

Comparative safety and efficacy of short ragweed extracts differing in potency and composition in the treatment of fall hay fever

Use of allergenically bioequivalent doses by parallel line bioassay to evaluate comparative safety and efficacy

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One high potency (HP) and two low potency (LP) commercial whole short ragweed (WSR) extracts were assayed for relative potency (RP) by antigen E (AgE-Amb a 1), RAST inhibition, and parallel line bioassay (PLBA). The RP of the HP extract (300 µg AgE) was equal to the reference WSR, but the LP extracts were only ~ 0.01 of the RP of the reference. Each extract was administered to fall hay fever patients with skin sensitivity to WSR ($\leq 10^{-3}$ µg/ml AgE for sum of erythema = 50 mm) to the maximum tolerated dose, or 0.5 cc of the concentrate, by either a 9- or 40-dose regimen. HP-treated patients had significantly lower symptom scores than untreated controls. No significant difference was noted in the total symptom scores between HP and LP extract-treated patients. Despite ~ 100 -fold differences in the RP of HP versus LP extracts, both produced similar frequencies of severe systemic and severe late phase local reactions. Furthermore, when RP of HP and LP extracts were estimated by PLBA, both reached similar peak dosages, similar dosage for IgG WSR antibody response, and comparable IgG WSR antibody levels, indicating that the WSR dose estimates based on RP were bioequivalent. The 9-dose HP regimen produced fewer late phase local and systemic reactions per patient than the 40-dose HP regimen. Most patients experiencing severe systemic reactions were among those most skin sensitive to WSR. The number of systemic reactions per injection was significantly higher at higher WSR doses. One LP extract produced a high incidence of systemic reactions in the initial three injections. With respect to HP, its RP varied ~ 10 -fold depending on the skin sensitivity of the patient to heat-stable ragweed allergens, demonstrating that it was compositionally different. However, when the dose of this extract was estimated by RP based on the skin sensitivity of each patient, the peak dose of the extract as well as the doses associated with late phase and systemic reactions were found to be similar to the HP doses. This indicated that immunizing dose estimates of compositionally different WSR extracts based on RP by PLBA were also bioequivalent.

Key words: allergic rhinitis; bioequivalent doses; fall hay fever; immunotherapy; safety; short ragweed; therapeutic efficacy.

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CLINICAL ASPECTS

To avoid the high incidence of systemic allergic reactions observed with maximum tolerated doses of HP and LP extracts, an HP extract was administered by an 8-dose weekly regimen to a fixed peak dose of 0.3 µg AgE (below the mean HP peak doses of 4.3 µg AgE and the mean HP systemic reaction dose of 0.9 µg AgE). The submaximal

dose (~ 0.001 of concentrate) produced no severe systemic reactions, and the total symptom scores were significantly lower than those of an untreated control group, but no IgG WSR antibody was detectable. A treated group receiving only $0.003 \mu\text{g}$ AgE (0.00001 of concentrate) had a total symptom score no different from the untreated controls. These data suggest that ragweed hay fever can be safely and effectively treated by submaximal doses of short ragweed in only eight injections and that comparative safety and efficacy of short ragweed extracts of differing potencies and composition can be evaluated by administration of bioequivalent doses based on RP estimated by PLBA.

Prior to Antigen E standardization, whole short ragweed (WSR) pollen extract concentrates contained levels of Antigen E varying as much as 100-fold, indicative of differences in manufacture and storage (13). This wide variation in potency is also present in other unstandardized allergenic extracts (3, 47). The relative safety and efficacy of commercial extracts that vary this widely have not been investigated, but safety and efficacy studies of high and low ("Rinkel") doses of the same short ragweed extract compared to each other or to a placebo have been carried out (41, 42). This study evaluated the safety and efficacy of low potency (LP) commercially available short ragweed extracts versus a high potency (HP) WSR when each was administered to a maximum tolerated dose or 0.5 cc of the concentrate (37). The safety and efficacy of a rapid versus slow maximal tolerated dose treatment regimen were also compared (36) as well as rapid treatment regimen, which used submaximal WSR doses (40). Because the relative potency (RP) of the short ragweed extracts was estimated by parallel line bioassay (PLBA), bioequivalent units could be estimated for each extract administered (33). This study therefore determined whether RP based on bioequivalent skin test doses using PLBA was also predictive of equivalent responses at bioequivalent immunotherapeutic doses (34).

MATERIAL AND METHODS

Patient population

Adult volunteers with fall hay fever of more than 2 years duration were selected for this study. Each patient completed a questionnaire

indicating the severity of the previous year's hay fever symptoms (0, absent; 1, mild; 2, moderate; 3, severe) for each month and the average number of tablets or sprays required per day for control of symptoms. Patients with moderate to severe symptoms in September (83%) who required daily medication (76%) were preferentially selected for this study. These patients reacted with a sum of erythema diameter (ΣE) of 50 mm following the intradermal injection of 0.05 ml of WSR, which contained $\leq 10^{-3} \mu\text{g}$ AgE (see *in vivo* methods). This skin sensitivity is predictive of patients whose hay fever symptoms are severe and whose symptom severity correlates with the ragweed pollen count (25). None of the patients had received immunotherapy in the past 2 years, and those with histories of fall asthma requiring medication were excluded. The control population was selected using the same criteria but received no treatment. Patients were stratified based on skin sensitivity and randomized into treatment groups. There were no significant differences found between groups in the severity of the prior year's symptoms and in the daily treatment required. This study was performed under a protocol approved by the Research on Human Subjects Committee of FDA.

Extracts and methods of analysis

Commercially available short ragweed pollen extracts (Table 1) Product A (1:10 w/v), Product C (10,000 PNU/ml), and Product D (1:20 w/v) were evaluated with respect to the FDA reference WSR (1:10 w/v, Lot E₃) by isoelectric focusing, radial immunodiffusion (RID) for Antigen E, RAST inhibition, and PLBA (39) Product E (1:20 w/v) was assayed for AgE by

Table 1
Short ragweed extracts used in treatment

Year of treatment	1978-1979			1980		1981
Product	Reference	A	C	A	D	E
Concentration	1:10 w/v	1:10 w/v	10,000 PNU/ml	1:10 w/v	1:20 w/v	1:20 w/v
Relative potency ^a						
RAST	[1]	0.76	0.032	ND	0.19	ND
PLBA	[1]	0.84	0.012	0.63	0.015 ^b	ND
Antigen E (µg/ml)	400	300 ^c	3.6 ^c	300 ^c	4.5 ^c	355 ^d

^a95 % lower and upper limits for RAST (0.47-2.12); 99 % upper and lower limits for skin test (0.60-1.66). Values within these limits are not statistically different.
^bLater testing of thermostable and thermolabile ragweed allergic patients indicated wide variability in the relative potency of this extract with respect to Product A from 0.015 to 0.172.
^cAntigen E calculated from the skin test relative potency (see Extracts and methods of analysis for method of calculation and for Antigen E content of Product A determined by radial immunodiffusion).
^dAntigen E determined by RID.
ND = Not determined.

RID. The extracts that had no detectable Antigen E by *in vitro* assay ($\leq 5 \mu\text{g/ml}$) were assigned an estimated Antigen E value based on RP by PLBA using the following formula: estimated dose of AgE in test concentrate = [(RP of test with respect to reference concentrate by PLBA) $\times$ (Dose of AgE in reference concentrate)]¹ (see Results).

