

Safety of Chitosan Bandages in Shellfish Allergic Patients

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ABSTRACT Background: In 2005, the Office of the Surgeon General mandated that every soldier carry a HemCon bandage. Made from chitosan, a polysaccharide derived from shrimp shells, this bandage effectively stops bleeding. There are no studies reporting the safety of this bandage in shellfish allergic patients. Methods: Patients who reported shellfish allergy were recruited. Initial assessment included a detailed history, IgE skin prick testing (SPT), and serum testing to shellfish allergens. Participants who demonstrated specific shellfish IgE underwent a bandage challenge. Results: Nineteen participants were enrolled; 10 completed the study. Seven (70%) were male and the average age was 44.8 + 10 years. Nine (90%) reported a shrimp allergy history and five (50%) reported multiple shellfish allergies. All participants completing the study had positive SPT and serum IgE testing to at least one shellfish; eight (80%) had shrimp positive SPT and ten (100%) demonstrated shrimp-specific IgE. No participant had a positive SPT to chitosan powder or experienced an adverse reaction during bandage challenges. No protein bands were visualized during gel electrophoresis analysis of chitosan powder. Conclusion: All participants tolerated the HemCon bandage without reaction. This is the first study demonstrating the safety of this bandage in shellfish allergic subjects.

INTRODUCTION

In September 2005, the OTSG approved the distribution of the HemCon bandage (HemCon Medical Technologies, Portland, Oregon) to deployed soldiers. This decision was followed by the addition of this bandage to the improved first aid kit in FY08.¹ The bandage is portable, lightweight, and stops arterial bleeding.^{2,3} The bandage is also latex free, can be stored at room temperature, has 3-year shelf life, and creates an antibacterial barrier upon application. The bandage is made from ChitoClear chitosan (Primex, Siglufjörður, Iceland), a naturally occurring compound found in the shells of the pink North Atlantic shrimp *Pandalus borealis*.⁴ An initial safety study demonstrated no reaction to ChitoClear chitosan skin testing in 221 random subjects including four who had a positive skin prick testing (SPT) to shrimp.⁴ However, the positive predictive value of a positive SPT in unselected individuals is <50%. Further, although there have been no reports of adverse reactions to the bandage, HemCon Technologies warns that “individuals with known shellfish allergies should exercise caution in the use of products containing chitosan.”⁴

Shellfish allergy is common, being reported in 2.8% of adults and 0.6% of children.⁵ Shrimp is the most common shellfish allergy followed by crab and lobster. Although symptoms are usually limited to cutaneous manifestations (e.g., urticaria, angioedema, flushing), anaphylaxis is reported in 20 to 40% of reactions.⁵ Shrimp allergy represents an IgE-mediated reac-

tion to the shrimp protein tropomyosin. Tropomyosin, a muscle protein, shares significant homology with other shellfish in the *Crustacea* family—hence, most patients who are allergic to one are usually allergic to all *Crustacea*.⁶ This study was conducted as there are no studies assessing the safety of the HemCon bandage in patients reporting a shellfish allergy.

METHODS

Participants

The study (protocol number C.2007.164) was approved by the Brooke Army Medical Center Institutional Review Board. Men and women aged 18- to 60-years old were approached during encounters in the allergy clinic or after responding to study advertisement within the hospital. Patients who reported a history consistent with shellfish allergy were offered study enrollment. Details regarding food allergy history were taken including duration of food allergy, last reaction, timing of reactions, and reaction symptoms. All participants with a history of asthma underwent pulmonary function testing; forced expiratory volume in one second < 80% was an exclusion criteria. Women performed a urine pregnancy test before SPT.

