

Experimental Assessment of the Allergenicity of a Psyllium-Containing Laxative

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The allergenicity of psyllium was investigated using the Guinea Pig Maximization Test. Four of 12 animals gave positive skin reactions. Subsequent inhalation challenge of the same animals yielded respiratory hypersensitivity reactions in four of the animals. Two animals gave both skin and respiratory responses. The mosaic of respiratory and dermatologic symptoms demonstrated by animals resembled the pattern of findings reported in clinical studies. Results of SDS-acrylamide gel electrophoresis, immunoblot and protein determinations of aqueous psyllium extracts failed to indicate the presence of protein. Spectrophotometric studies suggested that the extract was a gum with a molecular weight greater than 14,000 daltons.

Key words: allergy; animal model; asthma; bulk laxative; dermatitis; gum; psyllium.

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Psyllium, a member of the plantain family *Plantaginaceae*, is the active component in many stool bulk-forming laxatives. Currently, several million people daily use such preparations for relief of constipation and other gastrointestinal distress.

We and others have described respiratory and skin sensitivity to a psyllium-containing bulk laxative in three clinical populations: pharmaceutical workers, nurses dispensing psyllium preparations, and users of the product (1-5, 10-12, 16, 18, 20, 22). However, there have been no experimental determinations of the allergic potency of psyllium or of the nature of the allergenic component.

An experimental investigation was undertaken using guinea pigs to evaluate the potency of psyllium as an allergen. All of the clinically observed symptoms except severe anaphylaxis were reproduced by skin and inhalation challenge of sensitized animals. When compared with other materials, psyllium can be classified as a "mild" allergen.

MATERIAL AND METHODS

Animals

Animals used for both the Guinea Pig Maximization Test and for inhalation challenge, were male, English, smooth-haired guinea pigs (Hilltop Laboratory Animals, Scottdale, PA) weighing 350 to 430 g.

Psyllium extract

Aqueous extracts of psyllium were prepared by stirring 1 g psyllium in 185 ml phosphate buffered saline for 48 h at 10°C, then centrifuging the mixture at 480 g for 30 min to remove debris. The extract was lyophilized, dialyzed against distilled water (Spectropor membrane tubing, Spectrum Medical Industries, Inc., CA, USA, molecular weight cut-off 12,000-14,000 daltons) and re-lyophilized.

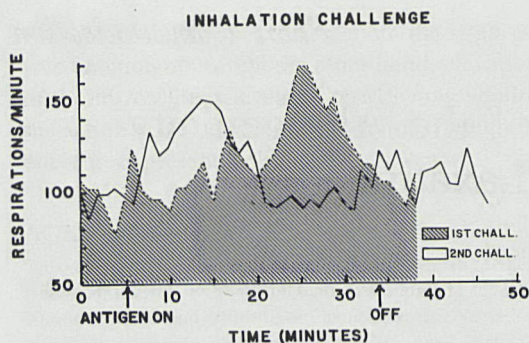


Fig. 1. Inhalation challenges of a guinea pig with aerosolized psyllium extract. Challenges were for 30 min (see arrows), chamber concentration averaged 11 mg/m^3 . The animal responded to the first inhalation challenge with an increase in respiratory rate after 20 min of exposure. Response to inhalation challenge, performed 1 week later, occurred by 10 min.

Guinea Pig Maximization Test (CPMT)

Twelve guinea pigs were exposed to psyllium by injecting a 2% solution of psyllium extract followed 1 week later by occlusive topical application of 5% psyllium in petrolatum (13). Dermal sensitivity was assessed by topical challenge to naive sites 14 days later. Reactions were recognized as swollen erythematous areas. The test was scored by the total number of reacting animals. Positive controls ($n = 4$) were animals exposed to the same quantity of picryl chloride (PiCl); negative controls ($n = 4$) were treated with saline in place of antigen.

Inhalation challenge

Approximately 1 week following the maximization test, the psyllium-exposed guinea pigs (and control animals) were challenged by inhalation of an aerosolized psyllium extract. The method of inhalation challenge has been described previously (7). The head of each animal extended through a latex dam into a 10 l Plexiglass inhalation chamber, while the body was held in an individual plethysmograph connected to a Gould 200 oscillograph. Psyllium extract was aerosolized as a 0.12% (w/v) aqueous solution through a Dautrebande aerosol generator. Airflow through the chamber was maintained at 20 l/min. Exposure con-

centrations, determined by sampling the inhalation chamber with an air sampling cassette (Millipore, 45 μm pore size), ranged between 9 and 20 mg/m^3 on different runs and averaged 11 mg/m^3 .

