with allergic reaction associated with dye exposure. Specific IgE antibodies to dye-HSA conjugates were demonstrated by Luczynska and Topping (1986) in 9 individuals with respiratory symptoms after dye exposure and RAST inhibition studies revealed that the antibody was mainly directed towards the hapten portion of the conjugate. In this study, in order to detect specific IgE antibodies to dye-HSA conjugate, we used the NFP disc as a solid phase and anti-IgE RAST tracer as a detecting antibody. The optimum antigen concentration, 0.1mg dye in 2mg/ml HSA, was the same ratio of dye to carrier protein as that used by Luczynska and Topping (1986). To prepare the solid phase for the dye-HSA conjugate, the immersion method of NFP discs we used was though to be simple and effective for RAST technique.

Some occupational agents that cause asthma are thought to do so through allergic mechanism involving specific IgE antibodies (Zeiss *et al.* 1980; Chan-Yeung 1982; Baur *et al.* 1984; Dykewitz and Patterson 1987). Current evidence suggested that many compounds of low molecular weight (less than 1000 daltons) act as haptens and must be conjugated to host proteins to generate immune responses (Zeiss *et al.* 1977; Karol *et al.* 1978; Cromwell *et al.* 1979; Patterson *et al.* 1979; Zammit-Tabona *et al.* 1983). In this study, six workers who showed positive results on bronchoprovocation test had specific IgE antibodies. However, patient CH, although he showed a dual type of bronchoconstriction after the inhalation of Black GR solution, had no specific IgE antibody. A case (PH) showed positive results on bronchoprovocation with orange GR but negative results on skin prick test and RAST to orange GR. A case (KS) showed positive results on bronchoprovocation with Red BBN but negative results on Red BBN RAST. Although we have to evaluate the sensitivity of reactive dye-RAST, in these three cases where specific IgE antibodies to offending dyes were not demonstrated, another mechanism such as a pharmacologic or other immunologic mechanism has been suggested to have a role in the pathogenesis of their asthma (Akiyama *et al.* 1984). Liss *et al.* (1988) noted that high total antibody binding against methylene diphenyldiisocyanate in exposed workers reflected a polyclonal humoral response that represented predominantly specific IgG. Although much less is known about the significance of IgG4 reaginic reactions in occupatonal asthma, it might be possible that this alternate method of allergenic sensitization could occur in situations where clinical sensitivity is suspected but specific IgE antibodies cannot be detected. Placebo and control periods are needed to rule out cases due to nonspecific or toxic irritative effects and further studies are needed to clarify the combination effect of allergenic substances and irritant vapours from chemical used in the dye process such as sulphur dioxide and hydrochloric acid.

Among the four reactive dyes, specific IgE antibodies to Black GR determined by skin prick test and RAST were most frequent. High specific IgE binding to Yellow GR was found in one case (Patient SE), although there was a negative response on skin prick test (Table 3). A patient (PK) also showed a negative response on skin prick test with Orange GR but high IgE binding to Orange GR. These differences might be because the skin prick tests were evoked by unconjugated dye.

In vitro cross-reactivity between 4 reactive dyes was observed in this study. We used RAST inhibition

studies with various concentrations of reactive dye-conjugates as inhibitors. Highest inhibition on Black GR-HSA conjugate discs was obtained with Black GR conjugate and minimal inhibitions were obtained with other dye-HSA conjugates, as shown in Fig. 2. HSA alone gave no inhibition. Fig. 3. illustrates that highest inhibition of Orange GR-HSA conjugate disc was noted with Orange GR-HSA conjugate and some inhibitions were noted with Black GR-HSA conjugate. These results might have occured because Black GR is known to contain 20% Orange-GR. It is suggested that there is no immunological cross-reactivity between four reactive dyes produced in that industry except the Black GR response on Orange GR discs, but further investigations are needed to provide information about the immunochemistry of these reactive dyes.

In conclusion, reactive dyes, which are biologically reactive chemicals, induced IgE mediate responses tested by skin prick tests and RAST in nine exposed workers. Most of their asthmatic symptoms were mediated by reaginic antibodies, but non-immunologic mechanisms were also suggested.

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