

Occupational Asthma Caused by FD&C Blue Dye No. 2

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ABSTRACT

We report the case of a 55-year-old male who experienced cough, dyspnea, wheezing, and nasal congestion immediately upon exposure to FD&C Blue Dye No. 2 (Indigotine) at work. The patient had worked for 10 years mixing and grinding powdered synthetic red, yellow, and blue dyes for use in foods; symptoms had occurred for 2 years and only with exposure to Indigotine ($C_{16}H_8N_2Na_2O_8S_2$), a free flowing blue powder. Prick testing to Indigotine (20 mg/mL) was negative. ELISA failed to detect specific IgE, IgA, IgM, or IgG to Indigotine-HSA conjugates. Bronchial challenge was done according to the method of Pepys et al. beginning with 4×10^{-4} lactose dilution of Indigotine powder. After 5 minutes of exposure to 4 gm Indigotine/100 gm lactose, the patient developed dyspnea and audible wheezing. At 20 minutes postexposure, there was a 20% decline in FEV₁ from prechallenge baseline; no late phase response was observed. A second bronchial challenge with sodium sulfate, the major nondye product additive was negative. To our knowledge, this is the first documented case of occupational asthma due to FD&C Blue Dye No. 2. The pathogenesis is uncertain but does not appear to be IgE mediated. (Allergy and Asthma Proc 17:31-34, 1996)

Occupational asthma (OA) has been defined as a "disease characterized by variable airflow limitation and airway hyperresponsiveness due to causes and conditions that are attributable to a particular occupational environment and not to stimuli encountered outside the workplace."¹ Over 200 causative agents have been identified in OA and of these, more than 140 are low molecular weight (LMW) chemicals.² Included among these LWM agents are

reactive dyes, which have frequently been implicated in cases of OA in textile workers.³⁻¹¹ In addition, OA has been reported in workers involved in the manufacture of carmine, a natural nonreactive dye.¹²⁻¹⁴ In this report, we describe the first case of OA caused by Indigotine, a synthetic nonreactive dye also known as FD & C Blue No. 2.

CASE REPORT

A 55-year-old white male presented for evaluation of a 2-year history of cough, dyspnea, wheezing, and nasal congestion while at work. He had been in good health before the onset of symptoms. There was no prior history of allergic rhinitis, food allergy, or asthma. There was a remote smoking history of approximately 25 pack years. The patient had worked for 10 years at a chemical plant where he ground and mixed powdered synthetic red, yellow, and blue dyes for use in food and drugs. His symptoms began at work, where he noted a marked exacerbation of wheezing, cough, rhinorrhea, and eye irritation almost immediately upon exposure to a blue dye powder. Symptoms lasted approximately 1-2 hours, were significantly improved over weekends, and were completely resolved during vacation periods. The patient denied exacerbation of his symptoms upon consumption of products containing blue dyes or aspirin. None of the patient's co-workers reported asthmatic symptoms in the workplace.

The initial physical examination was essentially normal, and no wheezing was noted. Results of routine laboratory studies revealed a mild eosinophilia of 660/cu mm. Baseline pulmonary function tests revealed an FEV₁ of 3.12 L/minute (97% predicted) and FVC 4.93 (119% predicted). A methacholine bronchoprovocation test performed on a day that the patient was not working demonstrated a PC 20 of 3.7 mg/mL.

The suspect causative agent was the blue dye Indigotine, a water-soluble free-flowing blue powder with sodium chloride and/or sodium sulfate as the principal noncolor agent comprising less than 15% total Indigotine powder. This dye is approved and indexed as FD&C Blue No. 2 (see Fig. 1).

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METHODS

Immunological Studies

A sample of Indigotine powder was obtained from the workplace. Indigotine powder (200 mg) was dissolved in 10 mL 0.9% NaCl to a final concentration of 20 mg/mL and sterilized by Millipore filtration (Costar, Cambridge, Mass.). Skin prick testing was performed with this solution, and a battery of common aeroallergens including *Dermatophagoides* sp., *Alternaria*, *Aspergillus* sp., grass, trees, cockroach, and ragweed, with histamine and saline controls. Two Indigotine conjugates were prepared. The first conjugate was made with carbodiimide (ECDI), which is frequently used in synthetic chemistry because of its ability to create peptide bonds, thereby allowing the formation of protein-protein conjugates. This was done by dissolving 100 mg Indigotine powder in 4.2 mL H₂O and then adding 50 mg human serum albumin (HSA). Next, 1 gm of ECDI was dissolved in 0.6 mL H₂O and then added to the dye-HSA solution. The mixture was dialyzed extensively in 0.15M NaCl at room temperature until the dialysate was free of color. The resulting Indigotine-ECDI-HSA conjugate was dark blue.¹⁵ A mock HSA-ECDI without Indigotine was prepared and dialyzed in the same manner. Subsequent spectrophotometric analysis of the Indigotine-HSA-carbodiimide conjugate revealed a hapten-protein ratio of 5 moles dye/mole HSA. The second antigen was prepared by directly combining Indigotine with HSA in the method of Levine and Ovary.¹⁶ However, the hapten-protein ratio of this antigen by spectrophotometry was significantly lower than the dye-HSA-carbodiimide conjugate with less than 1 mole dye/mole HSA.