To detect a respiratory response, a Statham pressure transducer was attached to each plethysmograph. Pressure changes, caused by inspiration and expiration of guinea pigs, were sensed by the transducers, amplified and displayed on the oscillograph. A decrease in tidal volume with an increase in respiratory rate is known to occur when sensitized guinea pigs respond to inhalation challenge (6, 17).

A positive response was a 47% increase in respiratory rate over the baseline rate measured during the 5 min prior to challenge (6). Animals were monitored continuously for respiratory status throughout challenge and for 5 min following exposure. Upon removal from the chamber, they were further examined for rhinoconjunctivitis and wheezing.

SDS-polyacrylamide gel electrophoresis

Electrophoresis was performed at 10°C for 3.5–4 h (30 mA), using a 7.5% gel (8). Proteins were visualized by staining with Coomassie Blue R-250 stain. For immunoblot (21), fractions were transferred to nitrocellulose then stained with antibody from a clinically sensitized patient (3) followed by peroxidase labelled anti-human IgE and IgG.

Proteolytic digestion of antigen

Fifteen mg of chymotrypsin (Sigma Chemicals) were incubated with 30 mg of psyllium extract

Table 1
Dermal reactions in the Guinea Pig Maximization Test

Antigen	Dermal reaction ¹	
	24 h	48 h
Psyllium	3/12	4/12
Neg. control	0/4	0/4
Pos. control ² (PiCl)	3/3	3/3

¹ A positive response is a red, swollen area.

² One death occurred during topical exposure.

for 4 h at room temperature in phosphate buffer, pH 7.6. The reaction was terminated using a serine inhibitor. The mixture was dialyzed extensively against distilled water and lyophilized yielding 45 mg.

RESULTS

Guinea Pig Maximization Test

Of the 12 guinea pigs exposed to psyllium, using the maximization procedure, three gave perceptible skin reactions at 24 h while four showed reactions at 48 h (Table 1). By comparison, each of the three surviving PiCl -exposed animals showed severe reactions at both time periods. None of the four saline-exposed control animals showed reaction at either time period.

Inhalation challenge

Four of 12 animals previously exposed to psyllium in the GPMT, responded with an increase in respiratory rate to inhalation challenge using aerosolized psyllium extract (Table 2). No reactions were obtained in any of the four adjuvant-injected guinea pigs (negative

controls) nor in two naive animals challenged at the same time. On re-challenge 7 days later, one of the four psyllium-exposed animals gave a positive reaction. Respiratory reactions to first challenge typically occurred about 20 min into challenge. By contrast, the animal which responded to the second inhalation challenge (#1484) showed reaction by 8–10 min of exposure (Fig. 1).

In three animals having positive respiratory responses, wheezing was noted immediately following the first inhalation exposure but was not noted on rechallenge (Table 2). Rhinitis was noted on first challenge in three (#1480, 1481, 1484) of the four animals displaying positive respiratory reactions. On re-challenge it was evident in all four of these guinea pigs. Rhinitis was especially marked in the animal (#1484) which responded on both occasions to inhalation challenge.

Characterization of the active component

The psyllium allergen had been assumed to be a protein. However, electrophoresis of the extract in SDS acrylamide gel, followed by staining yielded no protein band. Similarly, negative

Table 2
Symptoms of individual guinea pigs to the GPMT and inhalation challenges

Animal No.	GPMT ¹ (48 h)	Respiratory reaction ²		Wheeze		Rhinitis	
		1st	2nd	1st	2nd	1st	2nd
1478	-	-	ND	-	ND	-	ND
1479	-	-	ND	-	ND	-	ND
1480	+	+	-	+	-	+	+
1481	+	+	-	+	-	+	+
1482	+	-	ND	-	ND	-	ND
1483	+	-	ND	-	ND	-	ND
1484	-	+	+	+	-	+	+
1485	-	-	ND	-	ND	-	ND
1486	-	+	-	-	-	-	+
1487	-	-	ND	-	ND	-	ND
1488	-	-	ND	-	ND	-	ND
1489	-	-	ND	-	ND	-	ND

¹ GPMT: Guinea Pig Maximization Test (12).

