

Evaluation of papain/chymopapain cross allergenicity

Michael A. Sagona, Ph.D., George V. Bruszer, M.S., Lawrence Lin, Ph.D.,
Frederick C. Pearson, Ph.D., Kenneth Serkes, M.D., and
Carmine C. Mascoli, Ph.D. Morton Grove, Ill.

Recent clinical evidence suggests that papain and chymopapain may share common allergenicity. Patients that become sensitized to papain may subsequently experience an allergic reaction when they are exposed to chymopapain. This study demonstrates a cross antigenicity between the proteolytic enzyme preparations papain and chymopapain. Serum samples from six patients who demonstrated 4+ skin reactions to papain also revealed positive RAST ratios to both papain and chymopapain. In addition, serum samples from 12 clinically nonreactive patients who had discolysis with chymopapain demonstrated positive RAST results to papain as well as to chymopapain. (J ALLERGY CLIN IMMUNOL 76:776-81, 1985.)

Allergic reactions to papain have been reported in occupational settings. These allergic reactions are often respiratory in nature, typically manifested as asthma and allergic rhinitis.¹⁻³

Recently, however, nonoccupational sensitizations to papain have been observed. Mansfield and Bowers⁴ revealed a papain sensitization that resulted from the use of meat tenderizer, a sensitization that occurred via the gastrointestinal tract. Cogen et al.⁵ reported on a patient sensitized to papain by the daily consumption of the papaya fruit. Bernstein et al.⁶ demonstrated that a patient had become sensitized to papain through the use of a papain-containing contact lens cleaner. The last two investigators became aware of their patients' papain sensitivity because of allergic reactions during discolysis (chemonucleolysis) with the use of chymopapain.

Chymopapain, like papain, is a sulfhydryl protease derived from the latex of *Carica papaya*. Chymopapain is currently the drug of choice as an alternative to spinal surgery for herniated intervertebral lumbar discs. It has been estimated that approximately 1% of the patients undergoing chemonucleolysis experience severe allergic reactions.⁵⁻⁹ Investigators agree that

Abbreviation used

HSA: Human serum albumin

chymopapain sensitivity is IgE mediated.⁵⁻⁹ Skin testing and RAST evaluations have proved this conclusion to be correct.

In an earlier study we determined that IgE levels to chymopapain reached a maximum titer 2 weeks after discolysis.¹⁰ It is for this reason that samples obtained 2 weeks after treatment were used in this study for RAST and RAST inhibition.

MATERIAL AND METHODS

Serum samples

Serum samples were obtained from six patients who demonstrated sensitivity to oral challenge and also demonstrated a 4+ skin reaction to papain. Serum samples were also obtained from 12 clinically nonreactive chemonucleolysis patients. Samples were obtained 2 weeks after discolysis. All 12 discolysis patients had been treated at the same medical center, received the same dose of chymopapain, and had no known sensitivity to the drug. A pool of serum obtained from normal laboratory personnel with no known history of allergies to papain or chymopapain was used as the control sample.

Material

Materials used were chymopapain (Discase; Travenol Laboratories, Deerfield, Ill.), papain (Sigma Chemical Co.,

From the Department of Life Sciences, Travenol Laboratories, Inc., Round Lake, Ill.

Received for publication Dec. 21, 1984.

Accepted for publication April 6, 1985.

Reprint requests: Michael A. Sagona, Ph.D., Dept. of Life Sciences, Travenol Laboratories, Inc., Round Lake, IL 60073.

TABLE I. Results of testing with serum from donors with confirmed sensitivity to papain*

Patient	Test material	RAST ratio
A	Papain	2.2
	Chymopapain (Disease)	3.0
	HSA	1.0
E	Papain	3.3
	Chymopapain (Disease)	4.2
	HSA	1.0
F	Papain	15.7
	Chymopapain (Disease)	21.8
	HSA	1.0
G	Papain	5.1
	Chymopapain (Disease)	7.7
	HSA	1.0
H	Papain	4.8
	Chymopapain (Disease)	6.4
	HSA	1.0
J	Papain	1.9
	Chymopapain (Disease)	2.5
	HSA	1.0
Negative control	Papain	1.2
	Chymopapain (Disease)	1.0
	HSA	1.0

*Prick skin test 4+ to papain.

St. Louis, Mo.), and IgE Phadebas RAST (Pharmacia Diagnostics, Piscataway, N.J.).