This approach appeared justified based on the following line of reasoning: Products A (analyzed in 1978) and A+ (Product A re-analyzed in 1980) were not significantly different, either by quantitative skin testing [84% and 63% of the FDA reference respectively (mean = 73.5%)] or Antigen E assay [75% and 59% respectively of FDA reference (mean = 67%)]. The reproducibility of the Antigen E assay is $\pm 25\%$ (7). The reproducibility (99% limits) of quantitative skin testing is 60-166% of the reference when three tests are carried out (39). Be-

cause *in vitro* assays of the single antigens AgE and Rye 1 are highly predictive of the *in vivo* RP by PLBA ($r = 0.99$) (33, 39), the *in vivo* RP was used to estimate the single antigen content in those products where it fell below the limits of detection of the *in vitro* test. For example, because Products A and A+ had detectable Antigen E, these products should provide evidence to support this hypothesis. The FDA reference contained 400 $\mu\text{g AgE/ml}$ (39). The mean skin test potency for Products A and A+ is 73.5% and thus their estimated AgE content is 0.735×400 or $\sim 300 \mu\text{g/ml}$. The actual Antigen E content of these products is 0.67×400 or $268 \mu\text{g/ml} \pm 25\%$ (range 201-335). Thus the estimated AgE of Products A and A+ falls within the actual AgE limits found by *in vitro* analysis, providing evidence that the AgE content of extracts whose AgE is below the limits of *in vitro* detection could be estimated by use of quantitative skin testing (39) (see Results). Since Products A and A+ are equipotent they are both referred to as Product A (Table 1).

Following completion of the safety and efficacy studies comparing Product A with LP extracts Products C and D (1978-1980), AgE was assigned to each extract concentrate based on skin test RP (Table 1).

When moderate and low doses of Product E were compared for safety and efficacy (1981),

1 To avoid basing the potency of an extract solely on the content of a single antigen, such as AgE, the potency of the WSR reference can alternatively be designated by units reflecting its allergenic activity (e.g. 100,000 units). The bioequivalent dose of all other extracts can then be expressed in similar units with respect to the reference by multiplying their RP by 100,000 (33). These doses will be bioequivalent to the reference only if the RP is estimated using either a bioassay such as PLBA or an *in vitro* assay which is predic

the AgE dose was calculated from the RID value of the concentrate.

IgG ELISA

Measurement of allergen-specific IgG in patient sera was performed using an ELISA technique (12). Briefly, polystyrene microtiter plates were sensitized with WSR extract comparable to the reference extract. After overnight incubation at 4°C, plates were washed with a solution of normal saline containing 5% Tween 20. Patients' sera were then added to the wells at a constant volume and allowed to incubate for 3 h at room temperature. After washing, bound IgG was detected with an alkaline phosphatase-conjugated affinity purified anti-human IgG directed to the Fc fragment (Atlantic Antibodies, Scarborough, ME). P-nitrophenyl phosphate was used as the substrate, and color development was arrested with 3 M NaOH after 30 min. Optical density (OD) was read at 420 nm, and individual patient results were expressed as relative to the OD produced by a positive serum pool.

in vivo Methods

Quantitative skin testing to determine patient sensitivity was performed by injecting 0.05 ml of allergen intradermally in the volar surface of the forearm using three-fold serial dilutions. The sum of the longest plus mid-point orthogonal diameter defined the skin reaction at each dilution. The best-fit regression line [sum of erythema (ΣE) on $\log_3$ dose] was calculated using the first dose where $\Sigma E = 0$ or $\Sigma E \sim \Sigma W$ and the next three more concentrated serial doses. D_{50} , the dose required to produce a $\Sigma E = 50$ mm, was computed from the regression line $Y = mx + b$ when Y (ΣE) is 50 mm, m is slope (mm/ $\log_3$ dose), and x is $\log_3$ dose allergen (33, 39). Only lines where the correlation coefficient was ≥ 0.85 and the slope was ≥ 10 were used.

PLBA (33, 39) was used to determine the RP of each extract against the reference. In brief, a best-fit line for each extract and reference was

determined for each subject by finding four dilutions in triplicate producing sum of ΣE between 0 and 120 mm. A best-fit linear regression line using mean of ΣE on $\log_3$ dose was then calculated for each extract. In a valid test, the correlation coefficient for each line must be ≥ 0.85 and the slopes (≥ 10) not significantly different from the reference at $P = 0.01$. The computed distance between the reference and test extract best-fit lines is the RP. Geometric mean RP is based on a minimum of three valid assays in different patients.

Dose and administration

In 1978, a slow, conservative WSR regimen was started coseasonally with 1978 ragweed pollination and continued uninterrupted until terminated prior to the 1979 ragweed pollen season. This regimen was targeted to reach a 5 μ g Antigen E dose of Product A or 0.5 cc of Product C concentrate in 20 weekly injections using a variable dose regimen with 35% an average dose increment.

In 1980, WSR was given by a rapid schedule prior to the ragweed season and targeted to reach 5 μ g of AgE Product A or 0.5 cc of Product D concentrate in eight weekly injections, with 300% dose increments.

The highest dose administered was set arbitrarily at 0.5 cc of the concentrate or the maximum tolerated dose. Bioequivalent doses based on RP by PLBA were estimated at the end of the study.

In 1981, WSR was given by a rapid schedule prior to the ragweed season and was targeted to reach either 0.3 μ g or 0.003 μ g AgE in six weekly injections with 200% dose increments.

The criterion for proceeding from one dose to the next more concentrated dose was the absence of a systemic or severe delayed local reaction. In 1978–1979, doses were repeated or reduced to the preceding dose if the delayed local reaction, measured at 6 and 48 h, was > 50 mm in diameter.

Pollen counts. 1978 and 1979 pollen counts were obtained from a roto-slide sampler in Northern

Virginia ~ 36 m above ground. 1980 and 1981 pollen counts were obtained from a Pierre Cour sampler (9) in Bethesda, Maryland, ~ 50 m above ground.

Evaluation of safety and efficacy

Patients completed symptom diaries morning and evening. Every 12 h, sneezing, itchy nose/itchy throat, runny/stuffy nose, and eye symptoms were each scored as 0, none; 1, < 30 min; 2, 30 min to 2 h; or 3, > 2 h. Medication scores were determined by the number of tablets and/or capsules or sprays and/or drops used times a score assigned for each medication used. The sum of the combined symptom and medication score for each day was the total score. At the end of each week, an overall severity (0, none; 1 mild; 2, moderate; 3, severe; and 4, interferes with daily activity or sleep) score combining daytime symptoms (AM nasal, AM eye) and night-time symptoms (PM nasal, PM eye) was computed. The maximum possible total end-of-week severity score was 16. A total end-of-week severity score of 2 or less indicated minimal symptom severity.

In each ragweed season, a 21-day period surrounding peak pollination was selected to determine the mean total score for each patient.

Delayed reactions following each injection were recorded by the patient on an adverse effects reporting form. Patients measured redness and swelling with a millimeter rule at injection sites 6, 12, 24, 48, and 72 h postinjection and graded local discomfort, itch, and heat at the injection site as absent, mild, moderate, or severe. Subject sensations following injection were solicited and recorded by a physician at 15 min and assumed to be injection related. Patients recorded any unusual symptoms following their injections and any medications required for treatment of adverse reactions. Subjective adverse reactions were also assumed to be injection related. Systemic reactions tabulated in this manner are worst case estimates and not biased by the subjective evaluation of the investigator participating in the study. Reactions observed and treated by the physician were also

reported on the adverse reaction form to determine the total number of systemic reactions experienced.

Statistical methods

The primary statistical analysis of this paper is nonparametric. It is preferred because of the questionableness of the distributional assumption of normality and the relatively small number of observations.

For data analysis from two independent groups, the Mann-Whitney test (Wilcoxon test) was used to test the hypothesis of equality of medians. For more than two independent groups, the Kruskal-Wallis test was employed (18). When the medians were not equal and their ordering was known *a priori*, the Jonckheere test was employed (18).

