

IgE antibody-mediated shock reaction caused by topical application of chlorhexidine

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(Received in original form 25 October 1985; accepted for publication 21 November 1985)

Summary

A case of an anaphylactic shock following topical application of chlorhexidine preparation is reported. Specific skin-sensitizing antibodies against chlorhexidine were demonstrated in the serum from the patient by a passive transfer test. IgE antibodies against chlorhexidine were also detected by radioallergosorbent technique (RAST). Paper discs conjugated with chlorhexidine-HSA (human serum albumin) significantly bound the IgE antibodies. Furthermore, all of the sera from seven other patients with shock reactions following the topical application of chlorhexidine preparation also showed high RAST counts. Both chlorhexidine gluconate and chlorguanide which represents approximately half a molecule of chlorhexidine inhibited the reaction in a dose-dependent fashion. It is suggested that the shock reactions following topical application of chlorhexidine are mediated by IgE antibodies against chlorhexidine and that chlorhexidine and chlorguanide share the same antigenic determinant.

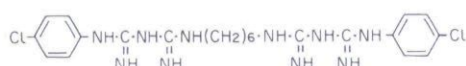
Introduction

Chlorhexidine is an antiseptic which is widely used for disinfection of skin, mucosa and medical instruments. It is both bacteriocidal and bacteriostatic at low concentration, to a broad spectrum of bacteria. The chemical structure is simple with a molecular weight of 505.48 daltons (Fig. 1). For clinical sterilization purposes chlorhexidine is used either as gluconate or hydrochloride salt. Adverse reactions due to chlorhexidine have been well documented [1-7], but anaphylactic shock following topical application of chlorhexidine has not been previously reported. This work presents a case of such an anaphylactic shock, with the demonstration of specific anti-chlorhexidine antibodies of the IgE class. Results of a RAST analysis in seven other similar patients are also reported.

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Chlorhexidine

1,1'-hexamethylenebis[5-(p-chlorophenyl)biguanide]

 $C_{22}H_{30}N_{10}Cl_2$ (mol wt 505.48)

Chlorguanide

1-(p-chlorophenyl)-5-isopropylbiguanide

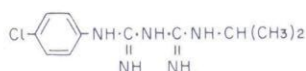
 $C_{11}H_{16}N_5Cl$ (mol wt 253.75)

Fig. 1. Chemical structure of chlorhexidine and chlorguanide.

Case report

A 24-year old male cosmetologist sustained an abraded wound on his left elbow, which was treated with a solution of 0.5% chlorhexidine gluconate in alcohol. Ten minutes later, itching of the left eye lid, generalized geographical urticaria, and hyperaemia of the palpebral conjunctiva developed, and 60 min later, the patient was cold and shivering, blood pressure had dropped, with subsequent loss of consciousness. He was admitted to hospital and emergency treatment, including oxygen inhalation, adrenalin, corticosteroids and aminophylline, was started. Full recovery occurred over a 48-hr period without any overt clinical sequelae. After 20 days, he was referred for further examinations. The only abnormal finding was an IgE level of 1750 IU/ml.

Previous medical history revealed an episode of generalized urticaria of unknown origin 3 years ago. As far as the patient knew, he had never received topical treatment with chlorhexidine, and there was no family history of atopic diseases.

Investigations

(1) Prausnitz-Küstner (P-K) test

The Prausnitz-Küstner test was done with the consent of his wife as a recipient. The serum was first checked for Hepatitis B surface (HBs) antigen and tested for syphilis (STS); both were negative. The recipient had no atopic diseases and her serum IgE level was within normal limits.

A skin-prick test using 0.02% CHG-saline solution (purchased from Imperial Chemical Industries (ICI), Cheshire U.K.) was first carried out with negative results. A 0.1 ml sample of the patient's serum was then injected intradermally into each of three sites (a, b, and c). The same quantity of normal serum was injected into three other sites (d, e, and f). After 24 hr, 0.02 ml of 0.0002%, 0.00002% CHG solution, or saline was injected respectively into the same sites, and reactions were assessed after 15 min. The greatest and smallest diameters of the weal and flare were recorded (Table 1).

Table 1. Prausnitz-Küstner (P-K) and P-K consumption test

Site	Serum*	First challenge		Second challenge	
		Concentration of CHG†	Weal‡	Concentration of CHG§	Weal‡
			Flare		Flare
a	Pt	0.0002%	$\frac{10 \times 10}{42 \times 42}$ pp(+)	0.0002%	$\frac{(-)}{12 \times 12}$
b	Pt	0.00002%	$\frac{9 \times 9}{35 \times 35}$	0.0002%	$\frac{9 \times 9}{35 \times 30}$
c	Pt	Saline	$\frac{6 \times 6}{8 \times 8}$	0.0002%	$\frac{11 \times 12}{40 \times 45}$ pp(+)
d	Normal	0.0002%	(-)	0.0002%	(-)
e	Normal	0.00002%	(-)	0.0002%	(-)
f	Normal	Saline	(-)	0.0002%	(-)

* 0.1 ml of the patient's serum or normal serum was intradermally injected into each site.