The antisera used was rabbit antipapain obtained from Cappel Laboratories, Cochranville, Pa.). Rabbit antichymopapain was produced by the following immunization schedule: a 1 mg/ml concentration of chymopapain (0.5 ml) prepared in Freund's complete adjuvant was injected subcutaneously and was followed 3 weeks later by a booster injection of 0.5 ml of the same concentration prepared in Freund's incomplete adjuvant.

RAST

Activation of paper discs. Whatman No. 50 qualitative filter paper discs (6 mm) were made and used as follows: Approximately 10 gm of the discs were allowed to swell 30 minutes in 200 ml of distilled water. A 5% w/v cyanogen bromide solution was added to the discs and stirred for 3 minutes at room temperature. After stirring, 40 ml of 1 mol/L NaOH was added dropwise, maintaining a pH range of 10.0-10.5. Immediately, 2.0 L of cold NaHCO₃ (0.005 mol/L at 4° C) was added, mixed well, and decanted. This step was repeated four times. The discs were then serially washed twice in 25% acetone, twice in 50% acetone, twice in 75% acetone, and four times with 100% acetone. After the final acetone wash, the solution was decanted, and the discs were allowed to dry. The discs were stored at -20° C.

Conjugation of chymopapain (Disease). Separate 20 mg/ml solutions of chymopapain (Disease) and papain were

TABLE II. Results of testing with serum obtained from chymopapain recipients 2 weeks after discolysis

Patient	Test material	RAST ratio
D-17	Chymopapain (Disease)	55.2
	Papain	7.2
	HSA	1.0
D-19	Chymopapain (Disease)	5.1
	Papain	3.9
	HSA	1.0
D-22	Chymopapain (Disease)	52.0
	Papain	11.5
	HSA	1.0
D-37	Chymopapain (Disease)	20.0
	Papain	12.4
	HSA	1.0
D-43	Chymopapain (Disease)	8.7
	Papain	5.6
	HSA	1.0
D-50	Chymopapain (Disease)	6.5
	Papain	2.5
	HSA	1.0
D-70	Chymopapain (Disease)	8.3
	Papain	2.9
	HSA	1.0
D-105	Chymopapain (Disease)	13.3
	Papain	4.8
	HSA	1.0
D-111	Chymopapain (Disease)	6.4
	Papain	6.7
	HSA	1.0
D-149	Chymopapain (Disease)	11.0
	Papain	10.8
	HSA	1.0
D-176	Chymopapain (Disease)	12.6
	Papain	4.4
	HSA	1.0
D-179	Chymopapain (Disease)	3.5
	Papain	3.2
	HSA	1.0
Negative control	Chymopapain (Disease)	1.4
	Papain	1.3
	HSA	1.0

prepared in borate buffer. The prepared paper discs were placed in a round-bottom, screw-topped tube. The respective solution was added, and the tube was rotated overnight at room temperature. The borate buffer was decanted, and the discs were washed three times in 1 mol/L of ethanolamine (pH 8.0). The paper discs were stored overnight in 1 mol/L of ethanolamine at 4° C. The following day they were washed three times in assay washing solution. After the washing solution was decanted, the discs were stored at -20° C. HSA (20 mg/ml) was conjugated to discs and used as the negative control.

RAST procedure. The RAST procedure developed by

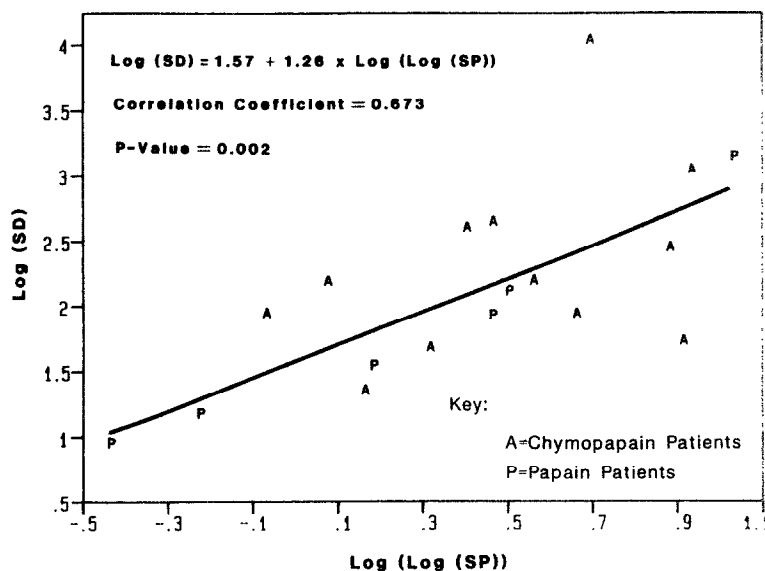


FIG. 1. The relationship between the sensitivity ratio of chymopapain (Discase) and the sensitivity ratio of papain (A, patient exposed to chymopapain [Discase]; P, patient exposed to papain).