When the Kruskal-Wallis test indicated that all the medians were not equal, a stepwise multiple comparison procedure was used to isolate which pairs differed. The particular procedure used at an experimentwise level of 0.05 is that of Ryan as applied to the appropriate nonparametric procedure (11). This stepwise approach can be applied with the tests of Kruskal-Wallis, Jonckheere, and the treatment versus control problem, as well as tests for equality of proportions based on the chi-square test or Armitage's test for ordered proportions(5).

In several instances, it is important to compare all treatments with a control in a one-sided test, as well as among themselves. This was accomplished by apportioning α ($= 0.05$) so that half was used for the one-sided nonparametric treatment versus control test (18) and half for all the treatment comparisons.

In a preliminary investigation, the parametric analysis of covariance was applied to total score data for each of the years to determine whether variables other than the treatments, such as age, duration of symptoms, previous treatment, and skin sensitivity, contributed to differences among the groups. In 1978 skin sensitivity and duration of symptoms were important covariates, but neither group (Product A or Product C) was found to differ with or without these

Table 2
Patient population characteristics

Year of treatment Regimen Product	1978-79			1980		
	Slow		Untreated	Rapid		Untreated
	A	C	Control	A	D	Control
No. of patients	12	13	18	13	12	12
Mean age (years)	37	34	37	32	40	35
Mean duration of fall symptoms (years)	16	21	17	13	20	23
No. of patients with prior treatment	3	5	9	3	3	8
No. of dropouts	1	0	NA	0	1*/3**	NA

*Unrelated to therapy. **Adverse reaction. NA = not applicable.

covariates. In 1979 skin sensitivity was also an important covariate, again not changing the inference about which groups differed. In 1980, however, the age of the patients and duration of symptoms were important covariates which were confounded with treatments (see Results). In 1981 no covariates were important.

The analysis of the data in Fig. 6 differed from the above described procedures in that the data at different dose levels were based on the same patients and hence not independent. There is a positive correlation for scores of two adjacent dosages for the same individual. Thus a Friedman-type dependent data analysis was

performed (18) to detect the first dose that differed in a positive direction from the no-dose background. For overall level α of 0.05, this was accomplished in five doses by using one-sided testing to compare dose 1 versus 2 at 0.0125; doses 1 and 2 versus 3 at 0.0125; doses 1, 2, and 3 versus 4 at 0.0125; and doses 1, 2, 3, and 4 versus 5 at 0.0125. The procedure stops at the first dose significant from those preceding it.

RESULTS

The properties of the extracts used for diagnosis and immunization in 1978 through 1980 are in Table 1. Product A was equipotent to the reference in all tests; Products C and D were approximately 0.01 of the reference by PLBA. Product C had no detectable Antigen E, and Product D had approximately 5 $\mu\text{g/ml}$ of AgE by the RID test, which latter value is at the lower limit of RID detection and therefore may not be accurate (2). To estimate the Antigen E content of Products C and D when their AgE were either below or at the lower limit of the *in vitro* test, an allergenically bioequivalent estimate of AgE was carried out by multiplying the respective RP of Products C and D with respect to Product A (see Extracts and methods of analysis). For example, since the skin test RP of Product C is 0.012 of product A, Product C can be estimated to contain 0.012 of the AgE content of Product A. Since Product A contains 300 $\mu\text{g/ml}$ of AgE in 1:10 w/v (see Extracts and methods of analysis), Product C is estimated to contain 3.6 $\mu\text{g/ml}$ of AgE in 10,000 PNU/ml.

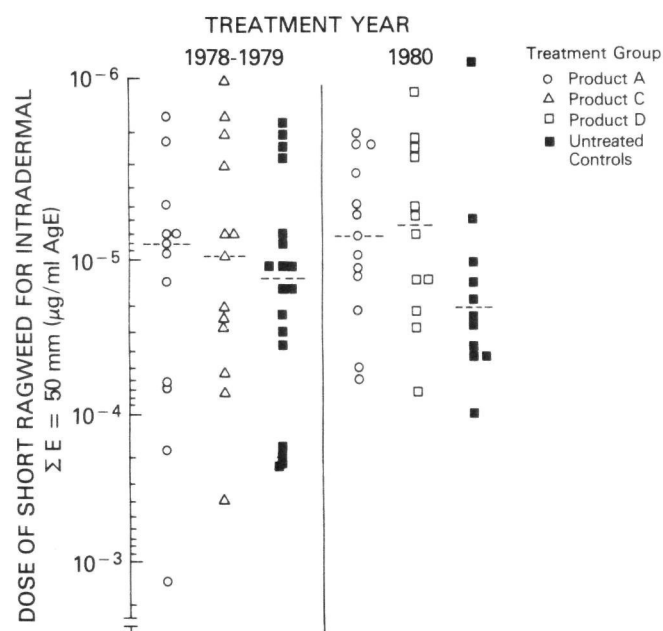


Fig. 1. Dose of Antigen E ($\mu\text{g/ml}$) in whole short ragweed extract for sum of erythema diameter (ΣE) equal to 50 mm in previously untreated patients. Patients on immunotherapy in 1979 and reimmunized in 1980 are not shown.