Serum for specific IgE, IgG, IgA, and IgM antibodies to Indigotine-HSA-ECDI and Indigotine-HSA was assayed using previously described ELISA methods.¹⁷ Mock HSA-ECDI and HSA were used as antigen controls. Optical density was determined at 410 NM by a Biotek ELISA miniplate reader (Winooski, Vt.). Tests were performed in triplicate. Sera from seven persons without occupational exposure to food dyes were used as controls.

Specific Bronchial Challenge Tests

A specific bronchoprovocation test was performed on a day when the patient was absent from work and free of symptoms. The patient's only asthma medication, a short-acting β -agonist, was withheld for 24 hours before the test.

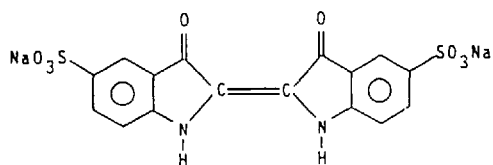


Figure 1. Structure of Indigotine (FD&C Blue No 2). This synthetic free-flowing blue powder is a mixture of disodium-3,3'-2,2'-bi-indolylidene-5,5'-disulfonate and disodium-3,3'-2,2'-bi-indolylidene-5,7'-disulfonate with less than 15% sodium chloride and/or sodium sulfate as the principal uncolored component.

Using the method described by Pepys,¹⁸ 4 gm of Indigotine powder was diluted in 100 gm lactose. Ten-fold serial dilutions were made to a final concentration of 4×10^{-4} Indigotine/100 gm lactose. Beginning with the 4×10^{-4} dilution, the patient sifted the powder mixtures between two traps in an open room for 10 minutes. Pulmonary function measurements of FEV₁ and FVC were obtained at baseline and repeated at 10, 15, and 20 minutes postexposure to each increasing concentration of the challenge agent until FEV₁ had declined 20% from baseline. The patient measured and recorded PEFR hourly for a period of 6 hours following the challenge test.

On a separate, nonwork day, a control bronchial challenge test was performed using a single dilution of 600 mg sodium sulfate in 100 gm lactose that was sifted for 10 minutes as above. This concentration of sodium sulfate duplicates the maximum exposure concentration the patient could have experienced at the dose of Indigotine powder that induced an asthmatic response during specific challenge. Pulmonary function tests were again obtained at baseline and 10–125 minutes postexposure. PEFR was again monitored by the patient for 6 hours after testing.

RESULTS

Skin prick testing was negative to the solution of Indigotine (20 mg/mL) as well as to a panel of common aeroallergens. Neither IgE, IgG, IgA, nor IgM antibodies specific for Indigotine-conjugates could be detected by ELISA. Because the patient's history and methacholine challenge results were suggestive of occupational asthma, a bronchial challenge test with Indigotine was performed. Results of specific inhalation challenge testing with Indigotine are shown in Figure 2. The patient began experiencing nasal congestion and rhinorrhea while sifting the 4×10^{-1} dye/100 gm lactose dilution, but did not have any lower respiratory symptoms. Within 5 minutes of exposure to 4 gms Indigotine/100 gm lactose, however, the patient developed dyspnea and audible wheezing. At 20 minutes postex-

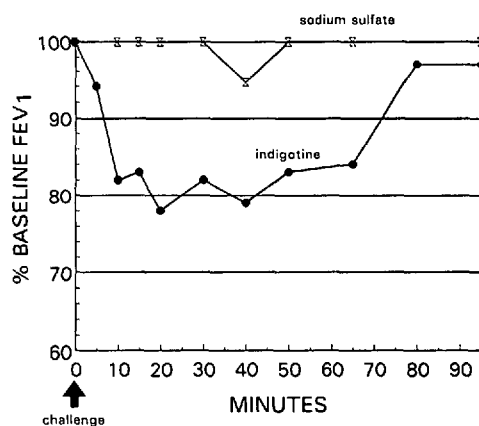


Figure 2. Results of specific bronchial challenge testing documenting a significant decline in FEV₁ after exposure to Indigotine but not control.

posure, there was a 20% decline in FEV₁ from prechallenge baseline, and the patient continued to have wheezing and dyspnea. The symptoms resolved without treatment, and the FEV₁ returned to baseline at 80 minutes postexposure. A late phase asthmatic response was not demonstrated as late as 6 hours after the immediate response. On a separate control day, the patient had no response after bronchial challenge with sodium sulfate, the principal noncolor component in the dye. It was recommended that the patient be restricted from exposure to the Indigotine dye in the workplace. Since initiation of these measures, there have been no significant asthma symptoms, nor has he required the use of asthma medication.

