

The safety and activity of polymerized ragweed: A double-blind, placebo-controlled trial in 81 patients with ragweed rhinitis

Leslie C. Grammer, M.D., Martha A. Shaughnessy, B.S.,
Michael I. Bernhard, Ph.D.,* Susan M. Finkle, B.A., H. Ruth Pyle, B.S.,*
Loui Silvestri, Ph.D.,* and Roy Patterson, M.D. Chicago, Ill., and Miami, Fla.

*Eighty-one patients with ragweed pollinosis were recruited for a double-blind, histamine placebo-controlled study of the safety, immunogenicity, and efficacy of 15 weekly injections of polymerized ragweed (PRW) immunotherapy totaling 1359 allergy units. Patients were paired on the basis of cutaneous end point titration to RAST standardized extracts of giant and short ragweed. One patient of each pair was randomized to receive PRW, and the other patient, a caramelized glucose histamine placebo. Symptom and medication score diaries were completed by 68 patients. All 68 patients received the full maintenance dose. No patient dropped out because of adverse reactions, and there were no systemic reactions. Except for one patient receiving placebo who developed mildly elevated liver function tests, there were no clinically significant changes in routine laboratory tests associated with injections. By Student's *t* test on log-transformed values, blocking antibody rose significantly in the patients receiving PRW but was unchanged in those receiving placebo. By Wilcoxon paired signed-rank test, the symptom and medication scores in the patients receiving PRW were significantly lower than scores in the patients receiving placebo. This study demonstrates the safety, immunogenicity, and activity of PRW in the treatment of ragweed pollinosis. (J ALLERGY CLIN IMMUNOL 1987;80:177-83.)*

Immunotherapy has been used in the treatment of pollinosis for more than seven decades since it was first described in the United States by Freeman and Noon.¹ During the past 40 years, there have been numerous clinical trials that have demonstrated the effectiveness of conventional immunotherapy with appropriate antigens in properly selected patients.²⁻⁴ However, even though immunotherapy is effective, there are several problems in its use that have caused various investigators to attempt to improve immunotherapy by modifying allergens. The first problem is the potential for systemic allergic reactions that have been reported to range between 8% and 30% per patient course.⁵ Although these reactions are rarely severe, death from anaphylaxis has been reported.⁶ The second problem is the amount of time that is required

Abbreviations used

PRW:	Polymerized ragweed
ALO:	Allergy Laboratories of Ohio
ESR:	Erythrocyte sedimentation rate
CBC w/diff:	Complete blood count with differential leukocyte count
U/A:	Urinalysis
BUN:	Blood urea nitrogen
LDH:	Lactic dehydrogenase
SGOT:	Serum glutamic oxaloacetic transaminase
SGPT:	Serum glutamic pyruvic transaminase
GGT:	Gamma-glutamyltransferase
AgE:	Antigen E
AU:	Allergy units

From the Section of Allergy-Immunology, Department of Medicine, Northwestern University Medical School, Chicago, Ill., and
*Key Pharmaceuticals, Inc., Miami, Fla.

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Reprint requests: Leslie Grammer, M.D., 303 E. Chicago Ave., Chicago, IL 60611.

to reach maintenance doses, at which time clinical improvement can first be expected. The final problem is that the overall duration of immunotherapy may be 3 or 4 years, and this may discourage patients from even beginning allergen immunotherapy at the present time.

An approach to the modification of allergens that has been extensively studied is that of polymerization

TABLE I. PRW immunotherapy schedule

Injection No.	PRW concentration (AU/ml)	Volume of injection (ml)	AU delivered	PNU delivered
1	25	0.10	2.5	30
2	25	0.25	6.25	75
3	25	0.50	12.5	149
4	250	0.10	25	298
5	250	0.25	62.5	746
6	250	0.50	125	1492
7	250	0.50	125	1492
8	250	0.50	125	1492
9	250	0.50	125	1492
10	250	0.50	125	1492
11	250	0.50	125	1492
12	250	0.50	125	1492
13	250	0.50	125	1492
14	250	0.50	125	1492
15	250	0.50	125	1492
Total			1359	16218

