Yonsei Medical Journal
Vol. 31, No. 1, 1990

□ Original Article □

# Reactive Dye Induced Occupational Asthma without Nonspecific Bronchial Hyperreactivity

**Hae Sim Park[1], Mi Kyung Lee and Chein-Soo Hong**

*Current asthma is often excluded by the presence of normal bronchial hyperresponsiveness. We report two asthmatic patients with normal bronchial hyperresponsiveness and one asthmatic patient with mild bronchial hyperresponsiveness (methacholine $PC_{20}$; 24 mg/ml) which was presumed to be caused by sensitization and exposure to Black GR, the most frequent sensitizer among reactive dyes. They all complaiend of lower respiratory symptoms after work as well as at the workstation. The bronchoprovocation test with Black GR revealed isolated immediate bronchoconstrictions in all 3 patients and all had high specific IgE antibodies to Black GR-human serum albumin conjugate. After one worker continued at work for 3 days, he experienced a marked drop of methacholine $PC_{20}$, and it returned to the pre-exposure level during 1 week. The other patient whose initial methacholine challenge was negative developed bronchial hyperresponsiveness on the first day after the dye bronchoprovocation, and returned to normal bronchial hyperresponsiveness on the third day. These findings suggested that patients with occupational asthma caused by reactive dye may not always have bronchial hyperresponsiveness to methacholine, and the screening program utilizing methacholine challenges may not always identify these patients.*

**Key Words:** Occupational asthma, reactive dye, bronchial hyperresponsiveness

Occupational asthma has been defined as variable airway narrowing causally related to exposure in the working environment to airborne dusts, gases, vapours or fumes (Newman-Taylor 1980). The majority of patients with symptomatic occupational asthma have demonstrable non-specific bronchial hyperreactivity (Lam *et al.* 1979). It is unclear at present whether it is the result of occupational exposure or a predisposing factor.

Some investigators (Hargreave *et al.* 1980; Smith *et al.* 1980; Stanescu and Frans 1982) reported several cases of occupational asthma caused by toluene diisocyanate (TDI) without nonspecific bronchial hyperreactivity. Our previous report described 2 of 20 asthmatic workers employed in dye industries who had a positive response on Black GR-bronchoprovocation test, but negative methacholine challenges.

This study describes our experience with three sensitized workers who demonstrated negative methacholine challenges and one worker with mild nonspecific bronchial hyperreactivity, but significant bronchoconstrictions on the bronchoprovocation test with Black GR, one of the most frequent sensitizers among reactive dyes according to our recent studies (Park *et al.* 1989; Park *et al.* 1989). And also, the changes of nonspecific bronchial hyperreactivity with exposure and avoidance to causative dye were observed.

Received November 14, 1989
Accepted December 15, 1989

Department of Internal Medicine, Yonsei University College of Medicine Seoul, Korea
Department of Chest Medicine[1], National Medical Center Jungku Eulgiro 6-ka 18-79, Seoul, Korea
Address reprint requests to Dr. C-S Hong, Department of Internal Medicine, Yonsei University College of Medicine, C.P.O. Box 8044, Seoul, Korea, 120-752

## MATERIALS AND METHODS

### Specific IgE to Black GR-Human serum albumin (HSA) conjugate by RAST assay

The Black GR-HSA conjugate discs were prepared and used to detect the specific IgE according to our previous studies (Park *et al.* 1989; Park *et al.* 1989) as follows: the dried discs were blocked with 10% newborn calf serum for 1 hour, and incubated with 50 μl of patient serum for 6 hours at room temperature. After a washing step, 50 μl of $^{125}I$ labelled anti-human IgE (Pharmacia, Uppsala, Sweden) was added and left for 18 hours at room temperature. After a repeated washing step, the bound radioactivity was

measured using a gamma counter (Packard). The results were expressed as RAST percent bindings, defined as a percentage of added counts per minute (cpm) bound to a reactive dye-HSA conjugate disc.

