

Activated charcoal forms non-IgE binding complexes with peanut proteins

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Background: Conventional management of peanut-induced anaphylaxis is composed of administration of epinephrine, antihistamine, and steroid and stabilization of airway, ventilatory, and circulatory function. Therapies directed toward slowing or preventing further absorption of peanut protein from the gastrointestinal tract after accidental ingestion have not been a routine part of management.

Objective: The purpose of this study was to determine the ability of activated charcoal to complex with peanut protein, thereby preventing its binding to either peanut-specific IgE or peanut-specific IgG.

Methods: Peanut protein was coincubated with micronized activated charcoal suspension at pH 3.5 or 7.4. Peanut protein complexed with charcoal was removed by centrifugation. Binding of residual peanut protein to peanut-specific IgG was measured by a sandwich ELISA assay. Also, ability of uncomplexed peanut protein to bind to peanut-specific IgE was determined by Western blot and by skin prick testing in subjects with peanut allergy.

Results: Activated charcoal (AC) formed complexes with peanut protein, effectively competing for binding with peanut-specific IgG in a sandwich ELISA assay. AC complexed efficiently with peanut protein at both neutral and acidic pH in as little as 60 seconds. AC was also able to remove IgE-binding peanut allergens from solution as determined by Western blot and by skin prick testing in subjects with peanut allergy. A ratio of 200 mg of AC to 1 mg peanut protein was required for complete removal of peanut protein from solution. AC was able to complex with peanut protein within food matrices such as ice cream and chocolate.

Conclusion: The data presented herein show that AC removes both IgE-binding and IgG-binding peanut proteins from solution rapidly at both neutral and acidic pH. These data suggest that administration of AC may be useful as an adjunct to slow or to prevent further absorption of peanut protein from the gastrointestinal tract after accidental ingestion by individuals with peanut allergy. (*J Allergy Clin Immunol* 2003;112:175-9.)

Key words: Peanut, complex, activated, ara h1, ara h2, charcoal, allergy, IgE, anaphylaxis, ELISA

Abbreviations used

TBS: TRIS-buffered saline

TTBS: TBS with 0.1% Tween 20

The routine approach to treatment of intoxication involves stabilization of the patient, toxidrome recognition, administration of antidote, and measures to enhance elimination of the toxin along with administration of supportive care.¹ In the context of food-induced anaphylactic reactions, routine management entails stabilization of airway, ventilatory, and circulatory function and administration of epinephrine, antihistamine, and steroid. Interestingly, one fundamental aspect of management of intoxication has not been applied to treatment of food-induced anaphylaxis. With some notable exceptions,² specific therapies are not routinely used to “decontaminate” the gastrointestinal tract—ie, to prevent further absorption of food allergens from the gut into the systemic circulation. In the absence of decontamination of the gastrointestinal tract, ongoing absorption of food allergens will contribute to perpetuation or worsening of the anaphylactic reaction.

Various strategies for gastric decontamination have been used to treat drug intoxications including emesis by syrup of ipecac, adsorption of toxins by activated charcoal, and whole bowel irrigation.^{3,4} Emesis is rarely used, whereas whole bowel irrigation is useful for lead, iron, and lithium poisoning.¹ Activated charcoal as a suspension has been shown to prevent absorption of a variety of drugs including diazepam, ibuprofen, citalopram, and fluoxetine.⁵⁻⁷ Volunteer studies have demonstrated that activated charcoal increases elimination of numerous drugs including amitriptyline, digoxin, nadolol, phenytoin, and sotalol.^{5,8} No reports were found of use of charcoal in management of allergic reactions resulting from accidental ingestion of food allergens.