Wide et al.¹¹ was used as follows: Each antigen-coupled (HSA, papain, and chymopapain [Discase]) disc was placed in a separate 12 by 75 mm tube. Serum (100 μ l) was added to each tube, and the tubes were rotated overnight at room temperature. The next day the discs were washed three times in assay washing solution. Anti-IgE ¹²⁵I (Pharmacia) was reconstituted with water, and 100 μ l was added to each tube, after which the tubes were rotated overnight at room temperature. The next day the discs were washed three times in assay washing solution and counted in a Packard 5110 autogamma counter.

The RAST ratio for IgE determination was

$$\frac{\text{Experimental counts per minute}}{\text{HSA control counts per minute}}$$

RAST inhibition

RAST inhibition, a modification of the RAST developed by Wide et al.,¹¹ was used as follows: RAST inhibition is performed in the same way as RAST but includes an additional step. Before the incubation of the discs, an overnight procedure involving 50 μ l of serum and 50 μ l of the inhibiting agent is performed. For controls, 50 μ l of saline is added to the serum in place of the inhibiting agent.

In this study chymopapain (Discase) (10 mg/ml), which had been stored at 4° C for 6 months, was used as the inhibitory agent.

The formula for RAST inhibition is

$$\frac{\text{Direct RAST} - \text{Inhibited RAST}}{\text{Direct RAST}} \times 100$$

Immunodiffusion

Immunodiffusion gels (Bio-Rad Laboratories, Richmond, Calif.) were cast onto hydrophilic plastic plates (Gel-

bond; FMC Corp., Rockland, Me.). Holes were punched in the usual hexagonal pattern. The center wells were filled with either antichymopapain or antipapain. The outer wells were filled alternately with papain and chymopapain at 1 mg/ml concentrations.

RESULTS

Table I reveals that six patients known to be sensitive to orally ingested papain and demonstrating 4+ skin reactions were RAST positive to both papain and chymopapain (Discase). Although these patients have known sensitivities to papain, the RAST ratios for chymopapain were higher in each case. Patient J revealed a positive RAST ratio for chymopapain (2.5) and a RAST ratio of 1.9 for papain. The RAST ratios presented are the means of duplicates tested. HSA served as the internal negative control for each patient. A normal human serum pool, drawn from personnel with no known history of allergies to either papain or chymopapain and who had been determined to be negative, was used as the negative control for papain, chymopapain, and HSA.

Table II demonstrates that 12 patients with high IgE titers to chymopapain 2 weeks after treatment also have positive RAST values to papain. One patient (D-111) revealed a higher ratio to papain (6.7) than to chymopapain (6.3). In general, however, most of the patients had higher RAST ratios to chymopapain than to papain.

Statistical analysis

One group of 12 patients was treated with chymopapain, and the RAST ratios to both chymopapain