† After 24 hr, 0.02 ml of each concentration of chlorhexidine gluconate or saline was intradermally injected into each site as the first challenge.

‡ The reactions were inspected after 15 min. The greatest and smallest diameters of the weal-and-flare were recorded. The pp shows pseudopodium.

§ 72 hr after the first challenge, 0.02 ml of 0.0002% chlorhexidine gluconate was injected into each site as the second challenge.

The sites (a and b) sensitized with the patient's serum and challenged with CHG solution showed positive weal-and-flare reactions, but the site (c) sensitized with the patient's serum and challenged with saline was negative, as were sites (d, e, and f) injected with normal serum.

Seventy-two hours following the initial challenge, 0.02 ml of 0.0002% CHG solution was injected intradermally into each site. The results are also seen in Table 1. It can be seen that the site (c) sensitized with the patient's serum and previously challenged with saline now developed a weal-and-flare. The site (a) sensitized with the patient's serum and previously challenged with a 0.0002% CHG solution showed now negative. The site (b) sensitized with the patient's serum and previously challenged with a 0.00002% CHG solution showed a positive reaction. All of the sites injected with normal serum developed no reactions as expected.

(2) IgE RAST

Chlorhexidine benzoate was conjugated with HSA (human serum albumin) by carbodiimide [8]. The chlorhexidine-HSA conjugates were then coupled to CNBr-activated filter paper discs. These discs were prepared and kindly supplied by ICI.

The RAST results were expressed as % RAST count, calculated as follows;

% RAST count:

$$= \frac{{}^{125}\text{I-anti IgE bound (cpm)} - \text{blank (cpm)}}{\text{total } {}^{125}\text{I-anti IgE (cpm)} - \text{blank (cpm)}} \times 100(\%).$$

The % RAST count of the serum from the shock patient was 30.5%, while that of the normal serum was 1.7%.

CHG solution was added to the patient serum in equal volumes and incubated at 37°C for 1 hr before testing in a RAST assay. As chlorhexidine is a symmetrical molecule, chlorguanide (ICI) which is marketed as the antimalarial 'Paludrine' and represents approximately half the compound, was also used in the inhibition assay.

Varying dilutions of the compounds were used and Fig. 2 shows the results obtained. Binding to the solid support was reduced by the free compounds in a dose dependent manner. The concentration of CHG solution which showed 50% inhibition

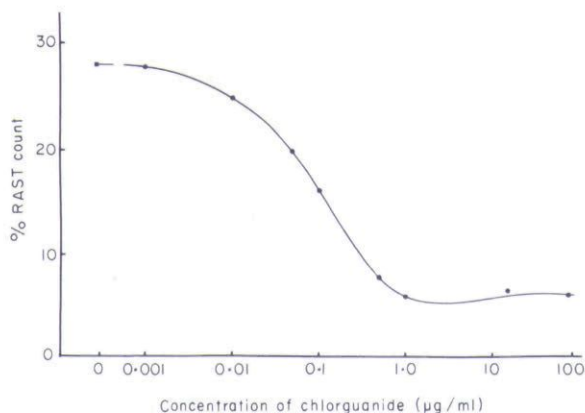


Fig. 2. Titer of specific IgE antibodies (RAST) and RAST inhibition by chlorhexidine. The concentration of CHG molecules necessary for 50% inhibition was 1.5 µg/ml (3.0×10^{-7} M).

was 1.5 µg/ml, that is 3.0×10^{-7} M. Chlorguanide reduced the % RAST count dose-dependently in the same manner as CHG (Fig. 3). The concentration of chlorguanide which showed 50% inhibition was 1.0 µg/ml, that is 4.5×10^{-7} M. The number of chlorguanide molecules necessary for 50% inhibition was almost the same as that of chlorhexidine.

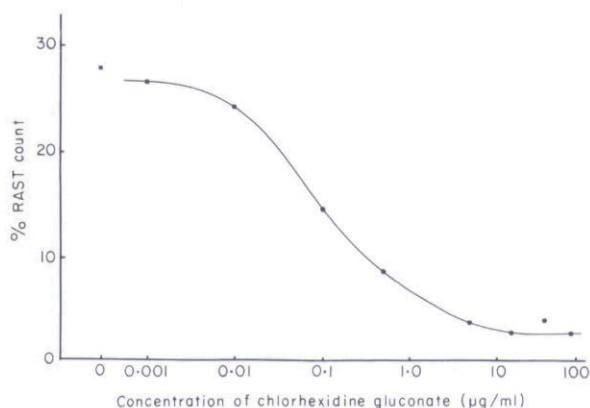


Fig. 3. Titer of specific IgE antibodies (RAST) and RAST inhibition by chlorguanide. The concentration of chlorguanide molecules necessary for 50% inhibition was 1.0 µg/ml (4.5×10^{-7} M).

Clinical course

After these investigations, a further episode of generalized urticaria but with no dyspnoea developed an hour after he took habitual 'lozenges'. It disappeared within 4 hr. A similar episode of urticaria but with asthma-like symptoms occurred when he used a 'lotion' as a skin disinfectant.