Statistical Analysis

This was an observational pilot study. After a review of the literature, there were no identified studies that determined an initial power analysis. 87% of individuals with an immediate shrimp allergy history, a positive IgE shrimp SPT, and a positive serum IgE shrimp will react to an oral shrimp challenge.⁷ The primary outcome is the probability of morbidity after 1 hour of skin contact with a HemCon bandage among shellfish allergic subjects. Because there is a possibility that all bandage wearing events will be negative, a Poisson distribution was used to calculate the 95% CI of the probability of an event for which all observations are negative. The upper limit of the

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95% CI was taken as the estimated probability of the event, calculated using the equation: 95% CI High = Maximum risk = $1 - 0.5^n$. With 30 negative observations the maximum risk is 14.2%; with 10 negative observations the risk is 36.9%.

Allergy Testing

Participants meeting study criteria had SPT and serum IgE testing. Oral and nasal antihistamines were stopped for 1 week before SPT. All SPT used the GreerPick (Greer Laboratories; Lenoir, North Carolina) pressed on the skin at a 90° angle by a single investigator (K.H.W.). SPT for each participant included a negative saline control, a positive histamine control (1 mg/mL; ALK Laboratories, Wallingford, Connecticut), and a shellfish panel consisting of crab, lobster, and shrimp (1:20 w/v; Hollister-Stier, Spokane, Washington). A prick-prick test using a chitosan slurry (0.15 g chitosan mixed with 0.6 mL 0.9% NaCl) was also performed. Participants were observed for 15 minutes, and SPT were interpreted by a single investigator (K.H.W.). Wheal and flare measurements were determined by the average of perpendicular diameters. A mean wheal diameter of >3mm compared to the negative control wheal defined a positive SPT. Participants who had negative skin testing to all three shellfish were removed from the study. Subjects with ≥1 positive shellfish SPT were evaluated for shrimp-specific serologic IgE with ImmunoCAP (Phadia, Uppsala, Sweden) IgE testing with additional serum stored at -70°C for immunoblot testing.

Bandage Application

A 2 × 2 inch HemCon bandage was moistened with water and placed on the anterior aspect of the mid forearm. The bandage remained adherent for 1 hour and then was gently removed with water. Participants used a 5-point Likert scale to assess discomfort with 1 representing "not bothersome at all," 2 "mildly bothersome," 3 "moderately bothersome," 4 "very bothersome," and 5 "as bothersome as I can imagine." Participants were telephoned 24 hours later to document any delayed symptoms.

Chitosan Analysis

Protein extraction was performed using 5 g of ChitoClear chitosan powder. This was added to 100 mL of a 0.125M ammonium bicarbonate with 1% acetic acid and stirred overnight at 4°C. The solution was centrifuged at 4,000 rpm for 30 minutes. The supernatant was filtered with the filtrate dialyzed in 3,500 Dalton cutoff dialysis membranes (Spectra/Por EZ-1 MultiDialyzer with Spectra/Por 3 membranes) (Krackeler Scientific Inc., Albany, New York) against 3 changes of double deionized water over a 24-hour period. The following day, the dialysate was drained and frozen at -20°C, then lyophilized with a Labconco FreeZone Freeze Dry System (Labconco Corporation, Kansas City, Missouri). Protein content of the lyophilized product was determined using the Modified Micro-Lowry Protocol against a 25 to 200 ng/mL bovine serum albumin standard curve.

A 10 to 20% gradient Tris-glycine polyacrylamide gel (PAGErGold Pre-cast Gels) was electrophoresed with a sodium dodecyl sulfate running buffer at 125v for 2 hours (Hoefer SE 250-Mighty Small II) (Hoefer Inc., Holliston, Massachusetts) with a 5- to 225-kD marker (ProSieve number 50547) (Lonza Rockland Inc., Rockland, Maine). Product was loaded at 12.5, 25, 50, and 100 µg per well.