**Methacholine bronchial challenge test**

The nonspecific bronchial hyperreactivity was determined by the previously described method (Chai *et al.* 1975). An aerosol of 0.9% NaCl, followed by doubled concentrations of methacholine (0.075 to 25 mg/ml) was inhaled. The forced expiratory volume in one second (FEV1) was measured 5 minutes after each inhalation and continued until FEV1 had fallen by 20% (calculated from the post-saline value). The provocative concentration of methacholine required for a 20% decrease in FEV1 ($PC_{20}$) was obtained from the dose-response curve.

**Bronchoprovocation test with Black GR**

The bronchoprovocation test was performed according to our previous studies (Park *et al.* 1989; Park *et al.* 1989) using Black GR solution which was prepared by dissolving Black GR in 0.4% phenolized saline. The FEV1 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 (Meiko Co., Japan) and a compressed air source. The subjects were asked to breathe the nebulized aerosol 5 times until their vital capacity was achieved. A phenolized saline solution was inhaled for a baseline value, and serial increments in antigen concentrations (0.01, 0.1, 1.0, 2.5 mg/ml) were given at 10 minute intervals until a 20% or greater decrease in FEV1 from the baseline value was recorded. The FEV1 and MMEF were measured frequently during the first hour, and then a pulmonary function test was performed every 9 or 10 hours after the challenge.

## SUMMARY OF CASES

Three workers employed in two dye industries located in Incheon, Korea were selected and the clinical and laboratory findings are summarized in Table 1.

Worker MY was a 38-year-old smoking man with no past or family history of atopy and asthma. For the previous 47 months he had worked at the dye industry. After 36 months, he began to experience shortness of breath and coughing with dye exposure. The chest PA was within normal limits and the initial methacholine bronchial challenge test was negative at that time. A skin prick test with Black GR revealed a strong positive response (7+), and the specific IgE level to Black GR-HSA measured by RAST was 9.0% of bound radioactivity. A bronchoprovocation test with Black GR demonstrated an isolated immediate bronchoconstriction after the inhalation of a 2.5 mg/ml Black GR solution. The following methacholine challenges revealed that nonspecific bronchial hyperreactivity developed 24 hours after the bronchoprovocation test, and returned to the normal range on the third day, as shown in Fig. 1.

Worker CY was a 32-year-old male working in another dye industry. He had worked for 20 months and was also a smoker. He showed a significant positive response (4+) on a skin prick test with Black GR, and the specific IgE level was high (30% of bound radioactivity). The methacholine bronchial challenge test showed a negative response, and the bronchoprovocation test with Black GR showed early bronchoconstriction after inhalation of the 2.5 mg/ml Black

**Table 1. Clinical and laboratory data of patients studied**

| Patients | Sex/Age (yrs) | Exposure Duration (M) | Atopy* | Methacholine $PC_{20}$ (mg/ml) | Skin reactivity to Black GR (A/H ratio) | Specific IgE to Black GR (bound %) | Bronchoprovocation test with Black GR |
|---|---|---|---|---|---|---|---|
| MY | M/38 | 47 | – | Negative | 7+ | 9 | Early response at 2.5 mg/ml |
| CY | M/32 | 20 | + | Negative | 4+ | 30 | Early response at 2.5 mg/ml |
| KM | M/30 | 20 | – | 24.0 | 2+ | 3 | Early response at 1.0 mg/ml |

Atopy* was defined as a positive responder to one or more antigens on a skin prick test with seven common inhalant allergens.
M: Months