Follow-up investigations afforded an opportunity to monitor the IgE antibody responses and the results are shown in Fig. 4. As can be seen there is a loss of activity with time. The counts showed 27.8% in 1982, 16.4% in 1983, 10.7% in 1984 and 11.1% in 1985. The last one is still higher than that of the mean normal sera ($2.3 \pm 0.3\%$).

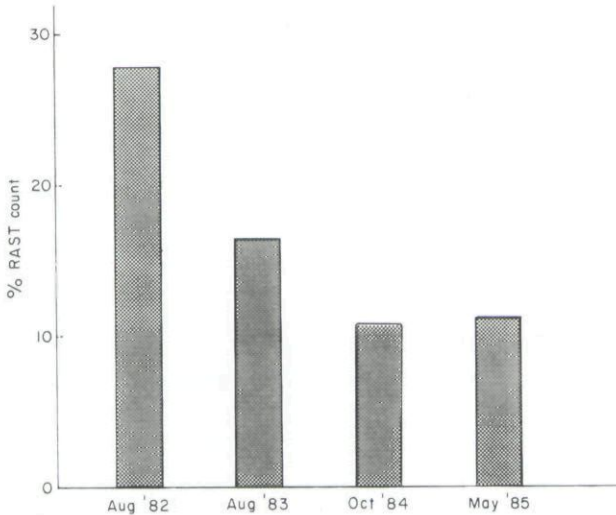


Fig. 4. Time course of specific IgE antibodies (RAST) titers of the serum from the patient.

IgE RAST and RAST inhibition assay on other seven sera

In Japan, over thirty cases of patients, who showed shock reaction following application of chlorhexidine, have been registered from 1974 to 1984 [9–12]. Seven sera from seven patients were referred to us. IgE RAST and RAST inhibition assay were carried out on these sera. The results are shown in Table 2. The mean % RAST count of the eight patients' sera was $15.6 \pm 3.1\%$, while that of the normal sera ($n=10$) was $2.3 \pm 0.3\%$. This difference was statistically significant ($P < 0.01$). The mean % RAST count of the patients' sera preincubated with the same volume of 15 $\mu\text{g/ml}$ CHG solution reduced to $4.5 \pm 1.0\%$.

Discussion

Chlorhexidine is used world-wide in a number of formulations for antiseptic purposes. Adverse reactions of chlorhexidine, toothstaining by dentifrices [1, 2], gingival bleeding by mouth-rinses [3], conjunctivitis by soft contact lens solutions [4, 5], and contact dermatitis by topical application [6, 7] are well documented and are not life-threatening. Anaphylactic reactions by topical application of chlorhexidine had not been reported.

In general, topical application of agents rarely induces anaphylactic reactions [13, 14]. Our study, we believe, is the first to demonstrate a specific skin-sensitizing

Table 2. IgE RAST and RAST inhibition assay by CHG on the sera from eight patients with CHG-induced shock

Case no.	Age	Sex	% RAST count*	RAST inhibition assay† (%)	Inhibition (%)
1‡	24	M	30.5	5.8	81.0
2	27	F	12.0	2.3	80.0
3	67	F	15.5	4.5	71.0
4	41	M	4.7	2.2	53.2
5	24	F	8.0	1.3	83.8
6	9	M	7.3	2.7	63.0
7	14	M	29.8	2.6	91.3
8	7	M	16.3	not examined	—
normal	—	—	2.3	—	—

* The % RAST counts of the sera from the patients with CHG-induced shock.

† The % RAST counts of the sera after preincubation with the same volume of 150 µg/ml CHG solution.

‡ Case no. 1 is our case.

antibody *in vivo* by a passive transfer test, and to detect anti-chlorhexidine IgE antibodies *in vitro* by RAST assay. Furthermore, the sera from seven other patients with shock reactions following topical application of the preparation also showed high RAST counts. The RAST counts were markedly reduced by pretreatment of the sera with chlorhexidine demonstrating the antigenic specificity. These results suggest that the anaphylactic shock reaction is induced in these patients by topical application of chlorhexidine mediated by specific IgE antibody against chlorhexidine.

Experiments using chlorguanide would suggest that chlorhexidine and chlorguanide share the same antigenic determinant. In our case, the patient had an episode of urticaria and asthma-like symptoms when he took habitual lozenges or used a lotion as a skin-disinfectant. It was likely that these preparations contained chlorhexidine as it is known to be incorporated into lozenges, lotions, gargles, disinfectants, agents for purulent infections, topical astringents, topical antiphlogistics, ectoparasitocides, emollients and toothpaste, which are easily obtained at drug stores and supermarkets [15]. Exposure to chlorhexidine by the use of these preparations may have accounted for the induction of specific antibodies. The elicitation event using 0.05% chlorhexidine gluconate would not be fortuitous.

Acknowledgments

We wish to thank Imperial Chemical Industries (ICI), Cheshire, U.K. for kind donation of CHG-conjugated paper discs. And we also thank Miss Mutsuko Miyamoto for her excellent technical assistance and secretarial work. This research was supported in part by a grant from the Manabe Medical Foundation.

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