
Case report

Anaphylaxis to isosulfan blue and cross-reactivity to patent blue V: case report and review of the nomenclature of vital blue dyes

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Background: Blue dyes used for lymphatic mapping in sentinel lymph node biopsy cause intraoperative anaphylactic reactions in up to 2.7% of patients. With increasing implementation of this technique, the incidence of anaphylaxis to these dyes can be expected to increase. In the literature, the chemically often unrelated and inconsistently designated dyes have been confused, adding to other inconsistencies in the nomenclature.

Objective: To demonstrate the nomenclature, chemical and physiologic differences, and allergenicity of the various blue dyes used in a medical context.

Methods: We describe a patient with an intraoperative grade IV anaphylactic reaction to isosulfan blue. Immediate-type hypersensitivity was proved by positive skin test reactions and CD63 expression to isosulfan blue and cross-reactivity to patent blue V.

Results: A review of the literature clarified the exact nomenclature of the blue dyes and the possible pitfalls of confusing nomenclature in the context of structurally closely related dyes with different allergenic properties. For the detection of type I hypersensitivity, intracutaneous tests are valuable tools. An IgE-mediated mechanism has been shown recently. In most cases, sensitization exists without known previous exposure in a medical context. This may be due to the widespread use of such dyes in objects of everyday life. Preoperative antiallergic medication use does not prevent anaphylactic reactions but apparently reduces their severity.

Conclusion: For better comparison and precision, the Chemical Abstracts Service number of the respective dye should always be given.

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INTRODUCTION

Anaphylactic and anaphylactoid reactions are rare events during anesthesia, although the true incidence of anaphylactic reactions and their morbidity and mortality remain poorly defined. These reactions may lead to death, even when appropriately treated, with a mortality of 3.5% to 4.7%. Incidence rates are known to be 0.5 to 1 in 10,000 (in Australia in 1993) to 1 in 13,000 (in France in 1996)^{1,2} in countries with well-organized documentation systems. Of the drugs most liable for inducing anaphylactoid or anaphylactic reactions during anesthesia, myorelaxants account for approximately 50%,³ followed by latex sensitization (12.1%) and antibiotics (15%).

Allergologic diagnostic approaches to these events are challenging for multiple reasons.⁴ Usually more than 1 possible elicitor of anaphylaxis has been administered at the same time. Some of the drugs in question, especially myore-

laxants and opiates, may cause anaphylactoid reactions owing to their strong histamine-liberating capacity, in addition to the possibility of an IgE-mediated reaction. Skin tests, which are usually a valuable diagnostic tool, may yield misleading results owing to uncertainties in distinguishing irritant from allergic skin reactions. Blue dyes, used for lymphatic mapping in the context of sentinel lymph node biopsy (SLNB) in cancer surgery, are rare causes of anaphylactic reactions. Because of the increasing implementation of this technique for new indications, eg, melanoma, breast carcinoma, bladder cancer, and cervical and endometrial cancer, the incidence of anaphylaxis to these blue dyes can be expected to increase.

By means of this case report of intraoperative anaphylaxis to isosulfan blue and an overview of the literature, we attempt to draw attention to this increasingly important group of dyes and its potential to cause intraoperative anaphylaxis. In addition, often these dyes are not correctly designated, and even from a chemical point of view misleading designations have been used, resulting in a mix up of the dyes in the literature.

CASE REPORT

A 70-year-old woman was scheduled to undergo a lumpectomy of the left breast and SLNB for suspected breast cancer.

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Hypertension was treated with enalapril. After the smooth induction of general anesthesia, the patient developed, shortly after the start of surgery, generalized erythema, tachycardia, a decline in blood pressure to 80/50 mm Hg, and conjunctivitis. After the administration of vasoactive substances and glucocorticosteroids and substitution of volume, her circulation returned to a stable condition and her shock symptoms regressed. The postoperative course was uneventful.

At the time of the anaphylactic reaction the patient had previously received propofol, enflurane, and thiopental sodium, as well as the muscle relaxant atracurium besylate for the induction of anesthesia and cefazolin sodium (a first-generation cephalosporin) as a prophylactic antibiotic. Mast cell tryptase levels were elevated immediately after the incident to 113 $\mu\text{g/L}$ (reference range, 1–13.5 $\mu\text{g/L}$), indicating an allergic reaction. At that point, an anaphylactic reaction grade IV to cefazolin, atracurium, thiopental, or latex was suspected.