of allergens into species of high molecular weight with the use of glutaraldehyde as a cross-linking agent. By gel filtration chromatography, given molecular weight ranges of polymers are selected. It has been demonstrated that, on a weight basis, PRW is significantly less allergenic⁷ and similarly immunogenic⁸ when it is compared to unmodified ragweed extracts. Because these properties of PRW would theoretically confer obvious potential advantages for immunotherapy, several clinical trials have been performed. In a multi-institutional trial of 157 patients, it was demonstrated that PRW can be administered safely in 15 weekly injections and that it reduces patients' symptom and medication scores.⁹ In a double-blind, placebo-controlled trial of 40 patients, we reported the safety, activity, and immunogenicity of PRW.¹⁰

The present double-blind, histamine placebo-controlled study was undertaken in order to extend our previous reports of safety, activity, and immunogenicity with more patients. Eighty-one patients were recruited for this study, and 68 completed the entire 6-month study.

MATERIAL AND METHODS

Patients

The eighty-one patients who entered the study had histories compatible with allergic rhinitis caused by ragweed for at least the past 2 years in the Chicago area. They also had at least 30 mm sum of erythema (sum of longest and orthogonal diameters) to prick testing with 100,000 AU/ml extracts of giant ragweed (lot No. HF25628) and short rag-

weed (lot No. HF26610) (Pharmacia Diagnostics, Piscataway, N. J.). All patients were 21 to 60 years of age, had not received immunotherapy for at least 3 years, and were otherwise healthy as determined by history, physical examination, ESR, CBC w/diff, U/A, and serum creatinine, BUN, LDH, SGOT, SGPT, GGT, alkaline phosphatase, and direct and indirect bilirubin.

Intradermal end point titrations to appropriate serial three-fold dilutions of giant ragweed and short ragweed extracts (100,000 AU/ml, lot numbers as above, Pharmacia) were performed. The end point was the last dilution to result in at least a 10 mm wheal and 20 mm flare (longest diameter). Prick tests to *Alternaria tenuis* (1:20, ALO, Columbus, Ohio) and *Aspergillus* mix (1:20, Hollister-Stier, Spokane, Wash.) were also performed. Patients were paired before immunotherapy on the basis of intradermal end point titration to giant and short ragweed, and when it was possible, patients were also paired on the basis of asthma history, mold sensitivity, age, and sex. After immunotherapy but before unblinding of the study, patients were again paired with identical criteria in order to maximize the number of pairs.

Preparation of PRW

PRW was prepared at ALO. Short and giant ragweed pollens were extracted with aqueous buffer, precipitated with $(\text{NH}_4)_2\text{SO}_4$, and fractionated on Sephadex G-15. Polymerization with glutaraldehyde was followed by chromatographic fractionation with Sepharose CL-4B and Sephacryl S-200 columns. ALO performed the sterility and safety tests required by the Office of Biologics Research and Review, Food and Drug Administration. The PRW used contained 250 AU/ml on the basis of RAST inhibition com-