RESULTS

Nineteen participants were enrolled; 10 completed the study (Fig. 1). Seven (70%) were male and the average age was 44.8 ± 10 years. Nine (90%) reported a clinical history of shrimp allergy and five (50%) reported multiple shellfish allergies (Table I). The median duration of food allergy was 31 years (range 14–42). The median time from the last clinical reaction was 12.5 years (range 0.75–30). All reported reactions occurred within 1 hour of ingestion, consistent with an IgE-mediated reaction. Subjects reported their most severe clinical reaction as either Mueller class 2 (40%) or 3 (60%).

All participants completing the study had positive SPT and serum IgE testing to at least one shellfish (Table I). Specifically for shrimp, eight (80%) were positive on SPT and ten (100%) had positive shrimp serum IgE testing with a median of 9.5 kU/L (range 0.51–51.7). The reaction severity (Mueller classification) did not correlate with shrimp-specific IgE levels ($p = 0.6$). SPT to chitosan was negative in all participants. Two

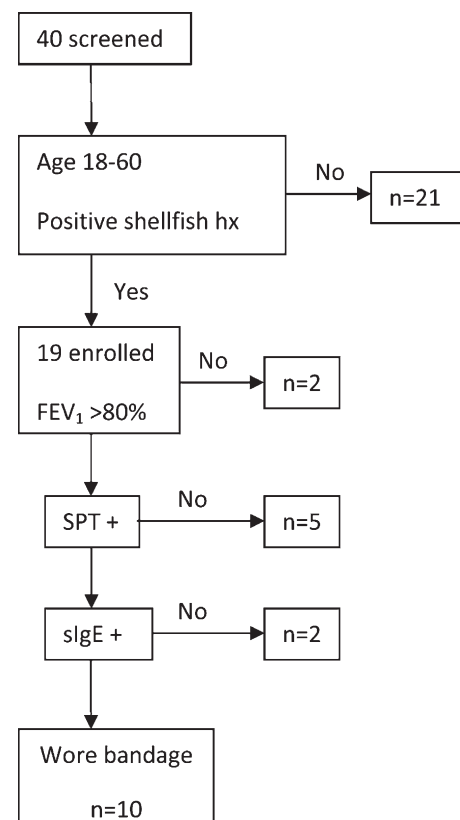


FIGURE 1. Study algorithm.

TABLE I. Clinical and Immunologic Characteristics of Participants

Age (Year)/Sex	Shellfish Allergy History				Skin Testing (mm)								Serum IgE (kU/L)		
	Crab	Lobster	Shrimp	Mueller Class ^a	Crab		Lobster		Shrimp		Chitosan		Crab	Lobster	Shrimp
					W	F	W	F	W	F	W	F			
47/M	No	No	Yes	II	14	20	15	25	15	30	0	3	7.1	4.18	9.24
54/F	Yes	No	Yes	III	4	10	3	5	0	3	0	3	0.4	0.38	0.51
55/M	Yes	Yes	Yes	III	15	30	10	25	10	18	0	1	8.8	10.4	9.85
54/F	No	No	Yes	II	11	20	12	20	12	20	0	1	5.1	1.97	7.23
49/F	Yes	No	Yes	II	6	50	6	30	6	30	0	3	22	31.1	24.6
52/M	No	No	Yes	III	11	30	11	15	11	20	3	3	35	40.7	34.2
39/M	No	No	Yes	II	6	15	10	25	10	23	3	0	37	39.2	51.7
37/M	Yes	No	No	III	4	13	0	3	2	4	0	5	0.5	0.72	1.27
36/M	Yes	No	Yes	III	8	25	9	26	7	30	2	4	1.1	1.69	2.1
25/M	No	Yes	Yes	III	6	28	3	10	5	30	0	3	32	34.4	34.8

W, wheal; F, flare. ^aMueller classification: I, Urticaria, pruritis; II, Angioedema, chest tightness, N/V, dizziness, abdominal pain; III, Dyspnea, wheeze, stridor, dysphagia, hoarseness; IV, Hypotensive collapse, LOC, incontinence, cyanosis.

participants had a 3-mm wheal to chitosan; however, their negative control SPT also demonstrated a 3-mm wheal. No participant experienced an adverse reaction to the bandage. Seven (70%) reported the bandage as “not bothersome” and 3 (30%) reported the bandage as mildly bothersome. No erythema, urticaria, or angioedema were observed at the bandage site. No participant reported any symptoms within 24 hours of the bandage challenge.