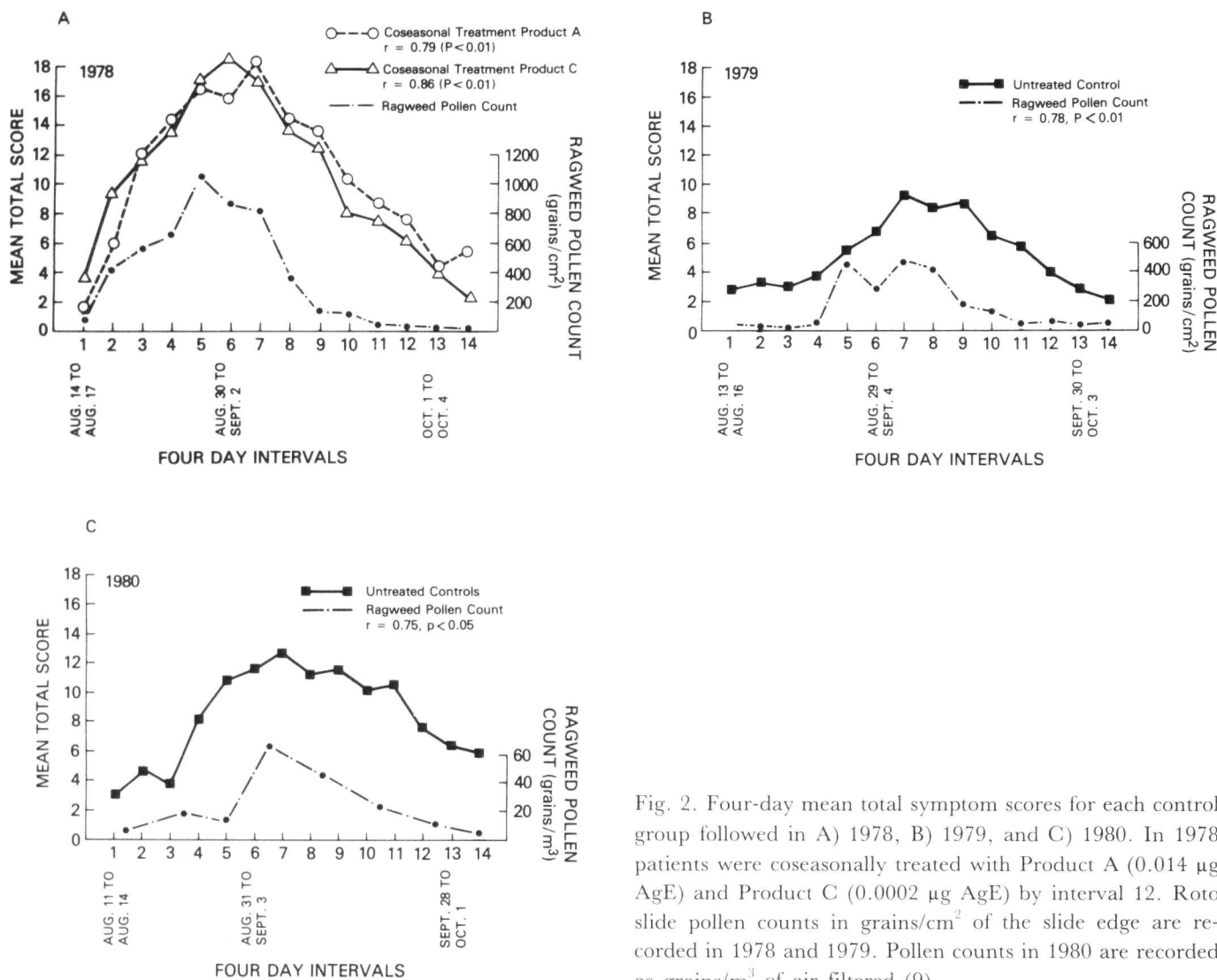


Fig. 2. Four-day mean total symptom scores for each control group followed in A) 1978, B) 1979, and C) 1980. In 1978 patients were coseasonally treated with Product A ($0.014 \mu\text{g AgE}$) and Product C ($0.0002 \mu\text{g AgE}$) by interval 12. Roto slide pollen counts in grains/cm² of the slide edge are recorded in 1978 and 1979. Pollen counts in 1980 are recorded as grains/m³ of air filtered (9).

To deliver $3.0 \mu\text{g og AgE}$ requires either 0.83 ml of $10,000 \text{ PNU/ml}$ of Product C or 1.0 ml of $1:1,000 \text{ w/v}$ of Product A. A similar calculation was done for Product D. By this method, the AgE content of each dose of Products C and D was calculated.

The demographic characteristics of the patients treated are shown in Table 2. They were not statistically different within years based on a Kruskal-Wallis nonparametric test, with the exception of age and duration of symptoms in 1980. Since no effect of age and duration of symptoms is seen on total score in 1979 and 1981, these differences were not thought to contribute to the treatment effects seen in 1980. Using the Kruskal-Wallis procedure there was no significant difference in skin sensitivity between treatment and control groups (Fig. 1) for 1978–1980.

The pattern of symptoms for each control group followed through the 1978–1980 ragweed seasons is shown in Fig. 2. In 1978 the treatment groups (Products A and C) did not significantly differ in mean total scores at each time period despite coseasonal treatment at end of pollination with $0.014 \mu\text{g AgE}$ of Product A and $0.0002 \mu\text{g AgE}$ of Product C, and their total scores were statistically correlated to the pollen count (Fig. 2A). Untreated control patients selected in 1979 and 1980 using the same criteria also showed a significant correlation of the mean group total scores to the pollen count in each season, confirming the utility of this patient selection method (Fig. 2, B and C). There is a marked impact of pollination intensity in 1978 and 1979 (Fig. 2, A and B) on the median total score between pollen seasons. The pollen count data of 1979 and 1980 are not

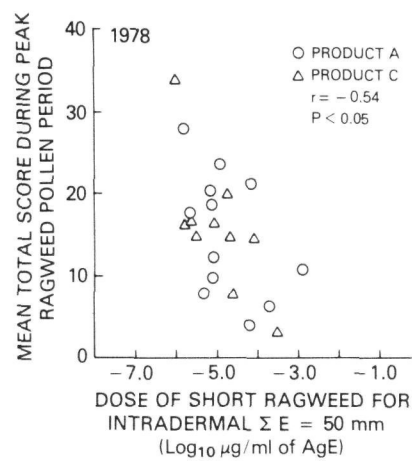


Fig. 3. Relationship of mean total symptom scores for each patient during peak ragweed pollenation in 1978 to skin sensitivity (μg/ml AgE in WSR for ΣE = 50 mm).

directly comparable because a different pollen sampling device was used.

The correlation of total symptom score with skin sensitivity during the peak pollenation was statistically significant in 1978 ($r = -0.54$, $P < 0.05$) (Fig. 3) but not during 1979 and 1980. This may be due to the lower total scores seen in those years compared with 1978 (Fig. 2, A-C).

In 1978, the 4-day mean interval 4–8 (Fig. 2A) and in 1979 and 1980 (Fig. 2, B and C) 4-day mean interval 5–9 defined the period of peak pollenation used to compute the mean total scores for treated and un-treated patients. These were plotted against the mean end-of-week severity score for the same period (Fig. 4). The predicted mean total score for patients whose end-of-week severity scores were 2 (minimal symptom severity) using a linear regression of total score on end-of-week severity was 4.4, which is very close to the total score seen at the beginning and end of ragweed pollenation (Fig. 2), that is, the level of non-ragweed related symptoms.

The effect of different dose regimens and extracts on the peak dose is shown in Table 3. Despite great differences in dose schedules between 1979 and 1980, the peak dose reached for Product A ($\sim 4.2 \mu\text{g AgE}$) in each year is comparable. Although Products C and D have peak and systemic reaction doses that are biased to slightly lower values than Product A because of lower potencies, the AgE estimates for the peak and mean doses associated with adverse effects vary only several-fold, despite the nearly 100-fold variability in estimated AgE content of

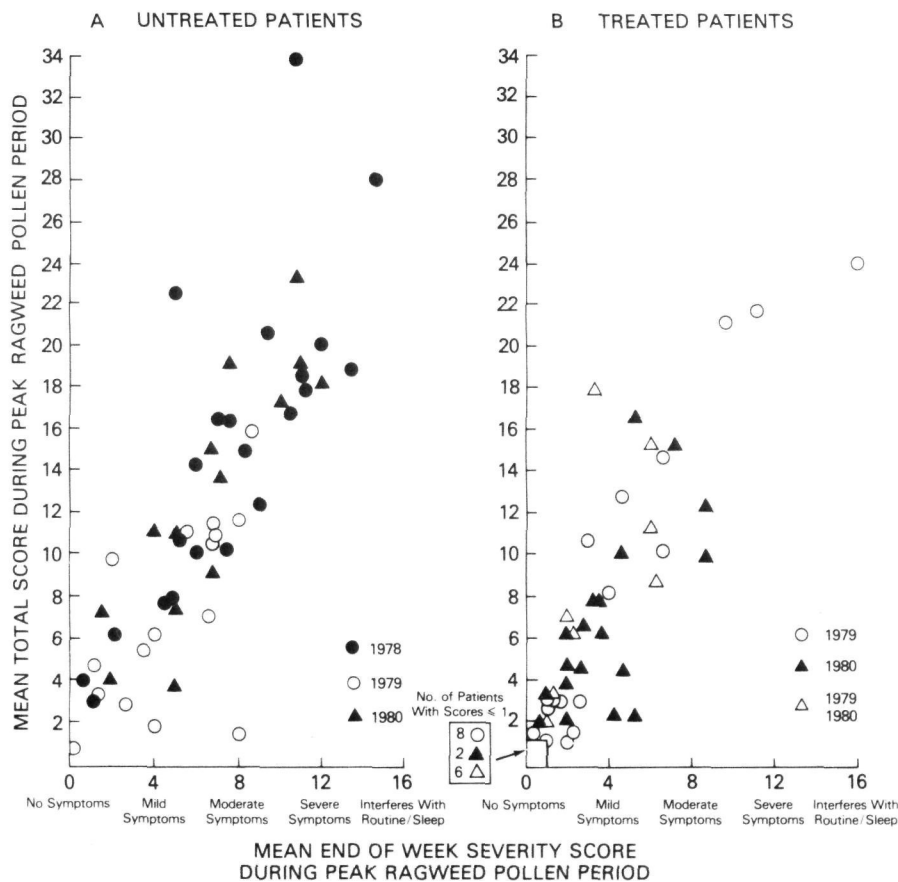


Fig. 4. Relationship of mean total symptom score during peak ragweed pollenation to mean end-of-week severity score computed for each patient for the same time period for A) control patients and B) high dose preseasonally treated patients. All patients were not previously treated except for patients treated in 1979 and 1980 (Δ).

the concentrates, demonstrating that the RP estimates using PLBA are also predictive of bioequivalent immunizing doses.