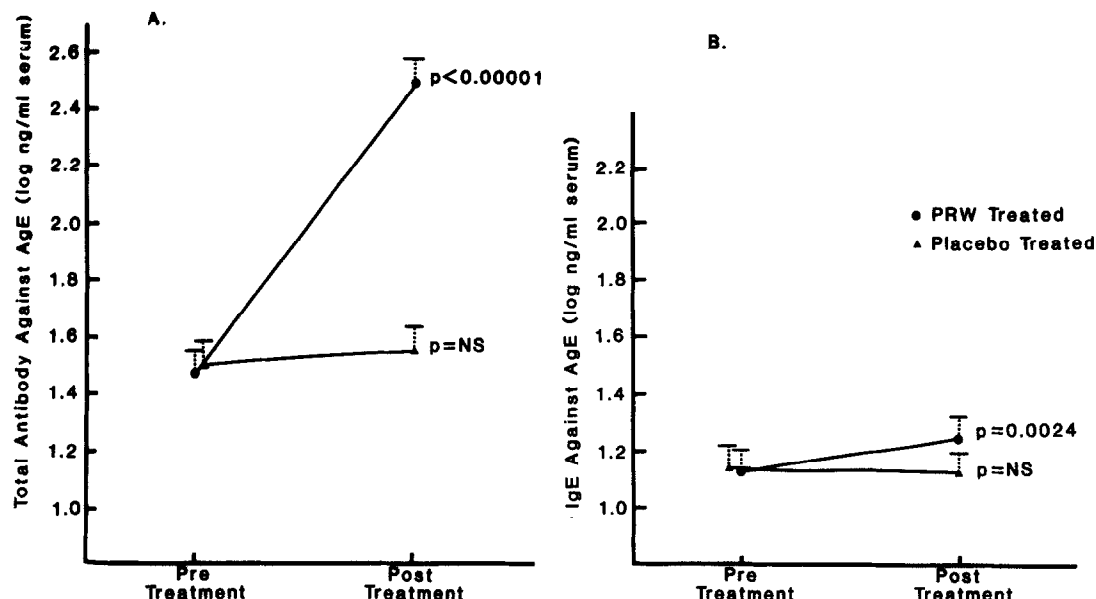


FIG. 1. A, Changes with injection in total antibody binding of AgE in the PRW- (●—●) and placebo-treated (▲—▲) patients. The vertical bars represent SEM. The rise is highly significant ($p < 0.00001$) in the PRW-treated patients but is not significant in the placebo-treated patients. B, Changes in IgE against AgE with injections in the PRW- (●—●) and placebo-treated (▲—▲) patients. The vertical bars are SEM. The rise in PRW-treated patients is statistically significant, whereas the change in the placebo-treated patients is not.

pared to the short ragweed E6 standard of the Office of Biologics Research and Review, and 2983 PNU/ml by the micro-Kjeldahl method.

Histamine placebo

Caramelized glucose and histamine phosphate, 20 $\mu\text{g/ml}$, were prepared by ALO to be used as the placebo. The PRW and placebo vials were visually indistinguishable. The vials were identified only by number, and instructions as to which vial each patient was to receive were supplied by an individual at ALO who was unaware of the patients' histories and who did not participate in any of the patient evaluations, safety, or efficacy analyses. No one at Northwestern, including treatment nurses, physicians, or technical staff, nor anyone at Key Pharmaceuticals, had access to the code.

Immunotherapy schedule

The patients received 15 injections in 15 weekly visits from late April to early August 1985. The schedule as presented in Table I is identical to that used in our previous double-blind trial.¹⁰ The placebo treatment schedule was identical in terms of volume. Both PRW and placebo were stored at 4° C. A total of 73 patients had at least one injection, and 69 patients completed the injection series. Patients were unable to complete injections caused by various compliance reasons such as job changes or inability to get time off from work. No patient dropped out because of reactions from immunotherapy.

Quantitation of reactions to injections

Immediate local reactions were recorded as millimeters of erythema and wheal 30 minutes after injection. Patients were queried and assessed at that time for symptoms of systemic allergic reactions. Delayed local reactions were recorded by patients on reporting forms at specified intervals up to 48 hours after each injection and were also reported to the treatment nurse before the next injection.

Clinical laboratory tests

Before the initial injection and after the final injection, each of the following tests was performed on each patient: U/A, CBC w/diff, ESR, serum creatinine, BUN, LDH, SGOT, SGPT, GGT, alkaline phosphatase, and direct and indirect bilirubin.

Immunologic studies

Each patient's blood was drawn for immunologic studies before and after the completion of injections. Sera were separated from cells and stored at -20° C until use.

Total serum AgE-binding capacity was determined by a modification¹¹ of the ammonium sulfate precipitation technique of Lidd and Farr.¹² In brief, 0.1 ml aliquots of sera were incubated with 1000, 100, and 10 ng of ¹²⁵I-labeled AgE. Saturated ammonium sulfate (40%) was used to precipitate antibody-bound AgE. The amount of AgE bound by 1 ml of serum was calculated and expressed as nanograms of AgE bound per milliliter of serum as a quantitative estimate of blocking antibody.

