Chemiluminescence-Based IgE Dot-Blot Assay in the Diagnosis of Anaphylaxis Caused by Prontosan

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Prontosan Wound Solution (B. Braun) is a topical wound disinfectant containing the antiseptic agent polyhexanide (0.1% polyhexamethylene biguanide), with 0.1% undecylenamidopropyl betaine as a surfactant. Cases of anaphylaxis associated with polyhexanide have been reported [1-3]. No cases of undecylenamidopropyl betaine allergy have been described to date.

Our aim was to use a chemiluminescence-based immunoglobulin (Ig) E dot-blot assay to diagnose a case of anaphylaxis caused by Prontosan administered to treat a skin ulcer.

A 91-year-old man developed a severe anaphylaxis episode after topical administration of Prontosan as an antiseptic to treat skin ulcers on the lower limbs. The episode was treated with intravenous fluid therapy, intravenous corticosteroid therapy, and antihistamines. Two days later, during a second, accidental exposure, the patient developed hives with pruritus, nasal obstruction, and ear plugging, which resolved with antihistamines. The patient had previously experienced urticaria episodes following topical administration of Prontosan. Although chlorhexidine had not been applied prior to the episode of anaphylaxis, the patient's daughter reported an episode of urticaria after administration of topical chlorhexidine during a subsequent admission to hospital. No other drug or environmental allergic sensitizations have been reported to date. The patient gave his written informed consent for the study to be performed.

The allergology work-up comprised prick-to-prick tests, which yielded positive results for Prontosan (6 mm) and negative results for chlorhexidine (mild erythema). Twenty healthy controls were also tested, with negative results. The diameters of the wheals corresponding to controls were 5 mm for histamine and 0 mm for saline solution. The ImmunoCAP determination for total serum IgE was 98.4 kU/L; specific IgE for chlorhexidine was negative (<0.10 kU/L).

A chemiluminescent dot-blot assay was performed to detect specific IgE for Prontosan and its components. The samples were previously coupled to bovine serum albumin (BSA) as a carrier molecule, as follows: 1 mL each of Prontosan (as is), polyhexanide (biguanide) (2.5%), and cocamidopropyl betaine (1%)—undecylenamidopropyl betaine could not be obtained—was mixed with 1% BSA in phosphate-buffered saline and incubated for 3 hours in the dark at room temperature and then left at 4°C overnight.

Given the absence of literature references that would enable us to calculate the correct amount of Prontosan and its components before loading the dot-blot assay, we tested several volumes of each compound. Based on the results of these previous experiments, we selected 10 µL polyhexanide and 500 µL of cocamidopropyl betaine. Concluding initially that polyhexanide was the cause of the reaction, we tested a range of Prontosan dilutions that contained a similar amount of polyhexanide to ensure a positive dot-blot signal; the best signal was obtained at 1250 µL. Thus, drop aliquots of Prontosan (1250 µL), polyhexanide (10 µL), cocamidopropyl betaine (500 µL), and BSA (10 µL) (Roche) as a negative control were placed onto a supported 0.45-µm nitrocellulose membrane (Bio-Rad) and incubated overnight with the patient's serum (diluted 1:5). Specific IgE was detected by incubation for 2 hours at room temperature with a monoclonal mouse antihuman IgE antibody conjugated with horseradish peroxidase (HRP) (Southern Biotech) at a 1:10 000 dilution. The reaction was developed with the WesternBright ECL HRP substrate (Advansta), and the chemiluminescence signal was acquired using an Alliance system (UVITECH).

As shown in the figure, the patient's serum reacted to Prontosan and polyhexanide but not to cocamidopropyl betaine. Polyhexanide belongs to biguanide antiseptics such as chlorhexidine, which is considered to be responsible for anaphylaxis [4,5]. Cross-reactivity has been reported between polyhexanide and chlorhexidine [3,5]. Thus, although the result of the specific IgE ImmunoCAP determination for chlorhexidine was negative, the patient's serum was also tested by dot-blot following the same protocol, and a negative result was obtained (data not shown).

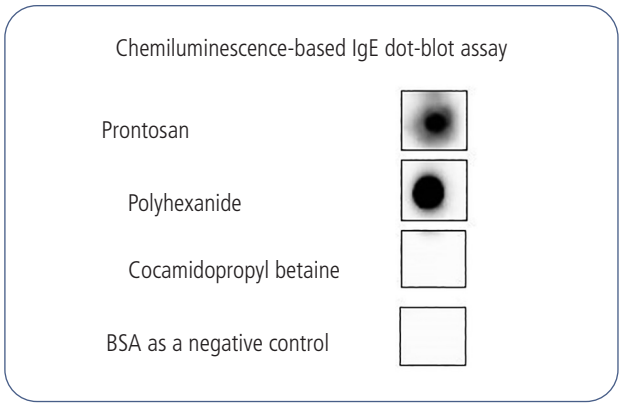

Figure. Chemiluminescence-based IgE dot-blot assay performed with the patient's serum to detect specific IgE to Prontosan and the components of its formulation, namely, polyhexanide (biguanide) and cocamidopropyl betaine—undecylenamidopropyl betaine was not available—with BSA as a negative control. BSA indicates bovine serum albumin.

The lack of commercial tests available for the diagnosis of allergic reactions caused by drugs or their components makes it necessary to use in vitro methods such as dot-blotting. This technique has previously demonstrated its ability to accurately identify the culprit reactive agent, as in other reported cases of anaphylaxis caused by folic acid, polyvinylpyrrolidone, carboxymethylcellulose, and macrogol 6000, among others [6-9]. However, not all molecules bind to the nitrocellulose or polyvinylidene difluoride membranes commonly used for dot-blotting, or the sample contains a sufficient amount of the molecules for detection; therefore, methods that offer greater sensitivity are required. Chemiluminescence immunoassays are applied owing to their high sensitivity to specific IgE in serum [10].

In conclusion, a chemiluminescence-based IgE dot-blot assay was a reliable tool for identifying polyhexanide as the causative agent in a case of anaphylaxis caused by Prontosan. A chemiluminescence-based IgE dot-blot assay should be considered an option when attempting to identify sensitization in an allergology work-up for drug allergy.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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