DISCUSSION

A wide variety of agents encountered in the workplace have been implicated in the etiology of OA. Agents which are high molecular weight proteins usually induce asthma *via* an IgE mediated mechanism. Although OA caused by some LMW proteins appears to be associated with specific IgE responses, most of these compounds induce asthma through an undefined mechanism.¹⁹

Among the LMW proteins that have been implicated in OA are reactive dyes. This is a large group of compounds that were first introduced into the textile industry in the 1950s.^{3,4} These colorants are water-soluble and are used extensively because of their brightness, ease of application, and color-fastness in both natural and synthetic fibers. The reactive dye molecule contains a color-forming component, usually an azo, anthraquinone, or phthalocyanine derivative and a fiber-reactive component such as a heterocyclic halogen group or an aliphatic chain with a reactive group. This reactive group could be mono- or dichlorotriazine, fluorotriazine, bromoacrylamide, or vinyl sulfone, for example. When exposed to an alkaline solution and heat, the reactive group forms a permanent covalent bond with the fiber, and does this at the fiber's hydroxyl, amino, or sulfhydryl group, depending on the particular fiber type. Reactive dyes have a molecular weight between 500 and 1000 daltons and are believed to act as haptens, combining with endogenous proteins to initiate immunological responses.^{3,4} OA caused by reactive dye was first reported by Alanko et al. in 1978.³ Using RAST, they were able to demonstrate high serum titers of specific IgE to four reactive dyes. Since then, other investigators have also reported cases of asthma, allergic rhinitis, and contact dermatitis caused by various reactive dyes.⁴⁻¹⁰ In these studies, many patients with dye-induced asthma not only had positive bronchial challenge responses, but also had specific IgE antibodies directed against dye-HSA conjugates. There remained, however, a significant number of workers who had similar respiratory symptoms associated with reactive dye exposure who did not have demonstrable IgE antibodies, suggesting another mechanism for reactive dye-induced

asthma. Park and Hong¹¹ investigated a group of such workers and found that a significant number had dye-HSA conjugate specific IgG detected by ELISA. The significance of these antibodies remains unclear.

The dyes and colorants used in the food, drug, and cosmetic industry are not reactive dyes and have generally not been implicated in OA. However, there have been rare reports of OA due to a nonreactive red dye used in food and cosmetics.¹²⁻¹⁴ Carmine is a natural red dye extracted from the dried body of the female *Coccus cactus* (cochineal), a parasitic insect found on certain species of cacti. The reported workers were all exposed to carmine during the extraction and manufacture process. Quirce et al. described a worker with OA and rhinitis who had specific IgE against both carmine and cochineal. Other workers who did not have specific IgE did have positive bronchial challenge tests to both carmine and cochineal, and were found to have specific IgG antibodies to both substances.¹² It should be noted that in all cases of OA caused by carmine, the worker was also sensitive to cochineal extract, implying that the immunological mechanism was directed against the organic insect protein.

In contrast, Indigotine (FD&C Blue No 2) is a synthetic, nonreactive chemical dye used as a colorant in the manufacture of food (e.g. cereal, ice cream, sausage, bakery, and confectionery products) and drugs (e.g., Stelazine®, Corgard®, Sinemet®). It is essentially a mixture of disodium-3,3'-2,2'-bi-indolylidene-5,5'-disulfonate and disodium-3,3'-2,2'-bi-indolylidene-5,7'-disulfonate with less than 15% sodium chloride and/or sodium sulfate as the principal uncolored component. To our knowledge, this case of OA caused by FD&C Blue No. 2 is the first report of a synthetic, nonreactive dye being implicated in OA. Several observations suggest an immediate hypersensitivity mechanism. The patient had a latency period of exposure before developing asthma in the workplace, was the only exposed worker to develop symptoms, and exhibited immediate bronchoconstriction after bronchial challenge with low concentrations of the antigen. Yet specific IgE antibody to Indigotine conjugates could not be demonstrated by ELISA. This may be related to the fact that Indigotine, like many LMW chemicals, is not intrinsically reactive with serum proteins, as evidenced by the inability of dye to conjugate spontaneously with HSA. Thus, the potential for antigenic epitope to form *in vivo* may have been reduced. Therefore, although we have presented evidence confirming a diagnosis of OA induced by FD&C Blue No. 2, the precise mechanism responsible for the induction of bronchial hyperresponsiveness is unknown.

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