Presently peanut is the most common cause of food-induced anaphylaxis and is the single most common cause of fatal anaphylaxis.^{9,10} Peanut allergy is now reaching epidemic proportions. Approximately 1% of the preschool age population in the United States and Britain is sensitized to peanut.^{11,12} Given the increasing prevalence of peanut allergy and the potential for ingestion of only trace amounts of peanut to cause life-threatening anaphylaxis, we undertook a study to examine the ability of activated charcoal to adsorb to peanut protein,

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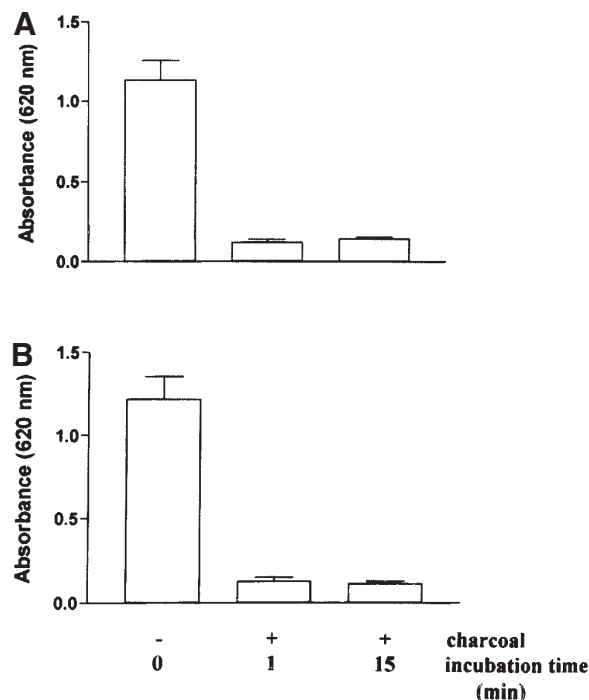


FIG 1. Removal of peanut protein from aqueous solution by activated charcoal. A, pH 7.4; B, pH 3.5

forming insoluble complexes. The efficiency of charcoal-peanut protein complex formation was assessed by 3 independent methods: by using a peanut-specific ELISA assay, by Western blot analysis, and by skin prick testing in individuals with peanut allergy.

METHODS

Defatted peanut protein was prepared by the method of Nordlee et al.¹³ Roasted peanuts were ground to a paste and defatted with acetone (2.5:1, v/w). The suspension was allowed to settle, and the acetone was decanted. The peanut paste was mixed with ethyl ether in the same proportion and allowed to settle, and the ether was discarded. This defatting procedure was repeated 5 times, and the resulting defatted peanut product was air dried overnight. Extract was prepared by adding 0.1 mol/L ammonium bicarbonate (pH 8.0, 3:1, v/w). The mixture was stirred for 20 hours at 25°C. The extract was clarified by centrifugation for 30 minutes at 20,000g at 2°C. The protein concentration of the supernatants was determined by the method of Bradford.¹⁴

Before use, a suspension of micronized activated charcoal in purified water (200 mg/mL; Pharma Science, Montreal, Quebec, Canada) was centrifuged at 10,000g for 2 minutes, and excess water was discarded. By using a stock solution of peanut extract (2.35 mg/mL), dilutions were prepared with 10 mmol/L PBS (pH 7.4). Dilutions of peanut extract (0.2 mg) were mixed with 0, 4, 10, 20, and 40 mg of charcoal at pH 7.4 or 3.5. Dilutions of peanut extract were coincubated with charcoal suspension for 1 or 15 minutes at 22°C.

Charcoal-treated peanut extracts were centrifuged twice at 10,000g for 2 minutes, and the resultant supernatants were assayed for residual peanut protein by specific sandwich ELISA (Quantitative Peanut Allergen Test; Neogen Corporation, Lansing, Mich). Samples were added to rabbit anti-peanut IgG antibody-coated wells. Unbound protein was washed away, and horseradish peroxidase-conjugated IgG

antibody was added. After a second wash, substrate was added. The reaction was allowed to develop for 10 minutes, and the absorbance of the resulting solution was read at 620 nm. Intra-assay (within run) and interassay (between run) precision coefficients of variation are as follows: for 100 ng/mL peanut protein, 12.6% and 14.8%, respectively; for 200 ng/mL peanut protein, 7.8% and 10.9%, respectively; for 400 ng/mL peanut protein, 6.2% and 8.9%, respectively; and for 1000 ng/mL peanut protein, 8.3% and 16.5%, respectively.