Results of skin prick and intracutaneous tests with propofol, thiopental, atracurium, benzylpenicillin, amoxicillin, and cefazolin in several concentrations; skin prick tests with latex-protein derivatives; and determination of specific IgE against latex, penicillins, and aminopenicillins were negative. A lymphocyte transformation test to benzylpenicillin, amoxicillin, and cefazolin did not show any stimulation. A serum sample from the patient was used for experimental determination of specific IgE to thiopental, propofol, and cefaclor (another first-generation cephalosporin), and the findings were negative. The basal mast cell tryptase concentration was elevated to 25.4 $\mu\text{g/L}$, suggesting an increased mast cell mass. However, no clinical symptoms of mastocytosis were present. The preliminary diagnosis was an anaphylactic reaction of unknown origin, possibly due to the suspected diagnosis of systemic mastocytosis. However, on repeated controls, serum mast cell tryptase levels ranged from 18 to 25 $\mu\text{g/L}$, thereby not fulfilling the criterion for systemic mastocytosis of being constantly greater than 20 $\mu\text{g/L}$.

A new careful study of the anesthesia protocol and the surgical report and repeated questioning of the anesthesiologist revealed that immediately before the start of surgery the patient was injected with several milliliters of isosulfan blue perilesionally as a marker for the lymphatic drainage system of the diseased tissue in preparation for the intended SLNB.

Skin prick and intracutaneous tests were then performed with isosulfan blue and patent blue V (concentration for intracutaneous tests: 1:1 to 1:10⁶ of stock solution [1%]) and methylene blue (1:1 to 1:10⁴ of stock solution [1%]). Methylene blue results were negative for all the tests, whereas isosulfan blue and patent blue V results were positive up to dilutions of 1:10⁵ of the stock solution, positive being defined as a wheal larger than the negative control after 15 minutes.

Informed consent was obtained from 9 healthy individuals who had never been exposed to isosulfan blue or patent blue V in a medical context and who served as controls for the intracutaneous tests. This testing was approved by the ethics committee of the Medical Faculty of the University of Basel.

Both dyes were injected in the upper back at 1:10 and 1:100 dilutions. After 15 minutes, the resulting wheals were documented using a digital camera (Coolpix 5000; Nikon USA, Melville, NY) through an optical device with an integrated scale. The wheal circumference was determined in triplicate, and the area was then calculated using the freeware NIH Image/J software V 1.30 win32 (National Institutes of Health). By statistical analysis, compared with a negative control (0.9% sodium chloride), a threshold dilution for intracutaneous tests of 1:100 of the stock solution for isosulfan blue and patent blue V was established, separating toxic-irritant reactions from true allergic reactions.

Specific IgE to isosulfan blue and patent blue V could not be detected using either ImmunoCAP (isosulfan blue) or radioallergosorbent test (patent blue V) techniques (Pharmacia, Uppsala, Sweden). Investigation of specific IgE to isosulfan blue using an enzyme-linked immunosorbent assay according to a recent publication⁵ was also negative. Results of sulfidoleukotriene release tests (CAST, Bühlmann Inc, Allschwil, Switzerland) were negative; however, CD63 expression (Flow-CAST Basophil Activation Test, Bühlmann Inc) was positive for both substances. Therefore, the final diagnosis was an intraoperative anaphylactic reaction due to immediate-type hypersensitivity to isosulfan blue with cross-reactivity to patent blue V, possibly aggravated by enalapril.

RESULTS

The dyes commonly used for SLNB are isosulfan blue and patent blue V, although other dyes, such as indocyanine green and fluorescein, have been investigated and are used in special situations.⁵ The chemical systematics of the standard dyes isosulfan blue and patent blue V are complicated, and the situation is aggravated by a misleading nomenclature that is sometimes contradictory even in the chemical expert literature. Figure 1 shows the chemical structure of the 2 molecules, which differ in the position of the substituted sulfonate. Both belong to the group of triarylmethane dyes and basically share the same formula of C₂₇H₃₂N₂O₆S₂, with patent blue V having an additional hydroxyl group.