² Positive respiratory reaction was an increase in respiratory rate at least 47 % above that displayed during the baseline period 5 min prior to challenge. Responses to first and second challenges are indicated. No positive reactions were observed in any of six control animals challenged at the same time.

results were obtained using the immunoblot technique when serum from a psyllium-sensitized patient (3) was used to provide antibody to psyllium. Lastly, the lack of weight loss during attempted proteolytic digestion gave further evidence for the non-proteinaceous character of the extract.

The ultraviolet absorbance spectrum of the extract showed a peak at 190 nm and had no absorbance in the 260–290 nm range. Ultraviolet absorbance spectra of galacturonic acid, rhamnose, and arabinose each gave a similar pattern with maximum absorbance at 190 nm.

DISCUSSION

Guinea pigs displayed a range of symptomatology following exposure to psyllium. Skin reactions were noted in some animals. Respiratory symptoms ranged from wheeze, cough and rhinitis to “rapid, shallow” breathing. These findings were compared with the clinical situation by compiling all reported cases of clinical psyllium sensitivity (Table 3). Clinically and

experimentally, the diversity of symptomatology is evident.

In the experimental study reported here, animals were injected with psyllium in the presence of Freund's adjuvant, then received topical exposure to the antigen. Challenge via inhalation exposure resulted in respiratory sensitivity reactions. Clinically, sensitization to psyllium appears to follow exposure via the inhalation route (1–5, 10–12, 16, 18, 20, 22). However, as in the animal study, reactions have been elicited by routes other than inhalation, i.e., ingestion of psyllium has produced severe anaphylaxis (11, 20, 22) and eosinophilia (16).

The allergenic component of psyllium has been assumed to be a protein (1, 20). Tests were conducted to characterize the nature of the psyllium allergen. Psyllium seed has been reported to contain 15–20 % protein and 9–10 % polysaccharide (15). Sugars include: L-rhamnose, D-xylose, L-arabinose and galacturonic acid-containing residues (9, 14). In this study, several independent assays failed to

Table 3
Survey of clinical reports of psyllium sensitivity

Year, country	Exposure	Findings	Reference
1970, USA	1 handler	asthma	1
1975, USA	3 pharm. workers	asthma, wheeze, sneeze, itch, BPC pos.	2
1979, Sweden	27 pharm. workers	wheeze, sneeze, itch, BPC pos.	4
1979, Denmark	10 hosp. handlers	wheeze, sneeze, itch, BPC pos.	12
1979, USA	1 hosp. handler	severe asthma	5
1980, USA	1 user	eosinophilia from ingestion	16
1982, USA	1 pharm. worker	cough, wheeze, SOB, pos. RAST and skin tests	18
1983, Sweden	6 hosp. handlers 9 pharm. workers	rhinitis, wheeze, SOB, itch, pos. RAST and skin test	10
1983, Finland	1 hosp. handler	anaphylaxis from ingestion	20
1984, Sweden	6 hosp. handler	respiratory symptoms following ingestion, RAST and skin test pos., gastrointestinal symptoms	11
1984, USA	1 hosp. handler	anaphylaxis from ingestion, pos. RAST and skin test, passive transfer	22
1985, USA	1 pharm. worker	wheeze, sneeze, SOB, itch, dermatitis, RAST pos.	3

BPC: bronchial provocation challenge with psyllium.

demonstrate any proteinaceous component, whereas, ultraviolet spectroscopy revealed an absorbance pattern resembling that of simple sugars. Our studies are consistent with the extract being a "gum".

Three classes of materials have been recognized as agents of allergenic response: proteins, polysaccharides, and haptens (19). Although it is not possible to give a chemical definition of a gum, it is closely related to carbohydrates. Based on our results, we conclude that the allergenic component has a molecular weight greater than 14,000 daltons and is likely not a protein. We do not know whether the psyllium allergen is a complete antigen in itself or whether, like many carbohydrates, it requires *in vivo* association with protein to form a complete antigen. However, it is clear that gums are recognized allergens (19) causing IgE responses in the pharmaceutical and printing industries.

In summary, this report describes experimental sensitization to psyllium followed by skin and inhalation challenges. Responses to challenge resembled, in their diversity, the responses of patients clinically sensitized to psyllium laxative. The psyllium extract appears to be a gum.

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