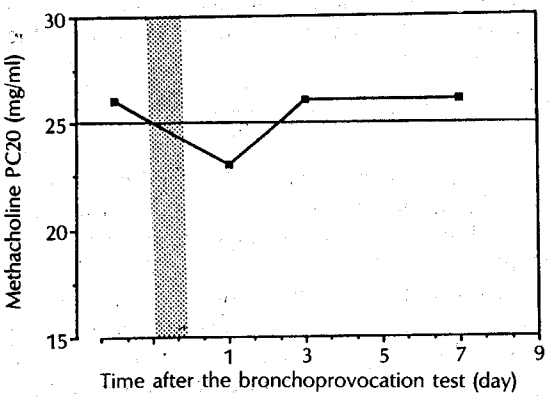


***Fig. 1.*** *Changes of methacholine $PC_{20}$ after Black GR-bronchoprovocation test ( ) in patient MY.*

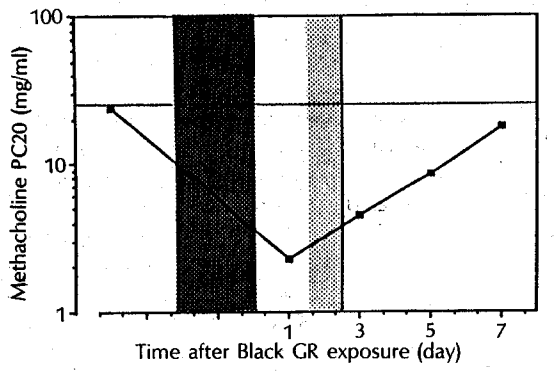


***Fig. 2.*** *Changes of methacholine $PC_{20}$ after Black GR exposure ( ) for 3 days and Black GR-bronchoprovocation test ( ) in patient KM.*

GR solution. The following methacholine challenges could not be performed because he refused them.

Worker KM was a 30-year-old male worker and he was also a smoker. He had worked for 20 months at the same dye industry with worker CY. A skin prick test with Black GR showed a 4+ response, and his specific IgE was 3.0 %. His initial methacholine $PC_{20}$ was 24 mg/ml and he also demonstrated an isolated immediate bronchoconstriction after inhalation of the 1.0 mg/ml Black GR solution. After working at his workstation with dye exposure for 3 days, his methacholine $PC_{20}$ markedly decreased to 2.4 mg/ml 24 hours after the dye exposure stopped and then increased to 4.5 mg/ml on the third day, 8.5 mg/ml on the 5th day, and to 18 mg/ml on the seventh day, as shown in Fig. 2. The Black GR bronchoprovocation test was performed again on the second day, but his methacholine $PC_{20}$ did not decline.

## DISCUSSION

The workers studied had clinical presentations of occupational asthma without bronchial hyperresponsiveness as other investigators have reported (Hargreave *et al.* 1980; Stanescu and Frans 1982). Butcher *et al.* (1977) reported that 8 of 11 TDI-sensitive asthmatic workers showed positive methacholine challenge tests, and O'Brien *et al.* (1979) noted increased bronchial reactivity to histamine in only 17 of 31 subjects sensitive to TDI. Our previous study (Park *et al.* 1989) revealed that 2 of 20 asthmatic workers who showed positive bronchoprovocation tests after inhalation of Black GR had negative methacholine challenge studies. These findings are interesting observations, since the question occurs as to whether underlying airway hyperreactivity, as demonstrated by sensitivity to methacholine, predisposes an individual to reactive dye asthma, and also as to whether it is present before the development of clinically over asthma. In this study, we could speculate that the Black GR-bronchoprovocation test could develop nonspecific bronchial hyperreactivity in a worker with a negative methacholine challenge test, and repeated dye exposure could decrease the methacholine $PC_{20}$ in a worker with initially mild nonspecific bronchial hyperreactivity. Furthermore, careful inquiry about whether he has ever experienced respiratory symptoms have ever been experienced with dye exposure might be needed to identify reactive dye induced occupational asthma patients whose initial methacholine challenge tests are negative.

The mechanism which would explain the airway response to Black GR cannot be ascertained from this study. Our previous studies suggested that more than one mechanism may be responsible for reactive dye asthma (Park *et al.* 1989; Park *et al.* 1989). Black GR, which has two reactive groups combined with tissue proteins, can also act as an allergen. The specific IgE, specific IgG and IgG4 antibodies to Black GR-HSA conjugate were demonstrated in patients with occupational asthma caused by reactive dyes in our recent studies (Park *et al.* 1989; Park *et al.* 1989; Park *et al.* 1989). In this study, our patients demonstrated significant bronchoconstrictions on Black GR-bronchoprovocation test. They all showed significant positive responses on a skin prick test with Balck GR, and had high specific IgE antibodies. These findings suggested that the immunological, probably IgE mediated, reaction was operative in their asthmatic symptoms after the dye exposure.