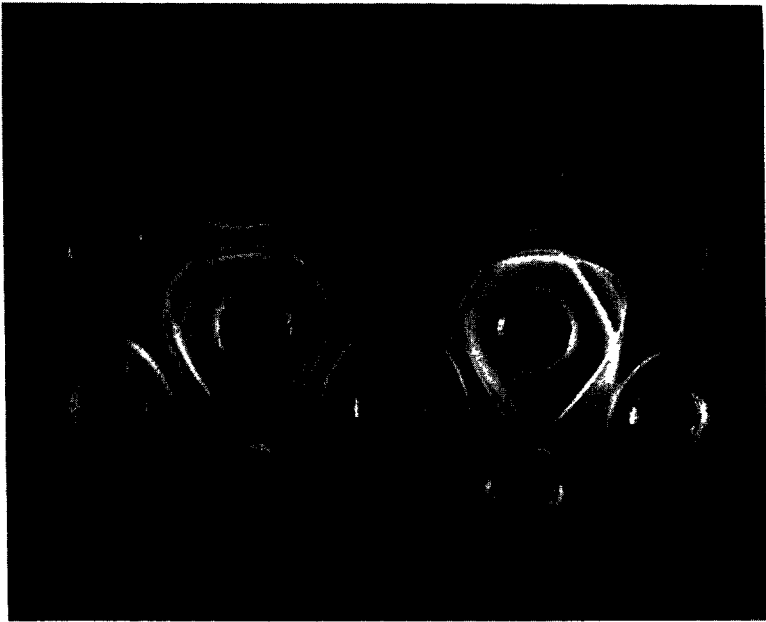


FIG. 2. Cross antigenicity of papain and chymopapain preparations with Ouchterlony immunodiffusion. *Center wells* contain antipapain (*left*) and antichymopapain (*right*). *Outer wells* are filled alternately with papain or chymopapain (*top wells* contain chymopapain; *bottom wells* contain saline).

TABLE III. RAST inhibition papain/chymopapain discs

Patient serums after 2 wk discolysis	Inhibitory agent	% Inhibition chymopapain (Discase) discs	% Inhibition papain discs
D-17	Chymopapain (Discase)	93.13	82.3
	HSA	− 31.1	− 9.2
D-19	Chymopapain (Discase)	65.3	44.4
	HSA	12.4	− 18.9
D-70	Chymopapain (Discase)	73.8	47.4
	HSA	− 49.8	− 35.1
D-105	Chymopapain (Discase)	85.4	54.8
	HSA	− 24.3	− 26.0

and papain were calculated. The other group (six patients) had been orally exposed to papain, and the RAST ratios to both chymopapain and papain were calculated. When the association between the sensitivity to chymopapain and the sensitivity to papain was studied, the RAST ratio rather than the original counts per minute was used in order to control and eliminate the variation caused by the negative control (HSA). In other words, such association was established free of the effect of HSA or by holding the effect of HSA constant. Before evaluating this association, it is necessary to examine whether or not this association depends on the two exposure routes (two groups of patients). This examination was conducted by means of the generalized linear model approach,

which tests for the equality of the two regression lines.¹² Each regression line represents the association for each group of patients. Results indicated that the regression lines of these two groups of patients were indistinguishable ($p = 0.6$). Therefore, the association was evaluated by combining the two groups of patients by means of the ordinary regression analysis. The final regression line is illustrated below.

$$\log SD = 1.57 + 1.26 \log \log SP$$

where

$$SD = \text{RAST ratio to chymopapain}$$

$$SP = \text{RAST ratio to papain}$$

The correlation coefficient (0.673) is highly sig-

nificant ($p = 0.002$). This indicates a strong relationship between the two RAST ratios for both groups of patients; that is, if patients were observed to have a high sensitivity to papain, they are likely to have a high sensitivity to chymopapain, regardless of the effect of HSA. This is true for both groups of patients. Note that the use of logarithmic transformation was simply to linearize the relationship. The results are summarized in Fig. 1.

Table III illustrates RAST inhibition by use of four high IgE titer serums from Table II. Chymopapain (Discase) and HSA were used as the inhibitory agents attempting to block the reaction with chymopapain and papain. The chymopapain (Discase) used was aged aseptically for 6 months in order to minimize any enzymatic activity. HSA used as the control inhibitory agent failed to block the reaction for either chymopapain (Discase) or papain discs. Chymopapain (Discase), however, was able to block the reactions with papain and chymopapain discs. Chymopapain (Discase) discs were inhibited anywhere from 65.3% to 93.1%. Papain discs were blocked from 44.4% to 82.3%. There were insufficient quantities of patient serums to test RAST inhibition with papain as the inhibitory agent. There were also insufficient quantities of serums from the papain-sensitive patients to carry out RAST inhibition with these serums with chymopapain and papain as the inhibitory agents.

Fig. 2 demonstrates that, with the use of the Ouchterlony technique and with antipapain as the antibody and papain and chymopapain as antigens, definite lines of identity and nonidentity exist between the two allergens. With antichymopapain as the antibody, once again links of identity and nonidentity exist between the two antigens. From this gel precipitation assay antigenic likenesses and differences are noted.

DISCUSSION

Papain is widely used in our society in the preparation of food, beer, and pharmaceuticals.^{3,4} Both severe and mild allergic reactions to papain have been demonstrated in occupational settings. These reactions range from papain-induced asthma to persistent itching and watering of the eyes.^{2,3} Recently, however, allergic reactions to papain have been found to occur outside the workplace. Mansfield and Bowers⁴ have discovered a patient who experienced a severe systemic allergic reaction after ingesting meat tenderizer. Further study demonstrated that the reaction was mediated by IgE antibody to papain food tenderizer. This may be the first reported case in which the sensitization appears to have occurred via the gastrointestinal tract. Cogen et al.⁵ demonstrated a sensitization to papain via the gastrointestinal route, apparently

caused by daily ingestion of the papaya fruit. Bernstein et al.⁶ also describe a patient sensitized to papain by use of a papain-containing contact lens cleaner.