Western blot

Untreated and charcoal-treated peanut extracts were mixed with SDS sample buffer containing 2-mercaptoethanol, heated at 100°C for 2 minutes, and a volume of extract equivalent to 25 µg of protein was applied to each lane of polyacrylamide gel. Vertical slab SDS-PAGE was carried out by the method of Laemmli¹⁵ in Mini-Protein II (Bio-Rad, Mississauga, Ontario, Canada). Resolving gels (8 × 7.3 cm) were 12% w/v polyacrylamide, with 4% w/v stacking gels. Gels were run at 150 V for approximately 55 minutes in Tris-glycine buffer.

Separated extract proteins were electrophoretically transferred to Hybond ECL Nitrocellulose membrane (Amersham, Oakwill, Ontario, Canada) in semi-dry Transblot apparatus (Bio-Rad) for 40 minutes at 18 V in Tris-glycine buffer, containing 20% methanol. Protein transfer was ascertained by efficient transfer of prestained marker proteins as well as by staining the membrane with ponceau solution.

After transfer, the membrane was washed thoroughly with TRIS-buffered saline (TTBS; 20 mmol/L TRIS, 500 mmol/L NaCl, pH 7.6) and blocked with a solution of 5% BSA in TBS, with gentle shaking for 2 hours at room temperature. The membrane was washed 3 times with TTBS (TBS + 0.1% Tween 20) and cut into strips corresponding to the sample lanes of the original gel. The blots were then probed for antigen-specific IgE binding to the protein bands. Strips were incubated overnight at room temperature on a rocking platform in a 1:20 or 1:4 dilution of sera from individuals with peanut allergy in 5% BSA in TTBS and then washed for 15 minutes with each of 3 changes of TTBS. The blots were incubated for 2 hours at room temperature at 1:2000 dilution of goat anti-human IgE conjugated with alkaline phosphatase (ε-chain specific; Sigma, Oakwill, Ontario, Canada). After a final set of washes (3 changes of TTBS for 15 minutes each), the strips were developed with Immune-Star substrate with Enhancer (Bio-Rad) for 5 minutes at room temperature, wrapped, and exposed to Hyperfilm ECL (Amersham).

Skin prick tests

For skin prick testing in individuals with peanut allergy, commercial peanut extract (10 mg/mL) (Omega, Montreal, Quebec, Canada) was treated with activated charcoal as described above. After 1 minute coincubation with 0, 4, 10, 20, and 40 mg of charcoal, peanut extracts were centrifuged twice at 10,000g for 2 minutes. Supernatants were applied to the volar surface of the forearm and introduced percutaneously with a 27-gauge needle. The resultant wheal and flare were allowed to develop for 15 minutes.

RESULTS

The ability of activated charcoal to form complexes with peanut protein was assessed at neutral and acidic pH. Activated charcoal was able to form complexes with peanut protein, which effectively competed for binding with peanut-specific IgG in a sandwich ELISA assay. Activated charcoal complexed efficiently with peanut protein at both pH 3.5 and 7.4 in as little as 60 seconds. The lower pH value was used to mimic gastric conditions. Residual peanut protein after charcoal coincuba-