Continued antigen exposure was associated with an increase in methacholine responsiveness in sensitive patients. Such increases have been recognized in association with late asthmatic responses which have been known to probably be a consequence of the allergic inflammatory reaction (Cartier *et al.* 1982; Boulet *et al.* 1983). Patterns of variable airway narrowing when the patient was at work suggested that he had late asthmatic responses which are known to be common in occupational asthma caused by a chemical sensitizer (Chan-Yeung 1982). These late responses are considered to be associated with an inflammatory reaction (Gleich 1982), and the occurrence of inflammation is dependent on the level of IgE antibodies in sensitive patients (Boulet *et al.* 1983). In this study, all patients experienced asthmatic symptoms during the night after work, as well as at the workstation, suggesting late responses. The following methacholine challenges revealed that repeated dye exposure could increase airway hyperresponsiveness.

Changes in bronchial hyperresponsiveness to histamine or methacholine have been described after late or dual asthmatic reactions with common inhaled allergens (Cockcroft *et al.* 1977; Cartier *et al.* 1982) or occupational allergens (Cockcroft *et al.* 1979; Cartier *et al.* 1984). The inflammation that accompanies late bronchoconstriction is assumed to be responsible for such changes (Mapp *et al.* 1985; Hargreave *et al.* 1985; Fabbri *et al.* 1987). Several studies (Cockcroft *et al.* 1977; Cartier *et al.* 1982; Mapp *et al.* 1986) have demonstrated that isolated immediated reactions after exposure to common allergens and to low molecular weight chemicals do not induce significant changes in nonallergic bronchial responsiveness. However, Fabbri *et al.* (1987) described one subject who showed an isolated immediate response, but had a change in methacholine $PC_{20}$ 2 hours after TDI exposure. Malo *et al.* (1989) described two patients who demonstrated isolated immediate bronchoconstrictions, but had very significant changes in methacholine $PC_{20}$. In this study the patient MY showed early asthmatic response on the Black GR-bronchoprovocation test, but nonspecific bronchial hyperreactivity developed on the first day after the bronchoprovocation test and disappeared on the third day. The mechanism that developed bronchial hyperresponsiveness in this worker is unclear. Cartier *et al.* (1986) described that a subject experiences a significant drop in methacholine $PC_{20}$ after inhalation of plicatic acid for 30 seconds even if there has been no evidence of airway obstruction. After a previous challenge with plicatic acid for 7 minutes, he did experience severe isolated late asthmatic responses associated with a significant change in methacholine $PC_{20}$. It is possible that more prolonged exposure to reactive dye in our workers would have induced late asthmatic reactions.

In conclusion, reactive dye induced occupational asthma can occur at a time when methacholine bronchial responsiveness is normal and an isolated immediate reponse induced by reactive dye might lead to nonspecific bronchial hyperreactivity.

## REFERENCES


Boulet LP, Cartier A, Thomson NC, Roberts RS, Dolovich J, Hargreave FE: Asthma and increases in nonallergic bronchial responsiveness from seasonal pollen exposure. *J Allergy Clin Immunol 71:399, 1983*

Cartier A, L'Archeveque J, Malo JL: Exposure to a sensitizing occupational agent can cause a long-standing increase in bronchial responsiveness to histamine in the absence of significant changes in airway caliber. *J Allergy Clin Immunol 78:1185, 1986*

Cartier A, Malo JL, Forest F, Lafrance M, Pineau L, St-Aubin JJ, Dobois JY: Occupational asthma in snow crab processing workers. *J Allergy Clin Immunol 74:261, 1984*

Cartier A, Thomson NC, Frith PA, Roberts R, Hargreave FE: Allergen-induced increase in bronchial responsiveness to histamine: relationsiip to the late asthmatic response and change in airway caliber. *J Allergy Clin Immunol 70:170, 1982*

Chan-Yeung M, Lam S, Koener S: Clinical features and natural history of occupational asthma due to western red cedar (Thuja plicata). *Am J Med 72:411, 1982*