The sodium salt of patent blue (Chemical Abstracts Service [CAS] No. 129-17-9) is, among many other names, also called sulfan blue, food blue 3, patent blue VF, and acid blue 1. Isosulfan blue (CAS No. 68238-36-8) is the 2,5-disulfo-phenyl structural isomer of patent blue (not patent blue V)

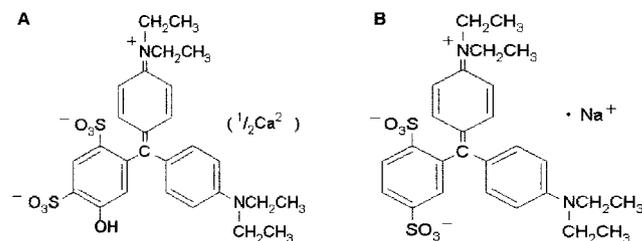


Figure 1. Chemical structure of patent blue V (A) and isosulfan blue (B).

and is sold under the trade name Lymphazurine.⁶ Patent blue V is predominantly provided as calcium-chelated dimer (CAS No. 3536-49-0) and can also be found under the name patent blue violet, food blue 5, acid blue 3, and disulfine blue. It is also known as E 131 and is still on the market as a food colorant, in contrast to the report by Quilquini et al.⁷ Patent blue V has a slightly different chemical structure containing an additional hydroxyl group at position 5.

Because of the close structural relationship of these vital dyes, cross-reactivity may be assumed and could be shown clinically and in the Flow-CAST in our patient. Recently, flow cytometric quantification of CD63-positive basophils has been shown to be a useful tool in the diagnosis of type I sensitization to patent blue V.⁸ For most patients, the mode of sensitization is not clear because almost all patients are exposed only once to one of the dyes in the context of lymphangiography or SLNB. Therefore, most patients have reacted at their first known exposure to such a dye. An as-yet unproven hypothesis states that sensitization against the vital dyes is facilitated by the common use of patent blue and other structurally closely related triarylmethane dyes in objects of everyday life, such as color textiles, cosmetics, detergents, paints, inks, antifreeze, cold remedies, laxatives, and suppositories.^{9,10}

In addition to a variety of case reports^{11,12} there are several retrospective and prospective studies of large numbers of patients on the frequency of allergic reactions to isosulfan blue and patent blue V. Montgomery et al¹⁰ calculated in their meta-analysis of several single-institution series, including their own of 2,392 patients, the incidence of allergic reactions to vital blue dyes in patients with breast cancer. For patent blue, the incidence is 0.6% to 2.7%, with a mean of 1.8%. For isosulfan blue, the incidence is 0.9% to 1.9%, with a mean of 1.4%. The cumulative number of patients included in these studies was 1,940 for patent blue and 4,247 for isosulfan blue. Most of the patients reacted mildly, with anaphylactic reaction grades I and II, with urticaria, blue hives, flush, and pruritus; however, severe hypotensive reactions do occur. The reactions are generally reported to respond rapidly to antiallergic measures.

In our patient, the long-term use of the angiotensin-converting enzyme (ACE) inhibitor enalapril may have aggravated the situation. In a recent retrospective analysis of 1,149 patients with anaphylaxis, Brown¹³ suggested that ACE inhibitors affect reaction severity, although it was not an independent predictor of any severe reaction feature. Other researchers¹⁴ report that inhibition of the metabolism of bradykinin by ACE inhibitors and the following buildup of bradykinin and substance P might predispose some individuals to anaphylaxis and to being more refractory to treatment if anaphylaxis develops.