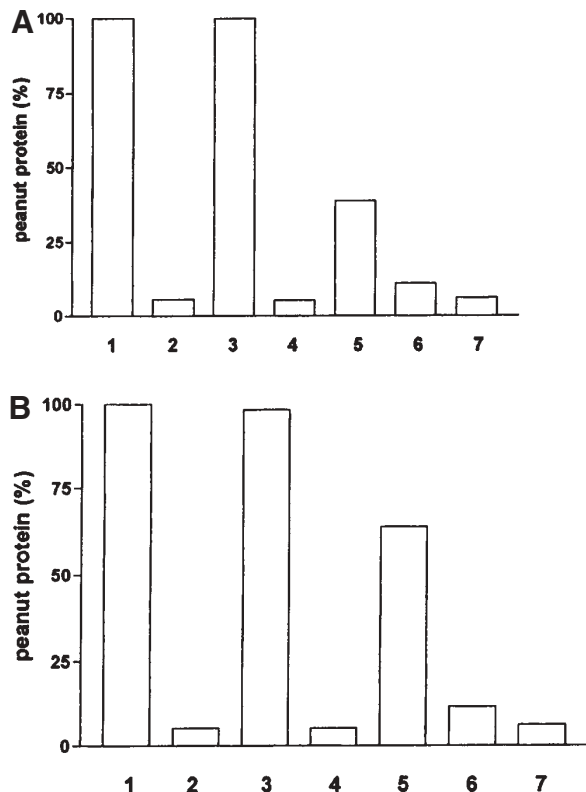


FIG 2. A, Binding of activated charcoal to peanut protein incorporated into a milk chocolate matrix. 1, peanut extract; 2, chocolate extract; 3, 1% peanut extract in chocolate; 4, peanut extract treated with charcoal (200:1); 5, 1% peanut extract in chocolate treated with charcoal (200:1); 6, 1% peanut extract in chocolate treated with charcoal (300:1); 7, 1% peanut extract in chocolate treated with charcoal (400:1). **B,** Binding of activated charcoal to peanut protein incorporated into a vanilla ice cream matrix. 1, peanut extract; 2, ice cream extract; 3, 1% peanut extract in ice cream; 4, peanut extract treated with charcoal (200:1); 5, 1% peanut extract in ice cream treated with charcoal (200:1); 6, 1% peanut extract in ice cream treated with charcoal (300:1); 7, 1% peanut extract in ice cream treated with charcoal (400:1).

tion was below the limits of detection of the ELISA assay (less than 100 ng/mL or 2.5 ppm). Residual peanut protein concentration after 15-minute incubation with activated charcoal was essentially unchanged as compared to 1-minute incubation. Removal of peanut protein from solution by charcoal was equally efficient at both neutral and acid pH (Fig 1).

The adsorptive capacity of activated charcoal for peanut protein was examined (Table I). Peanut protein was incubated for 60 seconds with different concentrations of charcoal at neutral pH. It was found that 40 mg of activated charcoal completely complexed with and neutralized 0.2 mg of peanut protein. Residual uncomplexed peanut protein was below the sensitivity of the ELISA (Table I). On the basis of these data, the optimal ratio of activated charcoal to peanut protein for efficient complex formation is 200 mg to 1 mg.

To test whether activated charcoal effectively adsorbs peanut protein when peanut protein was incorporated in