Both Kruskal-Wallis analysis and analysis of covariance using skin sensitivity as the covariate found no significant differences among products and dose regimens based on total scores at the peak of ragweed pollination for years 1978-1980 (Fig. 5). In years 1979 and 1980, based on a nonparametric treatment versus control test, both products yielded a significantly lower median total score than the untreated controls ($P < 0.05$), except for Product C in 1979. This lack of a significant difference between Product C and the untreated controls may be attributed to the lower total score of the untreated controls due to less intense pollination in 1979 or the relatively small sample size, which cannot statistically detect a smaller effect (46).

In 1979 subsequent Mann-Whitney testing of Products A and C failed to detect a difference and in 1980 the Kruskal-Wallis test found no difference among the treatments.

Despite the failure to detect AgE by *in vitro* assay, the administration of both Products A and C resulted in a comparable rise in specific IgG antibody against AgE and WSR at bio-

equivalent AgE doses based on PLBA (Fig. 6). This confirms that each product contained AgE and that the estimation of the AgE content of Product C with respect to Product A, based on its RP by PLBA, was reasonable. Based on a Friedman-type analysis (see Methods), the significant increases in 1979 antibody levels were detected after the administration of 0.24 µg of AgE in WSR (Fig. 6). The rapid dose regimen used in 1980 also produced significant increases in specific anti-WSR IgG antibody with no significant differences in antibody level seen between Products A and D. No significant association was found between total score during peak pollination and specific IgG against WSR, BSR, or AgE.

Adverse reactions from each product and dose regimen are presented in Table 4. Product C, administered to a lower peak dose than Product A in 1979 or Product D in 1980 (Table 3), induced fewer adverse reactions (Table 4), suggesting that the incidence of adverse reactions is dose-related.

Despite LP, Product D produced more systemic reactions than Product A, especially following the first three injections. It was determined that when Product D was initially

Table 3
Short ragweed injection schedules and short ragweed doses administered during treatment

Year of treatment Dose regimen Product	1978-1979 Slow		1980 Rapid	
	A	C	A	D
No. of injections to peak dose/patient	32	38	8	7
No. total injections/patient	34	40	9	7
GM initial dose (µg AgE)	0.002	0.00001	0.02	0.02 (0.20) ^a
GM peak dose (µg AgE)	4.3	0.82	4.2	2.7
Range µg AgE	0.60-90.6 ^b	0.18-1.80 ^b	0.12-150 ^b	0.09-11.41 ^b
GM systemic reaction dose (µg AgE)	2.3	0.23 ^c	0.9	0.6
Range (µg AgE)	0.15-11.94	0.04-1.8	0.04-9.9	0.07-9.1
GM dose producing systemic reaction requiring epinephrine (µg AgE)	0.9	0.16	1.5	0.64
Range (µg AgE)	0.15-4.5	0.06-0.45	0.12-9.9	0.10-3.11
GM severe local reaction dose (µg AgE)	0.1	0.1	0.2	0.4
GM large local (> 7.5 cm) reaction dose (µg AgE)	0.3	0.09	0.2	0.2

GM = geometric mean.

^aSee Results for explanation of discrepancy; relative potency of Product C was dependent on patient skin sensitivity.

^bUpper dose limit was dependent on maximum tolerated dose reached or 0.5 cc of Product A (1:10 w/v), Product C (10,000 PNU/ml), and Product D (1:20 w/v).

^cOne patient experienced two mild systemic reactions at a very low dose, 0.006 µg AgE. If this unusual patient is included, the geometric mean systemic reaction dose is 0.08 µg AgE, range (0.006-1.8).

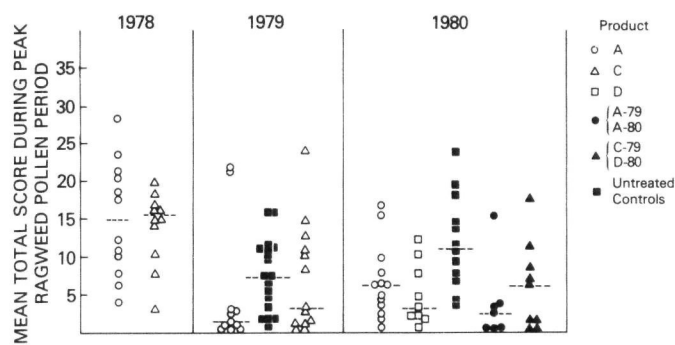


Fig. 5. Scattergram of mean total symptom score for each patient during peak pollenation. Two different populations of untreated patients ($\circ$, Δ) were studied in 1978–79 and 1980. All patients were previously untreated except for 1980 patients treated in 1979 and 1980 ($\bullet$, $\blacktriangle$). Coseasonal treatment of patients in 1978 (see legend Fig. 2) did not influence symptom scores in 1978 and treatment was continued uninterrupted into 1979.

assayed by PLBA, the patients selected were by chance less sensitive than most of the treated patients and primarily sensitive to thermolabile allergens (39). Reevaluation of Product D using patients with more representative skin sensitivity revealed that the RP of Product D with respect to Product A was patient-dependent, varying from 0.015 to 0.068 (range) in patients sensitive to thermolabile allergens but varying from 0.092 to 0.172 (range) in patients sensitive to both thermolabile and thermostable allergens (BSR); the latter RP approximates the RP by RAST inhibition (Table 1). Thus, the RP of Product D varied from about 0.1 to 0.01 of Product A, depending on patient sensitivity. The AgE content of Product D bioequivalent to Product A was estimated using the RP obtained from the best-fit line (Fig. 7). Since the original value of 0.015 for the RP of Product D with respect to Product A was used to estimate the dose administered, some highly sensitive patients received at least 10 times the anticipated dose, resulting in a high incidence of systemic reactions occurring in the first several injections (Table 4).

The effect of dose regimen length on safety and efficacy is illustrated by comparing Product A data in 1979 with 1980 data, since the products and sensitivity of patients were comparable. Despite the 75% reduction in number of injections in 1980, the peak doses reached in

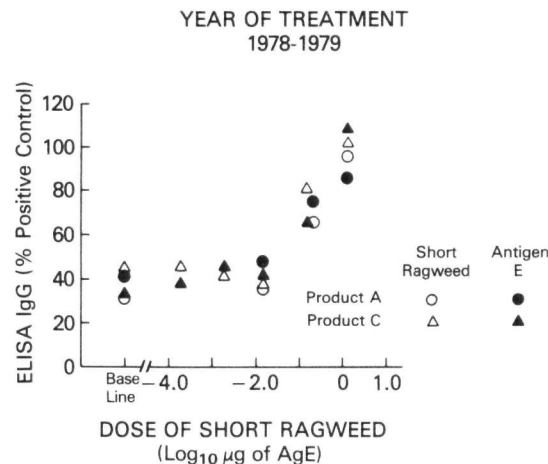


Fig. 6. Relationship of specific IgG antibody titer directed against whole short ragweed (WSR) and Antigen E to dose of WSR ($\mu\text{g AgE}$) administered during 1978–1979.

1979 and 1980 were comparable, as well as the doses that induced systemic and local reactions (Table 3). Although the frequency of adverse reactions (% patients) was similar for each dose regimen, the number of adverse reactions per patient (Table 4) was reduced at least 40% with the short dose regimen. Furthermore, the 1980 short dose regimen was efficacious (Fig. 5).