Chai H, Farr RS, Froelich LA, Mathison DA, Rosenthal RR, Shelter AL, Spector SL, Townley RG: Standardization of bronchial inhalation challenge procedure. *J Allergy Clin Immunol 56:323, 1975*

Chester EH, Martinez-Catinchi FL, Schwarz HF: Patterns of airway reactivity to asthma produced by exposure to toluene diisocyanate. *Chest 75:229, 1979*

Cockcroft DW, Cotton DJ, Mink JT: Nonspecific bronchial hyperreactivity after exposure to western red cedar. *Am Rev Respir Dis 119:505, 1979*

Cockcroft DW, Ruffin RE, Dolovich J, Hargreave FE: Allergen-induced increase in nonallergic bronchial reactivity. *Clin Allergy 7:503, 1977*

Fabbri LM, Boschetto P, Zocca E Milani G, Pivirotto F, Pleban M, Burlina A, Licata B, Mapp CE: Bronchoalveolar neutrophilia during late asthmatic reactions induced by toluene diisocyanate. *Am Rev Respir Dis 136:36, 1987*

Gleich GJ: The late phase of the immunogloblin E-mediated reaction; a link between anaphylaxis and common allergic disease. *J Allergy Clin Immunol 70:161, 1982*

Hargreave FE, O'Byrne PM, Ramsdale EH: Mediators, airway

responsiveness, and asthma. *J Allergy Clin Immunol 76:272, 1985*

Hargreave FE, Ramsdale EH, Pugsley SO: Occupational asthma without bronchial hyperresponsiveness. *Am Rev Respir Dis 131:513, 1980*

Lam S, Wong R, Chan-Yeung M: Nonspecific bronchial reactivity in occupational asthma. *J Allergy Clin Immunol 63:28, 1979*

Malo JL, L'Archeveque J, Cartier A: Significant changes in nonspecific bronchial responsiveness after isolated immediate bronchospecific reactions caused by isocyanates but not after an altered reaction caused by plicatic acid. *J Allergy Clin immunol 83:159, 1989*

Mapp CE, Polato R, Maestrelli P, Hendrich DJ, Fabbri LM: Time course of the increase in airway responsiveness associated with late asthmatic reactions to toluene diisocyanate in sensitized subjects. *J Allergy Clin Immunol 75:568, 1985*

Mapp CE, Di Giacomo GR, Omini C, Broseghini C, Fabbri LM: Late, but not early, asthmatic reactions induced by toluene diisocyanate are associated with increased airway responsiveness to methacholine. *Eur J Respir Dis 69:276, 1986*

Newman Taylor AJ: Occupational asthma. *Thorax 35:241, 1980*

O'Brien IM, Newman-Taylor AJ, Burge PS: Toluene diisocyanate induced asthma. II. Inhalation challenge tests and bronchial reactivity studies. *Clin Allergy 9:7, 1979*

Park HS, Kim YJ, Lee MK, Hong CS: Occupational asthma and specific IgE antibodies to reactive dyes. *Yonsei Med J 3:289, 1989*

Park HS, Hong CS, Kim JW: The significance of specific IgG and IgG4 to reactive dye in exposed workers. *Clin Exp Allergy* (submitted)

Park HS, Lee MK, Hong CS: Specific IgE, IgG and IgG4 to Black GR in asthmatic patients exposed to Black GR. *Kor J Med Asso 32:1197, 1989*

Park HS, Lee MK, Kim BO, Lee KJ, Roh JH, Moon YH: Clinical and immunological evaluations of reactive dye-exposed workers. *J Allergy Clin Immunol* (submitted)

Ryan G, Latimer KM, Dolovich J, Hargreave FE: Bronchial responsiveness to histamine: relationship to diurnal variation of peak flow rate, improvement after bronchodilator and airway caliber. *Thorax 37:423, 1982*

Smith AB, Brooks SM, Blanchard J, Bernstein IL, Gallagher J: Absence of airway hyperreactivity to methacholine in a worker sensitized to toluene diisocyanate. *J Occup Med 22:327, 1980*

Stanescu DC, Frans A: Bronchial asthma without increased airway reactivity. *Eur J Respir Dis 63:5, 1982*