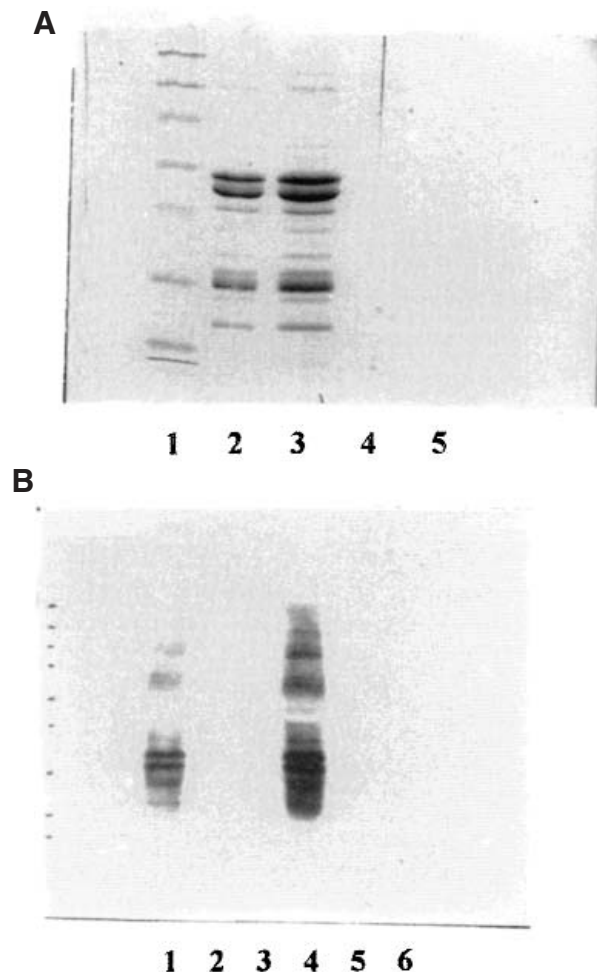


FIG 3. A, Coomassie blue R stained 12% SDS-PAGE gel. Lane 1, molecular weight standards; lane 2, peanut extract 12.5 µg total protein; lane 3, peanut extract 25.0 µg total protein; lane 4, peanut extract (25 µg) treated with activated charcoal, pH 7.4; lane 5, peanut extract (25 µg) treated with activated charcoal, pH 3.5. **B,** Immunoblot with peanut-specific IgE. Lanes 1 and 4, peanut standards; lanes 2 and 5, peanut extract treated with activated charcoal; lanes 3 and 6, peanut extract treated with activated charcoal; lanes 1, 2 and 3, treated with sera from patients with peanut allergy (1:20 dilution); lanes 4, 5 and 6, treated with sera from patients with peanut allergy (1:4 dilution).

low concentration in a food matrix, mixtures of peanut protein with milk chocolate or vanilla ice cream were tested. Extracts of chocolate and ice cream alone did not show any detectable peanut protein by ELISA (Fig 2). The ELISA assay was able to accurately detect peanut protein added to either chocolate or ice cream (Fig 2), indicating that the chocolate or ice cream food matrix did not interfere with the assay. Activated charcoal removed peanut protein from the 2 food matrices in a concentration-dependent manner. Whereas a significant effect was seen by using 200 mg of activated charcoal per mg of peanut protein in the food extract, complete adsorption and removal of peanut protein solution were seen with a ratio of activated charcoal of 400 mg per 1 mg of peanut protein.



FIG 4. Skin test reactivity in patient with peanut allergy by using peanut extract treated with activated charcoal. From *right to left*, peanut extract treated with 0, 20, 30, 40, 50, and 100 mg activated charcoal per mg peanut protein.

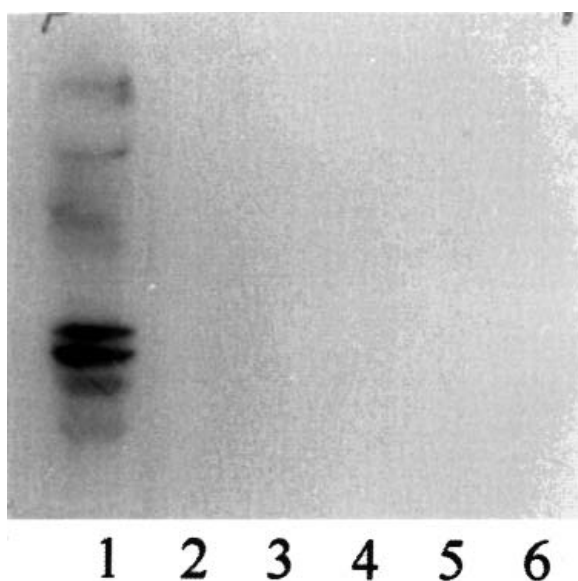


FIG 5. Western blot analysis of skin test solutions used in Fig 4. Peanut extract treated with 0 mg activated charcoal per mg peanut protein, *lane 1*; with activated charcoal:peanut protein (20:1), *lane 2*; with activated charcoal:peanut protein (30:1), *lane 3*; with activated charcoal:peanut protein (40:1), *lane 4*; with activated charcoal:peanut protein (50:1), *lane 5*; with activated charcoal:peanut protein (100:1), *lane 6*.

Activated charcoal binds peanut protein and prevents its recognition by a peanut-specific IgG in a quantitative ELISA assay. We next examined the ability of charcoal to complex with peanut protein in solution to remove IgE-binding proteins as determined by Western blot. Coincubation of solutions of peanut proteins with activated charcoal in ratios of 200 mg charcoal to 1 mg of peanut protein at both acidic and neutral pH resulted in complete removal of IgE-binding proteins (Fig 3).