The effect of patient sensitivity on the severity of systemic reactions is illustrated in Table 5. Further analysis has shown that patients with severe reactions were statistically more skin-sensitive than patients with no reactions in 1979 and different from patients with mild or no reaction in 1980 ($P < 0.05$).

The number of patients with systemic reactions and the ratio of these reactions per injection at high and low WSR dose levels of AgE were tabulated for each previously untreated patient (Product A, 1978–1979 and 1980) and each previously treated patient (A*, 1980) (Table 6). A sign test, conditional on those patients with an adverse reaction, found that the proportion of adverse reactions per injection was larger for the high dose than the low dose ($P < 0.002$). No discernable dose patterns were apparent for systemic reactions requiring epinephrine or for Products C or D, Product C having a low incidence of systemic reactions at all doses and Product D a high incidence at all doses. However, ragweed doses $\leq 0.3 \mu\text{g AgE}$ produced systemic reactions requiring epine-

phrine in only 7.5% of the patients treated in 1979 and 1980. Skin sensitivity may play a role here, too, since the single patient who reacted at doses $\leq 0.03 \mu\text{g}$ AgE had the highest degree of skin sensitivity and was also one of the two patients in 1980 who reacted at doses $\leq 0.3 \mu\text{g}$ AgE; the other 1980 patient who reacted at a low dose did not have an extremely high skin sensitivity. No protective effect of prior immunotherapy on the percent of patients with systemic reactions, the ratio of systemic reactions to injections (A + , Table 6), or the percentage of patients with systemic reactions requiring epinephrine (Table 4) was observed. No association was found between degree of skin test sensitivity and peak dose reached.

In summary, the peak dose of Product C containing $0.8 \mu\text{g}$ of AgE produced total symptom scores not significantly different from the total symptom scores of patients who received Product A containing $4.3 \mu\text{g}$ of Antigen E (Fig. 5). The Product A short dose regimen resulted in fewer adverse reactions per patient than the Product A long dose regimen (Table

4). Thus it was decided to administer WSR containing $0.3 \mu\text{g}$ AgE, a submaximum tolerated dose, in a short dose regimen to determine whether it would have fewer adverse reactions without loss of efficacy. The dose was also anticipated to produce an IgG WSR rise (Fig. 6).

Forty-three patients with appropriate histories (24 previously treated) were randomized and stratified based on skin sensitivity to AgE (mean sensitivity for each group $\leq 10^{-5} \mu\text{g/ml}$ AgE in WSR for $\Sigma E = 50 \text{ mm}$) and, if previously treated, on their 1980 total score during peak pollination to control for the carry-over effect of prior therapy. Each patient received either a moderate dose of Product E (Table 1) containing $0.3 \mu\text{g}$ AgE or a low dose containing $0.003 \mu\text{g}$ AgE in six weekly injections just prior to the ragweed season. Six previously untreated and three previously treated patients served as untreated controls. Doses and adverse reactions in 1981 are shown in Table 7, along with previously observed ranges. Systemic and severe local reactions per patient were dramatically reduced and, more importantly, severe systemic

Table 4
Adverse reactions

Year of treatment	1979		1980	
Regimen	Slow		Rapid	
Product	A	C	A	D
I. Systemic reaction				
No. of patients	12	13	20 ^a	22 ^a
% Patients	75	54	75	50
% Patients requiring epinephrine	25	15	30 ^b	23 ^b
No. patients reacting in first three injections	0	0	2	7
Total no. reactions	23	11	19	32
Reaction/injection	0.056	0.021	0.11	0.22
Reaction/patient	1.9	0.85	0.95	1.52
II. Severe delayed local reactions				
% Patients	50	23	25	25
Total no. reactions	15	8	5	5
Reaction/injection	0.037	0.015	0.028	0.034
Reaction/patient	1.25	0.62	0.25	0.24
III. Large local (> 7.5 cm) reaction				
% Patients	50	15	47	50
Total no. reactions	20	5	20	15
Reaction/injection	0.049	0.01	0.11	0.10
Reaction/patient	1.67	0.39	1.0	0.71

^aIncludes patients previously treated with high and low potency extracts in 1979 who opted for reimmunization in 1980.
^bThe % patients requiring epinephrine in 1980 who had previously been treated in 1979 with Product A was (2/7) 28.5% and Product C (1/10) 10%.

reactions were eliminated (Table 7). The systemic reactions experienced at these low doses were mild, short-lived, and did not require medication. The incidence of large local reactions in the 0.3 µg AgE (moderate dose) group was comparable to that seen in 1980. Based on an ordered alternative nonparametric test with stepwise multiple comparisons, total scores during 1981 peak pollination (Fig. 8) were significantly lower for the moderate dose group compared with either the low dose group or the untreated groups ($P < 0.02$). The proportion of patients with minimal symptoms (total scores of ≤ 5.0) using an analysis based on ordered proportions (moderate dose $\leq$ low dose $\leq$ control) was found to differ in that moderate dose $<$ low dose and moderate dose $<$ control at $P = 0.04$.

Despite the reduction in symptoms and the administration of 0.3 µg AgE, no significant increase in IgG antibody against WSR was seen during the rapid dose regimen in 1981.

DISCUSSION

This study demonstrates how estimating allergenically bioequivalent doses using PLBA was used to evaluate comparative safety and efficacy of high and low potency extracts and extracts that are compositionally different. Once bioequivalent doses were estimated, the influence of patient sensitivity, intensity of pollination, and specific IgG responses on the comparative safety and efficacy of short ragweed extracts of varying potency and dose regimens could be determined.

Product A was the most potent of the three extracts and was equivalent to the Reference, while Product C had approximately 1% of the RP of Product A. However, the administration of both products at bioequivalent AgE doses (Fig. 6) based on PLBA generated equal IgG responses and similar adverse and beneficial clinical responses at similar doses. The data therefore indicated that Product C could be considered a dilution of Product A and that the RP based on skin test was predictive of bioequivalent doses used in immunotherapy.

Product D was also much less potent than Product A but it was compositionally different

because its RP varied with the patient tested (Fig. 7). Since a single RP could not be assigned to Product D that was applicable to each patient, bioequivalent doses of Product D with respect to Product A were estimated based on its RP in each patient (Fig. 7). The RP of Product D was higher in patients sensitive to thermostable ragweed allergens (BSR) than in patients sensitive only to thermolabile allergens (Fig. 7), indicating that it may have been degraded, possibly from improper storage (1). Despite these compositional differences, Product D was equally immunogenic as Product A and produced similar adverse and beneficial clinical responses when bioequivalent doses of each were administered, demonstrating that bioequivalent doses based on skin test RP (PLBA) were also bioequivalent at immunotherapeutic doses. Administration of bioequivalent doses therefore permits comparison of the relative safety and efficacy of widely disparate extracts from the same source independent of differences in allergen potency.

Product D differed from Products A and C in that occurrence of systemic reactions could not be predicted on the basis of a single RP estimate since its RP varied with each patient. This was evident only when RP was determined by skin-testing selected patients. The patient dependence of RP may also not be evident by RAST inhibition since a pooled serum is used, masking individual RP differences (33), nor can it be predicted from a single antigen assay such as AgE. Thus, standardization, although reducing variability in potency, will not necessarily preclude compositional differences unless additional assays of compositional identity are also carried out. The safest approach for physicians in switching extracts or in immunizing patients using extracts from different sources is to evaluate the skin response to the two different extracts.

Although only two LP extracts were examined in this study; it is possible that extracts, especially those stored following dilution, may be further degraded than Product D since dilute extracts degrade more rapidly (24). The safety and efficacy of extracts with more extensive degradation are not known.