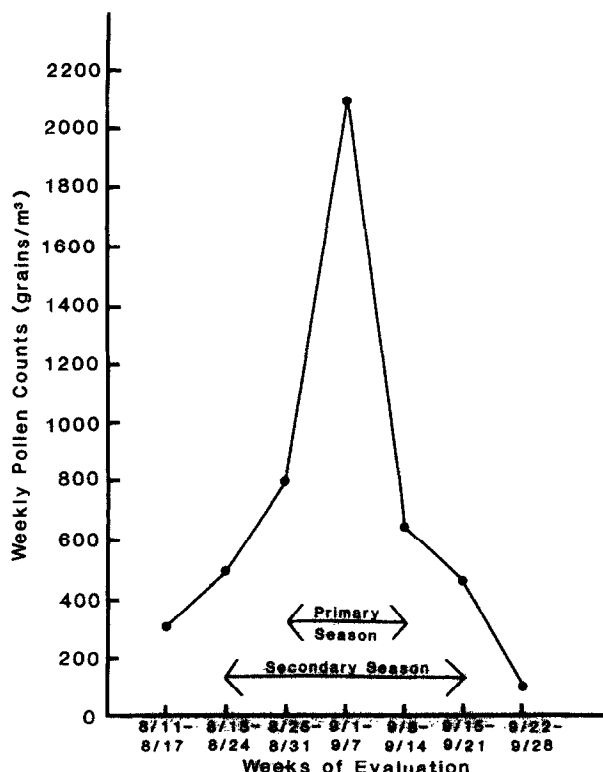


FIG. 2. Ragweed-pollen counts were obtained daily by a rotating arm impactor method, summed each week, and plotted. The units are pollen grains per cubic meter. The weeks encompassing the primary and secondary pollen seasons are illustrated.

AgE binding by IgE was measured by the polystyrene tube radioimmunoassay technique previously reported.¹³ Briefly, polystyrene tubes were coated with human myeloma IgE. Rabbit antihuman IgE was added to form an immunosorbent. After a 48-hour incubation with patient serum, the tubes were washed and ¹²⁵I-labeled AgE was added in excess. After another 48 hour incubation, tubes were washed and counted so that the nanograms of IgE against AgE in 1 ml of serum could be determined.

Symptom and medication scores

Sixty-eight patients completed symptom and medication score diaries three times daily from August 4, to October 19, 1985. The symptoms, quantified 0 to 3, were nasal congestion, nasal discharge, sneezing, ocular pruritus, cough, and wheeze. Patients also listed all medications taken. The total symptom-medication score was the summation of symptoms from all categories, each of which was given equal weight, and of weighted medication scores. Medications were weighted by assuming that administered medication would improve each applicable symptom by one point. For example, an inhaled β_2 -agonist was weighted as one point because it affects one of the quantified symptoms, wheeze. The rhinitis symptom-medication score was the total score minus asthma symptom-medication scores. Data for patients who were missing information or who were out

of the Chicago area, were filled statistically if the missing data did not comprise >25% of the period being analyzed. Patients missing >25% of the data were excluded from seasonal symptom and medication score analysis but not the safety or immunologic studies. Patients missing 4 or more days from a week were excluded from analysis of that week.

Ragweed-pollen counts

From the first week in August through the second week in October, ragweed-pollen counts were obtained daily by Grant Hospital by use of a rotating arm impactor.

Statistical analyses

All statistical analyses were performed at Key Pharmaceuticals, Inc., Miami, Fla., except for statistical analyses of immunologic data that were performed at Northwestern with paired Student's *t* test on log transformations of the data.

RESULTS

Patients

The median cutaneous end point titer of patients to short ragweed was 0.007 AU/ml and 0.06 AU/ml to giant ragweed. Thirty-two patients were prick positive to *Alternaria tenuis*, and 13 patients were positive to *Aspergillus mix*. Twelve patients had a history of mild asthma requiring only occasional bronchodilators for control.