Amount of product extracted from chitosan powder was low, ranging from 13.7 to 67 µg/mL with low total weight yield. Gel electrophoresis with Coomassie Blue R250 yielded a good marker separation and stain, but there were no protein bands for the chitosan powder; therefore, no Southern blotting of the participant’s serum was performed.

DISCUSSION

This is the first study to our knowledge that prospectively studied the safety of the HemCon bandage in a group of shellfish allergic subjects. The need to ensure the safety of this bandage is significant as over 700,000 HemCon bandages have been distributed, and chitosan-based products are being applied for emergency and dental care.⁴ Interestingly, no case reports have been published regarding the use of this bandage in a soldier who has a concurrent shellfish allergy. Further, although an initial safety study showed negative skin tests to chitosan in 221 participants, this was an unselected patient group. Within this group only four (1.8%) participants had a positive SPT to shrimp and there were no details regarding the clinical history or serum IgE testing—both important aspects when determining the predictive value of an isolated positive skin test.

We hypothesized that study subjects would not experience a reaction to chitosan based on the extraction process. During extraction of shellfish exoskeletons, acid destroys carbohydrate side chains removing proteins such as tropomyosin while leaving chitin and calcium. Chitin can then be either converted to chitosan during deacetylation or to the more popular product glucosamine. Acid leaching may not be

complete, potentially allowing intact tropomyosin to remain. Both the HemCon bandage and over-the-counter oral glucosamine products carry warnings to use caution in shellfish allergic persons. Two recent studies, however, have demonstrated that glucosamine is well tolerated in shellfish allergic patients without adverse reaction.^{8,9} One study conducted a double-blind placebo controlled food challenge with a 1,500-mg shrimp-derived glucosamine tablet in 15 shellfish allergic patients.⁸ All subjects tolerated the tablet without reaction. A second study examined six adults with a history of shellfish allergy; all tolerated an oral challenge to 500-mg glucosamine without reaction.⁹ We extracted only insignificant protein quantities and no tropomyosin. Similarly, HemCon has not found shrimp protein in chitosan used in their bandage; chitosan samples are analyzed with mass spectrometry analyses and is able to detect ppb (Dr. Simon J. McCarthy, HemCon, personal communication, 2010).

One limitation of our study was that we did not perform oral food challenges to verify shellfish allergy. This was not done for two reasons. First, unlike other food allergies, the combination of a positive clinical history and positive IgE testing for shellfish correctly identifies 87% of patients with shellfish allergy on oral challenge.⁷ Second, most subjects in this study reported anaphylaxis during previous reactions, an outcome that we wanted to avoid. Another limitation was the number of subjects enrolled. Statistically, ten consecutive negative bandage challenges resulted in a maximum risk of 36.9%. To determine a 95% CI with $p = 0.5$, 384 subjects would be required. The 19 subjects enrolled in this study represented a 2-year recruitment effort within two major military hospitals. Finally, the bandage was applied to undamaged skin (i.e., not bleeding). Antigen uptake and presentation to dendritic cells and mast cells may have been decreased without deliberate skin injury before bandage application.

CONCLUSION

Recognizing these limitations, we feel the negative findings in this study are important and applicable. With a shellfish

allergy prevalence of 2.8% in adults, safe application of chitosan-derived products holds great interest. This is the only study to assess the safety of a chitosan bandage in shellfish allergic patients. We found that all subjects tolerated the bandage without reaction. Although larger cohort studies should be considered, the results from this study are encouraging and consistent with two previous studies demonstrating the safety of other chitin-derived products in patients allergic to shellfish.

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