In comparison with other safety and efficacy studies, the peak doses reached in this study are somewhat lower than peak doses administered by Van Metre et al. by a slow weekly dose regimen, but comparable to those found in the clustered regimen (41). This may be due to differences in the dose regimens and criteria for proceeding to subsequent doses in the two studies. We could not detect a dose-dependent difference in efficacy during coseasonal treatment with two different regimens of WSR containing $\leq 0.01 \mu\text{g}$ AgE and differing 70-fold in peak dose. Studies have shown that lower coseasonal doses are also ineffective (42). The question of whether higher coseasonal doses can be administered safely and effectively remains to be determined.

When high peak doses were administered with Products A and D, the rates of adverse systemic and local reactions per patient and injection, as well as percent of patients with systemic reactions, were similar to that observed by Van Metre et al. (41). Their systemic reaction dose usually exceeded $1.0 \mu\text{g}$ AgE, which was well above their large local reaction dose ($> 5.0 \text{ cm}$ diameter reaction) (41). Also, their ratio of median symptom scores of high dose

treatment patients to placebo controls during peak pollination exceeds two-fold, as does ours, illustrating the value of using similar symptom scoring systems, similar assays of patient sensitivity, and a common bioequivalent unit of potency.

The short dose regimen (Product A) designed to rapidly reach a maximum dose produced fewer adverse reactions per patient than the long dose regimen (Product A). The smaller number of injections in the short dose regimen limited the cumulative number of potential adverse exposures of patients and was thus safer than the long dose regimen, confirming a similar observation by Golden et al. (15). Nevertheless, the percentage of patients with severe adverse reactions was similarly high in the slow and rapid regimens when maximum tolerated dosage was administered, necessitating evaluation of the safety and efficacy of submaximal doses of short ragweed.

The use of short ragweed extracts assigned a bioequivalent unit of potency permitted a rational selection of doses below the maximum tolerated and systemic reaction doses for evaluation of safety and efficacy.

A dose of WSR containing $0.3 \mu\text{g}$ AgE was administered in a total of eight weekly injections and was demonstrated to be safer than the maximum tolerated dose and more efficacious than either a very low dose or no treatment. This dose may more accurately reflect those used in clinical practice where the incidence of systemic reactions was estimated to be less than 1% (21). It also produced a high incidence of large local reactions ($> 7.5 \text{ cm}$) indicating that this dose retained substantial allergenic activity.

The search for the optimal safe and efficacious dose is not of recent origin. Rinkel's tech-

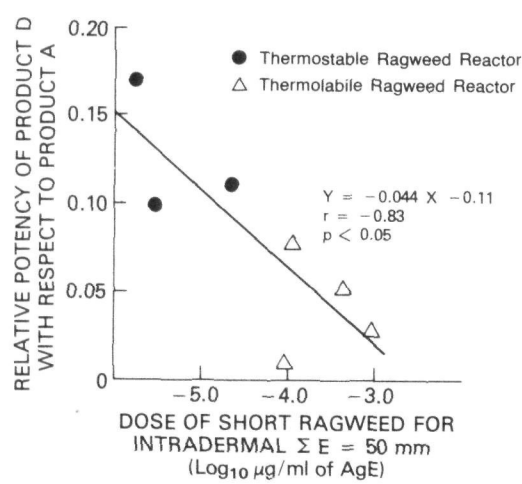


Fig. 7. Relationship of the relative potency of Product D with respect to Product A based on patient skin sensitivity to WSR ($\mu\text{g/ml}$ AgE for $\Sigma E = 50 \text{ mm}$). Patient reactivity to thermostable (TS) and thermolabile (TL) short ragweed allergens is shown. Thermostable ragweed reactors were defined as patients with $\Sigma E > 40 \text{ mm}$ following puncture testing with bifurcated vaccinating needles using WSR 1:100 w/v and boiled short ragweed (BSR) 1:40 w/v (2). Thermolabile ragweed reactors reacted to unboiled WSR 1:100 w/v, but not to BSR 1:40 w/v.

Table 5. Relation of percentage of patients with severe systemic reactions to degree of skin sensitivity

Skin sensitivity to WSR			
[Log ₁₀ AgE			
(μg/ml) for ΣE = 50 mm]	≥ -4.20	-4.20	≤ -5.20
No. of patients requiring epinephrine for systemic reaction	2	4	9
Total no. of patients	13	9	13
% With severe systemic reaction	15.4	44.4	69.2

Table 6

Effect of high and low dose of Product A on the number of systemic reactions/injection (Sys Rxn/Inj)

Year of treatment Product	1978-1979 A		1980 A		1980 A*	
WSR dose (µg AgE)	≤ 0.3	> 0.3	≤ 0.3	> 0.3	≤ 0.3	> 0.3
No. of patients	1		1 ^a		1	
¹ Sys Rxn/Inj	3/21	1/5	1/7	0/4	2/6	1/1
No. of patients	8		8		4	
² Sys Rxn/Inj	0/155	19/120	0/32	8/36	0/9	5/23
No. patients	3		3		2	
³ Sys Rxn/Inj	0/46	0/63	0/20	0/18	0/5	0/15

*Patients treated with Product A in 1979 and 1980
^aOne other patient had Sys Rxn/Inj = 2/6 at ≤ 0.3 µg AgE with no high dose administered
¹Patients in this row had Sys Rxn at low WSR dose.
²Patients in this row had Sys Rxn at high WSR dose.
³Patients in this row did not react regardless of dose.

nique for determining the optimal dose in highly sensitive ragweed patients is not effective, but based on our data the median Rinkel dose of 0.001 µg AgE (42) is well below the maximum tolerated dose. Others have suggested that doses just above those that produce local reactions (20) (0.1 µg AgE in our studies for large local reactions) or that are one-tenth of the maximum tolerated dose (19) (0.42 µg AgE in our studies) may be efficacious and safe. Our moderate dose of 0.3 µg AgE falls within this dose range and is consistent with the observation that submaximal immunotherapeutic doses of short ragweed (0.11 µg AgE) are associated with significantly decreased nasal sensitivity to inhaled ragweed (16) and therefore may be expected to be both effective and safe (35).

The inability to detect a specific IgG response in 1981 after only 8 weeks of treatment using a peak dose of short ragweed of 0.3 µg AgE is consistent with prior studies in which median dose of short ragweed for a threshold IgG response was higher (0.56 µg AgE) and required a longer median time interval (10 weeks) (10). Other investigators have noted that IgG antibody response is not associated with efficacy. A review of IgG literature by Reisman (30) and active (28, 45, 48) and passive (49) immunization studies indicates that many studies fail to find association between levels of specific IgG and efficacy. Several studies also report patients

with absent specific IgG antibody following immunization, but clinical protection is present (14, 48). Consequently, attempts at establishing efficacy should continue to monitor symptom changes and should not rely solely on IgG antibody responses as a measure of efficacy. Examples of the absence of a specific IgG response despite efficacy (and the lack of efficacy of passive humoral immunization) clearly raise the probability of another mechanism, probably cellular (23, 31), to explain the beneficial effect of immunotherapy.

The mechanism of safety and efficacy of modified extracts whose major claims are reduced systemic reactions, large increases in specific IgG, and comparable efficacy to high dose monomer treatment, remains to be elucidated (17, 26). One explanation may be due solely to residual allergenicity rather than enhanced immunogenicity. These modified allergens are claimed to have low allergenic potency by skin test (0.01 to 0.001 of the unmodified extract) (29) and histamine release (0.0017 of the unmodified extract) (26). Our approach to estimation of bioequivalent doses permits a comparison of the allergenic dose of the modified extract bioequivalent to that of the unmodified extract. For example, if the RP of the modified short ragweed extract is 0.0010 with respect to the reference used in this study, it would have the bioequivalent potency in AgE of 0.40 µg/ml

(0.0010 × 400 µg/ml); very close to the dose shown in this study to be safe and efficacious. Despite the low residual allergenic activity of modified extracts, this activity may still be 10 to 100 times the “Rinkel” dose (42). Thus the small amount of retained allergenicity in modified allergens may be sufficient to account for safety and efficacy.