At the initiation of the study as well as after dropouts occurred, there were no statistically significant differences between patients receiving placebo and those receiving PRW in terms of any of the following analyses: Student's *t* test analysis of IgE antibody against AgE or total antibody against AgE (before treatment); Wilcoxon rank sum test of age, giant ragweed or short ragweed end point titers; chi-square analysis of mold reactivity, asthma history, or sex. Of the 35 original pairs, 20 were retained, five were lost to dropout, and 10 new pairs were formed with original pairing criteria. Reforming of pairs, final data review, and all analyses were performed before breaking the study code.

Antibody data were analyzed for all patients who completed injections, and safety data were analyzed for all patients who received any injections.

Quantitation of reactions

Of 37 patients receiving placebo, 19 (51%) had at least one immediate local reaction. Six patients had at least one immediate local reaction consisting of erythema and wheal, whereas 13 patients had erythema alone. On the average, placebo-treated patients had 5.9 mm erythema and 0.1 mm wheal per injection. No patient receiving placebo had a dosage modification because of a large local reaction.

Twenty-six (72%) of 36 patients receiving PRW had

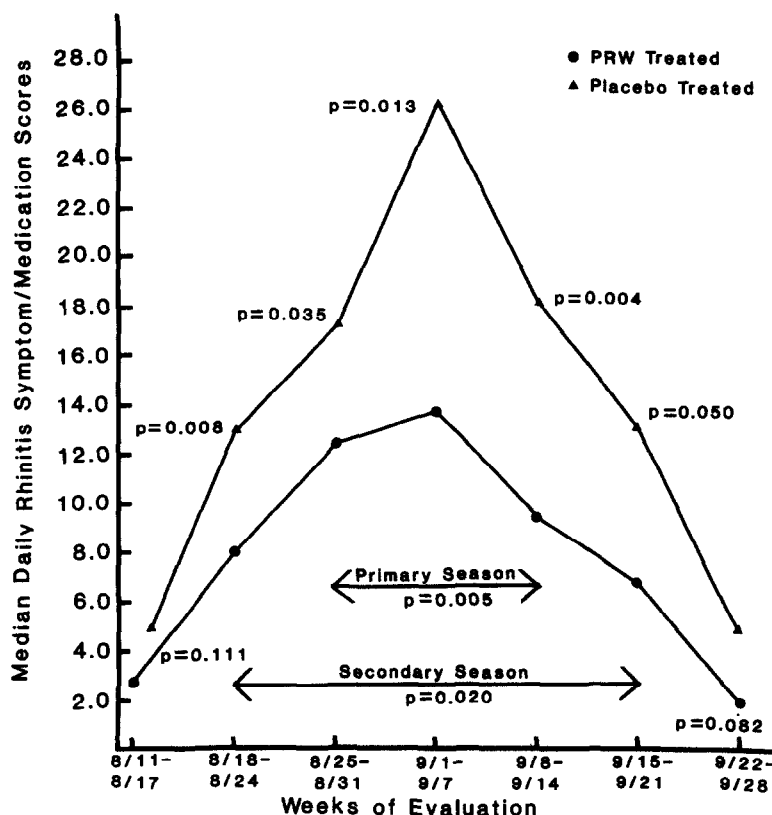


FIG. 3. The median daily rhinitis symptom/medication score indices are plotted for PRW- (●—●) and placebo-treated (▲—▲) patients. The *p* value is presented for the primary pollen season, the secondary pollen season, and for each week.

at least one immediate local reaction. Eighteen patients' reactions were characterized by erythema and induration; eight patients had erythema alone. PRW-treated patients averaged 11.3 mm erythema and 0.9 mm wheal per injection. Five patients receiving PRW had a total of seven large local reactions that required dosage modification. These patients required a total of five additional injections.

None of the patients experienced a systemic allergic reaction.

Clinical laboratory tests

No patient receiving treatment developed any significant change, as compared to results before injection, in tested laboratory parameters U/A, CBC w/diff, ESR, serum creatinine, BUN, LDH, SGOT, SGPT, GGT, alkaline phosphatase, and direct and indirect bilirubin. One patient who received placebo injections had slightly elevated liver functions after the conclusion of injections.