Biphasic courses of the reaction have sometimes been described. They may be attributed to the slow release of the dye from the subcutaneous tissue or the lymphatic tissue and the half-life of the dye in the body of several hours. Blue or green serum, urine, or skin discoloration for up to 24 hours

after injection of the drug indicate as much. The cause of biphasic reactions is not yet understood completely. Sampson et al¹⁵ and Lee and Greenes¹⁶ discuss in studies of children and adolescents a relatively long time between initial anaphylaxis and the administration of epinephrine and its correlation with the occurrence of a second anaphylactic (biphasic) episode. In a study¹⁷ of 639 patients who underwent SLNB for breast cancer using isosulfan blue, 7 anaphylactic reactions occurred, 2 of which were biphasic. The 2 patients had recurrences during postoperative monitoring (6 and 8 hours after surgery) and again responded well to antiallergic treatment.¹⁷ Quilquini et al⁷ described a patient with a second episode of severe anaphylaxis 3 hours after the first. In that case, patent blue V was the causative agent.

Raut et al¹⁸ investigated the use of preoperative prophylaxis with 100 mg of hydrocortisone (4 mg of dexamethasone), 50 mg of diphenhydramine, and 20 mg of famotidine in 448 patients with SLNB using isosulfan blue. They observed allergic reactions in 0.7% of these patients, all of them grade I. No episodes of hypotension were noted. They concluded that preoperative prophylaxis reduced the severity but not the overall incidence of adverse reactions to isosulfan blue.¹⁸ The complication rate regarding wound healing doubled under this treatment, but it did not reach statistical significance. No biphasic anaphylaxis was seen with prophylactic therapy, perhaps because of the overall less severe reactions with prophylactic therapy. Severe reactions and early onset after exposure to the allergen seem to be risk factors for a biphasic response.

Several incidences have been reported of transient, false lowering of pulse oximetry findings. Coleman et al¹⁹ postulated that the absorption maximum of isosulfan blue at 646 nm interferes with measuring of the absorption of oxyhemoglobin at 660 nm using conventional pulse oximeters, indicating a technical problem.

Skin tests, especially intracutaneous tests, are valuable tools for diagnosing type I sensitization to isosulfan blue and patent blue V. We demonstrated that a 1:100 dilution of the stock solution (1%) was not irritant after intracutaneous injection in 9 healthy individuals. This is in analogy to studies by Laurie et al,²⁰ who reported negative intradermal test results in 8 healthy individuals and 1 patient with breast cancer with 1:100 dilutions of the stock solution.

Woehrl et al²¹ recently demonstrated specific IgE antibodies against isosulfan blue in patients with previous anaphylactic reactions to isosulfan blue. However, they did not succeed with the serum of our patient. Despite this, an IgE-mediated mechanism can be assumed, although older publications suggest pseudoallergic mechanisms.²² Given the relatively small molecular weight of isosulfan blue, it is likely to act as a hapten. Approximately 50% of the isosulfan blue in aqueous solutions is weakly bound to serum proteins, which allows for its characteristic lymphatic tropism.¹⁹

Methylene blue is sometimes mentioned as another dye successfully used for lymphatic mapping.^{8,23} The methylene blue known under CAS No. 61-73-4 (anhydrous methylene

blue) or CAS No. 7220-79-3 (methylene blue trihydrate), however, is only approved for intravenous administration for the treatment of methemoglobinemia and hemolysis because it may cause necrosis on subcutaneous administration. It has the total formula $C_{16}H_{18}ClN_3S$ and is the trihydrate of the 3,7-bis(dimethylamino) phenazathionium chloride. According to Tsopelas and Sutton,²⁴ methylene blue does not bind to plasma proteins, having no sulfonic acid groups, and, therefore, is not taken up by lymph but diffuses directly into blood capillaries. Unfortunately, the exact name of the product used is not always given so that the question of possibly another nomenclature problem cannot be answered. However, methylene blue is structurally not related to isosulfan blue or patent blue V, and, therefore, cross-reactivity is not to be expected.

CONCLUSION

Anaphylactic reactions are a dreaded intraoperative complication. As SLNBs are increasingly performed in patients with various malignant tumors, the likelihood of anaphylactic reactions to vital blue dyes increases. Incidences of 0.6% to 2.7% for anaphylactic reactions to either isosulfan blue or patent blue V necessitate awareness of the risk on the part of the surgeon and the anesthesiologist. Preoperative antiallergic prophylaxis apparently reduces only the severity of the reactions and not the number of adverse events, with a consecutive increase in postoperative wound problems. Preoperative intracutaneous testing, which can easily be performed, should, therefore, be taken into consideration as a potential diagnostic procedure.

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