TABLE I. Adsorptive capacity of activated charcoal for peanut protein

Activated charcoal (mg)	Peanut protein (mg)	Relative ratio of activated charcoal/peanut protein (mg:mg)	Residual peanut protein (ng/mL)
0	0.2	0:1	>1000
4	0.2	20:1	>1000
10	0.2	50:1	650
20	0.2	100:1	250
40	0.2	200:1	<100

Removal of IgE-binding proteins by charcoal was further assessed by skin test reactivity *in vivo* in patients with peanut allergy. Commercial peanut extract was treated for 1 minute with progressively larger amounts of charcoal (0, 20, 30, 40, 50, and 100 mg per 1 mg peanut protein), and supernatants were used for skin testing. A typical result is shown in Fig 4. Whereas control (untreated) peanut extract showed marked skin test reactivity at 15 minutes in a patient with peanut allergy, substantial reduction in skin test reaction was seen after a 1-minute coincubation even with 20 mg of charcoal per 1 mg of peanut protein. Complete loss of skin test reactivity was seen at higher concentrations of activated charcoal. When solutions of peanut protein used for skin prick testing were analyzed by Western blot, the results of skin prick testing corresponded well with the results of Western blot analysis (Fig 5).

DISCUSSION

The principle of gastric decontamination to prevent absorption of specific food allergens or to enhance their elimination has not been applied to peanut-induced anaphylaxis. Because only trace amounts of peanut protein are sufficient to induce life-threatening anaphylaxis¹⁶

and because ongoing absorption of peanut protein from the gastric lumen may contribute to the potential lethality of anaphylaxis, we examined the ability of activated charcoal to form complexes with peanut protein, preventing binding to IgG and IgE. Activated charcoal efficiently bound to and removed peanut protein from aqueous solution in as little as 60 seconds at both neutral and acid pH. The ratio of activated charcoal to peanut protein resulting in essentially complete removal from solution was 200:1. When peanut protein comprised 1% by weight of a food matrix such as milk chocolate or ice cream, the amount of activated charcoal required to achieve complete removal of peanut protein from solution was 400:1, indicating that the food matrix competes with peanut protein for binding to sites on activated charcoal. However, when peanut is present in trace concentrations, consistent with contamination of a prepared food, activated charcoal effectively binds and removes peanut protein from the food matrix. Because peanut protein can induce objective manifestations in anaphylactically sensitive individuals in doses as low as 1 mg¹⁶ and because activated charcoal suspension is generally available at a concentration of 200 mg/mL, relatively small volumes of charcoal suspension should be useful in slowing or preventing further absorption of peanut protein into the systemic circulation. The weight of a peanut may range from 120 to 150 mg. Because peanuts are approximately 26% protein by weight (data not shown), a volume of 31 to 39 mL of activated charcoal suspension would be required to effectively complex with protein in the equivalent of 1 peanut.

In treating intoxications, activated charcoal is generally most effective when administered soon after ingestion of the toxins. Delayed administration of activated charcoal may lead to potentially increased morbidity. Prehospital administration of activated charcoal may result in substantial time saving. In 2 studies examining the time to administration of activated charcoal in poisoned patients, the time from first encounter with paramedics to administration of activated charcoal in the emergency department was 5.0 to 111 minutes^{17,18} as compared to 111 minutes when activated charcoal was administered on arrival in the emergency department. Both studies demonstrated that prehospital administration of activated charcoal could result in earlier and possibly more efficacious therapy. Similarly, self-administration of activated charcoal (in the form of either a suspension or a film-coated tablet) after accidental peanut ingestion, perhaps concomitant with self-administration of epinephrine, in patients with peanut allergy could result in decreased morbidity and mortality.

We have shown that activated charcoal complexes with peanut protein, preventing its binding to either IgG or IgE antibodies. Complex formation occurs within seconds at both neutral and acidic pH. This approach has the potential to delay or inhibit absorption of peanut protein after inadvertent ingestion. The data presented herein suggest that administration of activated charcoal may be useful as an adjunct to the present standard management of peanut anaphylaxis.

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