Immunologic studies

Changes in blocking antibody to AgE after PRW and placebo injections are illustrated in Fig. 1, A. In

the PRW-treated patients, there was a statistically significant ninefold rise in total antibody binding of AgE ($p < 0.00001$), whereas there was no change in patients treated with placebo.

The change in IgE against AgE in placebo- and PRW-treated patients is depicted in Fig. 1, B. There was a statistically significant 1.3-fold rise in PRW-treated patients but no change in placebo-treated patients.

Seasonal ragweed-pollen counts

Ragweed-pollen counts were obtained daily, summed weekly, and are illustrated in Fig. 2. The primary pollen season was defined as the peak pollen week, the week before, and the week after. The secondary pollen season was defined as the peak pollen week, the 2 weeks before, and the 2 weeks after.

Symptom and medication score indices

The median weekly rhinitis symptom-medication scores in the paired placebo- and PRW-treated patients are illustrated in Fig. 3. The principal efficacy analysis was performed with the primary and secondary pollen season-rhinitis combined symptom-medication scores

(Fig. 3). The scores of the PRW-treated patients are significantly lower than those of the placebo-treated patients. In addition, the PRW-treated patients had statistically lower rhinitis symptom-medication scores in all 3 weeks of the primary pollen season and in all 5 weeks of the secondary pollen season. The *p* values for the median weekly total symptom-medication scores are similar except for the fifth week of the secondary season, which is not significant.

DISCUSSION

This clinical trial was designed to evaluate further the safety, immunogenicity, and efficacy of the 15 injection dosage schedule of PRW used in previous trials.^{9, 10} In terms of safety, there were no systemic reactions in spite of the large dose, 16,218 PNU, administered during 15 weeks. This is an important finding in that the primary safety risk for patients receiving conventional allergen immunotherapy is anaphylaxis, and this risk has been reported to range from 8% to 30% per patient course.⁵ The cumulative number of patients treated with PRW is 299 with 4351 injections.^{9, 10} There have been three mild systemic reactions in two patients, giving a reaction rate of 0.7% per patient and 0.07% per injection.^{9, 10} Large local allergic reactions were infrequent. Of the 532 injections administered in this study, there were only seven repeated or reduced injections caused by large local reactions. Also, in terms of safety, no patient receiving PRW had any clinically significant change in any measured laboratory parameters U/A, CBC w/diff, ESR, serum creatinine, BUN, LDH, SGOT, SGPT, GGT, alkaline phosphatase, and direct and indirect bilirubin.

Immunogenicity of PRW was demonstrated in that there was a statistically significant ninefold rise in total antibody binding of AgE in the PRW-treated patients but no change in the placebo-treated patients. This rise is comparable to the rises in previous PRW trials wherein the rises were tenfold to fortyfold,^{9, 10} and in conventional immunotherapy trials.^{14, 15} In earlier clinical trials, PRW was compared to monomer immunotherapy, and comparable degrees of improvement in clinical symptoms and increase in AgE binding were observed.^{8, 16}

This trial reconfirmed our previous studies of the safety and efficacy of a 15-injection regimen of PRW for the treatment of ragweed pollinosis. In both the primary and secondary pollen seasons, the rhinitis scores for symptoms and medications were significantly lower in PRW-treated patients than in placebo-treated patients as analyzed by one-tailed paired Wilcoxon signed-rank test. The greatest improvement in

scores between PRW-treated and placebo-treated groups occurs at the peak of the pollen season.

PRW has now been studied in man for 9 years; it has also been standardized.¹¹ The two principal criteria for standardization are that PRW must have a molecular weight $\geq 200,000$ daltons as determined by gel filtration chromatography and it must have a RAST-inhibition value of 250 AU/ml. We have demonstrated that RAST inhibition values correlate with cutaneous reactivity of PRW.¹⁷

In conclusion, PRW is safe, immunogenic, and has demonstrated activity when it is administered in a 15-injection regimen. PRW-treated patients experienced no systemic reactions and no changes in laboratory parameters while they developed significant increases in total antibody binding to AgE. Finally, symptom and medication scores of PRW-treated patients were significantly lower than scores of placebo-treated patients.

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