Chemiluminescence-based IgE dot-blot assay to diagnose a case of anaphylaxis caused by Prontosan

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.18176/jiaci.0933
Key words: Anaphylaxis. Biguanide. Chemiluminescence. IgE. Polyhexanide.


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 previously described [1-3]. No cases of undecylenamidopropyl betaine allergy have been reported.

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

A 91-year-old male developed a severe anaphylaxis episode related to topical Prontosan administration employed as an antiseptic for the healing of skin ulcers in the lower limbs. Administration of intravenous fluid therapy, intravenous corticosteroid therapy, and antihistamines to reverse the anaphylaxis were required. Two days later, an episode of hives with pruritus and nasal and otic packing occurred during a second accidental exposure, which ceased with antihistamines. The patient had previously experienced urticaria episodes following topical Prontosan administration. Although no chlorhexidine cures had been documented prior to the anaphylaxis, an urticaria episode after topical chlorhexidine administration on subsequent admission was noted by his daughter. No other drug or environmental allergic sensitisations are known, to date. Written informed consent was obtained from the patient to perform the study.

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

A chemiluminescent dot-blot assay was developed 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 of each, Prontosan (as is), polyhexanide (biguanide) (2.5%), or cocamidopropyl betaine (1%) (employed because undecylenamidopropyl betaine was impossible to obtain), 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 that there were no previous references in the literature to calculate the correct amount of Prontosan and its components to load in the dot-blot assay, several volumes of each compound were tested. Based on the results of these previous experiments, 10 µL polyhexanide and 500 µL cocamidopropyl betaine were selected. We then drew the preliminary conclusion that polyhexanide was the cause of the reaction, so we tested a range of Prontosan dilutions that contained a similar amount of polyhexanide that returned 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 detection was performed by incubation 2 hours at room temperature with a monoclonal mouse anti-human IgE antibody conjugated with horseradish peroxidase (HRP) (Southern Biotech) at 1:10,000 dilution. The reaction was developed with the WesternBright ECL HRP substrate (Advansta), and the chemiluminescence signal was acquired with an Alliance system (UVITECH, Cambridge).

As shown in the figure, the patient’s serum reacted to Prontosan and polyhexanide and not to
undecylenamidopropyl betaine. Polyhexanide belongs to biguanide antiseptics such as chlorhexidine, which has been reported to be responsible for anaphylaxis cases [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).

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 utility in identifying the precise responsible reactive agent, as described for other reported cases of anaphylaxis caused by folic acid, polyvinylpyrrolidone, carboxymethylcellulose, or macrogol 6000, among others [6-9]. However, not all molecules bind to the nitrocellulose or polyvinylidene difluoride membranes commonly used for dot-blotting, or they are in a sufficient amount in the sample to be detected; thus, methods that offer greater sensitivity are required. Chemiluminescence immunoassays are being applied due to their high detection 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 to identify sensitisation in an allergological workup of drug allergy.

The patient signed an informed consent to publish the study of his case.
Acknowledgements

We thank Roxall Medicine Spain for supplying us with polyhexanide and cocamidopropyl betaine to perform the dot-blot assay.

Funding

The authors declare that no funding was received for the present study.

Conflicts of Interest

The authors declare that they have no conflicts of interest to disclose.

References


Chemiluminescence-based IgE dot-blot assay performed with the patient’s serum to detect specific IgE antibodies to Prontosan and the components of its formulation, polyhexanide (biguanide) and cocamidopropyl betaine (given that undecylenamidopropyl betaine was not available), with BSA as a negative control.