Haverly et al.¹³ recently reported that of 412 patients with varied but confirmed allergies, 1.2% were sensitive to papain. A number of patients sensitized to papain have experienced severe systemic allergic reactions to chymopapain during discolysis.

Results of this study clearly indicate that IgE-specific antibodies from either papain- or chymopapain-sensitized individuals do cross react. This cross antigenicity may occur in two ways: (1) Papain and chymopapain could contain small amounts of cross contamination because both are taken from the same source. (2) Papain and chymopapain could share similar antigenic determinants in their protein structures. Eder and Arnon¹⁵ have used antibodies prepared to both chymopapain and papain to demonstrate that the two substances are very similar.

Further studies, such as crossed immunoelectrophoresis and crossed radioimmuno-electrophoresis, need to be performed in order to delineate shared antigens.

Because of the cross antigenicity between papain and chymopapain demonstrated both in this study and in other recent studies,^{5,6} it is likely that a patient sensitized to papain may also experience an allergic reaction when the patient is exposed to chymopapain. It is therefore important for patients considering discolysis to be tested for such sensitivities. In vivo tests are currently available to test for specific IgE antibodies and, hence, can better predict these sensitivities to chymopapain.

Recently, concern has been expressed regarding the degree of sensitivity of the in vitro assays.⁵ Skin testing has proved so far to be the best indicator for patients with potential for anaphylactic reaction to papain and possibly to chymopapain.^{4,7}

The serum samples provided by Dr. L. E. Mansfield are gratefully acknowledged.

REFERENCES

1. Flindt MLH: Respiratory hazards from papain. *Lancet* 1:430, 1978
2. Tarlo SM, Shaikh W, Bell B, Cuff M, Davies GM, Dolovich J, Hargreave FE: Papain-induced allergic reactions. *Clin Allergy* 8:207, 1978
3. Novey HS, Marchioli LE, Sokol WN, Wells ID: Papain-induced asthma—physiological and immunological features. *J ALLERGY CLIN IMMUNOL* 63:98, 1979
4. Mansfield LE, Bowers CH: Systemic reaction to papain in a nonoccupational setting. *J ALLERGY CLIN IMMUNOL* 71:371, 1983
5. Cogen F, Goldstein M, Zweiman B: Skin tests in chymopapain anaphylaxis. *J ALLERGY CLIN IMMUNOL* 73:179, 1984 (abst)

6. Bernstein DI, Gallagher JS, Grad M, Bernstein IL: Local ocular anaphylaxis to papain enzyme contained in a contact lens cleansing solution. *J ALLERGY CLIN IMMUNOL* 74:258, 1984
7. Rajagopalan R, Tindal S, Macnab I: Anaphylactic reactions to chymopapain during general anesthesia: a case report. *Anesth Analg* 53:191, 1974
8. Watts C: Complications of chemonucleolysis for lumbar disc disease. *Neurosurgery* 1:2, 1977
9. Bernstein IL: Adverse effects of chemonucleolysis. *JAMA* 250:1167, 1983
10. Sagona M, Bruszer G, Nelson J, Mascoli C, Serkes K: Reactivity of IgE and IgG serum levels to chymopapain after chemonucleolysis. *J ALLERGY CLIN IMMUNOL* 75:659, 1985
11. Wide L, Bennich H, Johansson SGO: Diagnosis of allergy by an in vitro test for allergen antibodies. *Lancet* 2:1105, 1967
12. Graybill FA: Theory and application of the linear model. North Scituate, Mass., 1976, Duxbury Press, pp 291-302
13. Haverly RW, Mansfield LE, Ting S: The incidence of hypersensitivity to papain in an allergic population; confirmed by blinded food challenges. *J ALLERGY CLIN IMMUNOL* 73:179, 1984 (abst)
14. Hall BB, McCulloch JA: Anaphylactic reactions following the intradiscal injection of chymopapain under local anesthesia. *J Bone Joint Surg [Am]* 65:1215, 1983
15. Eder J, Armon R: Structural and functional comparison of antibodies to common and specific determinants of papain and chymopapain. *Immunochemistry* 10:535, 1973