Even very low doses of allergen may produce systemic adverse reactions whose frequency is dose-related (Tables 6 and 7). Claims of the absence of such reactions with immunotherapy should be accepted only after demonstration that the methodologies used to detect systemic reactions are suitably rigorous. Our techniques

for detecting systemic reactions use a worst case analysis of data, are independent of physician bias, and thus our data may not be comparable to data from other studies using less rigorous methods.

Although we detected no association between degree of skin sensitivity and final peak dose reached, other investigators who have treated patients with a wider spectrum of skin sensitivity have noted that highly sensitive patients tolerate lower doses than less sensitive patients (8, 44). Since we studied only highly sensitive patients, the doses found to be efficacious may be biased to lower values and may not be applicable to less sensitive patients who have less

Table 7
Dose and adverse reactions following submaximal dose immunotherapy

Year of treatment Regimen	1981 Rapid		1979-1980
Product	E	E	A, C and D Range*
¹ GM initial dose (µg AgE)	0.0001	0.01	(0.00001-0.20)
¹ GM peak dose (µg AgE)	0.003	0.30	(0.82-4.3)
No. of patients	21	22**	
No. injections/patient to peak dose	5	6	(7-38)
No. total injections/patient	8	8	(7-40)
Dropouts	3 +	0	
I. Systemic reactions			
¹ GM systemic reaction dose (µg AgE)	0.0002	0.06	(0.23-2.3)
% Patients with systemic reactions	9.5	23	(50-75)
% Patients requiring epinephrine	0	0	(29-46)
Total no. reactions	4	5	(11-32)
Reaction/injection	0.023	0.028	(0.021-0.22)
Reaction/patient	0.20	0.23	(0.85-1.9)
II. Severe delayed local reactions			
¹ GM severe local reaction dose (µg AgE)	0.0001	0.098	(0.1-0.4)
% Patients	5	18	(23-50)
Total no. reactions	2	4	(5-15)
Reaction/injection	0.01	0.02	(0.015-0.037)
Reaction/patient	0.095	0.18	(0.24-1.25)
III. Large local reaction (> 7.5 cm)			
¹ GM large local reaction dose (µg AgE)	0.0012	0.093	(0.09-0.3)
% Patients	19	40.9	(15-50)
Total no. reactions	6	21	(5-20)
Reaction/injection	0.036	0.12	(0.01-0.11)
Reaction/patient	0.29	0.96	(0.39-1.67)

¹Geometric mean.
*From Tables 3 and 4.
**Includes one patient originally on low dose but inadvertently given moderate dose.
+ Personal reasons.

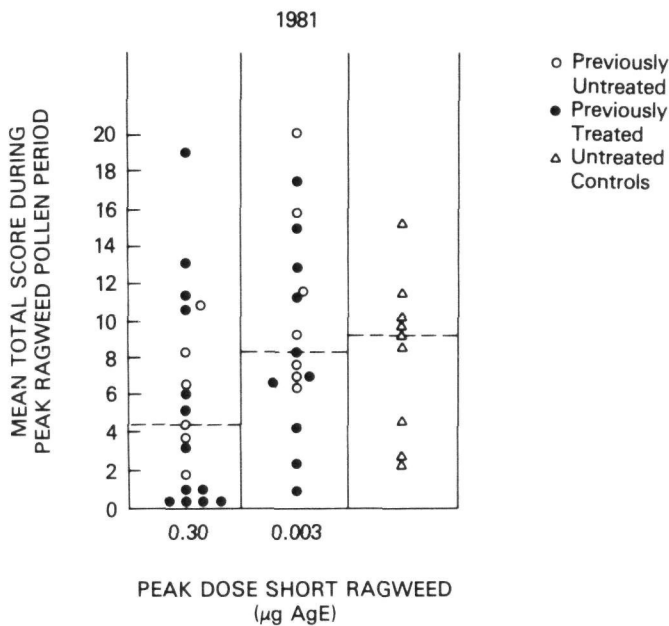


Fig. 8. Scattergram of mean total symptom score of each untreated (○) and each previously treated (●) patient during peak pollenation in each treatment group in 1981.

intense symptoms and so are more difficult to study (25).

This study supports the importance of precisely stratifying patients based on skin testing in order to predict symptom severity (Fig.3) and probability of severe systemic reactions (Table5) (38). Such stratification also permits comparison of populations between studies. Norman et al. (25) have previously demonstrated a significant association between skin sensitivity and symptom severity. Connell (8) has noted a positive association between skin sensitizing antibody titer and incidence of constitutional reactions during treatment, and Vander Veer et al. (44) reported a positive association between degree of skin sensitivity and incidence of constitutional reactions. In this study, small differences in skin sensitivity was observed to have significant effects on total symptom scores (Fig.3), treatment response (see Statistical methods), and on incidence of severe systemic reactions (Table5).

Because skin testing is such a critical patient selection parameter, accurate and precise methods for evaluating skin sensitivity are essential. The estimated variability of D_{50} , the $\log_3$ dose of allergen producing a 50 mm sum of erythema response is approximately two-fold (95 % limits)

when one patient is tested (33). Therefore, this method can discriminate between patients who differ more than two-fold in their skin sensitivity. A proficiency method for evaluating the accuracy and precision for estimating D_{50} has been developed (33). Because puncture testing has been estimated to be approximately 1,000–10,000-fold less sensitive than intradermal testing (4, 33, 39), patients selected solely by puncture test using a potent WSR containing 400 $\mu\text{g/ml}$ AgE may select patients reacting to as much as 0.04 $\mu\text{g/ml}$ AgE ($0.0001 \times 400 \mu\text{g/ml}$ AgE). Based on Fig. 3 and Table 5, such patients would bias a study population to patients who may be expected to have less severe symptoms prior to therapy and a lower incidence of severe systemic reactions during therapy. If these patients are then stratified and randomized based on wheal size to a single puncture dose where patients with similar sized wheals may differ in endpoint sensitivity to the same allergen by 2–3 $\log_{10}$ (32), erroneous conclusions concerning therapeutic outcome and safety may arise due to inaccurate assessment of patient sensitivity prior to randomization. Stratification of patients based on intradermal titration utilizing erythema responses detected these differences in this study.

Other prognostic factors in selecting hay fever patients of comparable symptom severity are seasonal asthma (6) and previous treatment (22); both contribute to symptom scores that are less than similarly selected controls. Thus, in order to use 1979 and 1980 treated patients in the 1981 study, patients were stratified and randomized based on their 1980 total scores. Patients whose previous fall hay fever symptoms were moderate to severe, required medication and were comparably distributed between treatment and control groups (see Patient population) further insured that there was no baseline bias in study groups due to disparate histories of clinical symptom severity or medication use that may confound the interpretation of treatment effects.

The importance of ragweed pollen intensity on symptom severity between years is also illustrated in this study (Fig.2, A and B) and supports observations with grass (27) and rag-

weed (43) pollen. In 1978 the peak pollen count was approximately twice that in 1979. Also, the median total symptom score of 1978 patients was approximately twice the median total score of untreated controls in 1979. Thus, in evaluating symptoms between years, comparability of both patients and pollen intensity are critical. When small sample sizes are used in this and other allergy studies, failure to detect a difference between controls and treated patients does not prove that the treatments are without effect, since the statistical power of these studies is low (46). Thus, the efficacy of low doses ($< 0.3 \mu\text{g}$) of short ragweed therapy will require larger sample sizes. Similarly, the observations of dose-dependence and the prognostic value of D_{50} on safety and efficacy observed in this study require confirmation by other investigators.

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- [Erratum: SR Ref in Fig.5 of reference 39 should be labeled SR Ref.
- C
- D
- The text in reference 39 referring to Fig.5 should make reference to Product D rather than Product C.]
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