A Comparison of Passive Cutaneous Anaphylaxis Guinea Pig Responses Using an Intravenous or an Intradermal Route for Antigen Challenge

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Abstract

The passive cutaneous anaphylaxis method has been used to evaluate residual antigenicity in castor bean pomace. This paper compares the results obtained using two routes of antigen challenge, intradermal (ID) and intravenous (IV). Passively sensitized guinea pigs were challenged with untreated and steam treated castor meal extract. An analysis of variance of the data shows there was greater variability between pigs within dose for the IV assay method compared to the ID technique. The ID assay also showed greater precision of the potency estimates.

Introduction

The castor beans and their components have a variety of potential agricultural and industrial uses. For over a century, the extracted oil has been valued as a safe and very efficient cathartic. In more recent years, other commercially valuable properties of the oil have been recognized. The oil and its derivatives are used in paints, varnishes and lacquers, plastics and plasticizers, lubricants, surfactants, as well as cosmetics and pharmaceuticals (1). These industrial uses have caused an increase in oil production resulting in a large commodity demand.

The meal, however, contains some relatively stable and powerful allergens as well as toxic substances such as ricin and ricinine. Ricin is easily detoxified by heat. The percentage of ricinine in castor is small relative to the toxicity so that it presents no particular problem for feed use as long as moderate levels are fed.

The allergens have been known to cause disabling symptoms to some workers. With the advent of the solvent extraction process, the problem became more acute. The light dusty powder is easily transported by winds from factory and shipping areas into the surrounding community. The pomace has been known to cause widespread and severe allergy in the areas where it is produced or handled (2).

Experimental Procedures

Efforts to deactivate the allergens in pomace require a sensitive, reliable evaluation technique. It is the object of this paper to present data so that a quantitative measure of the biological response could be made using the intravenous (IV) and the intradermal (ID) route of antigen challenge. We have used a guinea pig assay employing the passive cutaneous anaphylaxis (PCA) test (3,4).

Castor Meal Preparation

A single lot of production run castor meal was used for this series of experiments. Each batch was processed with steam under varying conditions of time and pressure. The details of the process yielding optimum deactivation levels will be reported in another paper.

Antigen Extraction

A 10 g sample of each batch was obtained for the antigen assay. Two hundred milliliters of water was added to each sample. The suspension was adjusted with HCl to pH 5 and boiled for 1½ hr. The slurry was filtered through an 0.8 μ Millipore filter (4). In the past, we lyophilized the liquid extract and cor-

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TABLE I

Analysis of Variance

Source of variance	Degrees of freedom	Mean squared deviations	F_{calcd}[a]	$F_{.05}$[b]
ID Test				
Time	2	159.7689	61.34	3.18
Pressure	3	65.3033	25.07	2.79
Time × pressure	6	14.4181	5.54	2.29
Log dose linear	1	2414.1870	926.82	4.03
Time × log dose	2	16.6370	6.39	3.18
Pressure × log dose	3	22.0240	8.46	2.79
Time × pressure × log dose	6	4.5303	1.74	2.29
Residual regressions	26	39.3192	15.09	1.72
Pigs/dose	51	2.6048		
IV Test				
Time	2	147.8265	29.60	3.30
Pressure	3	140.9768	28.23	2.90
Time × pressure	6	9.9221	1.99	2.40
Log dose linear	1	1081.1800	216.53	4.15
Time × log dose	2	33.8979	6.79	3.30
Pressure × log dose[c]	2	2.3228	0.47	3.30
Time × pressure × log dose[c]	4	35.1979	7.05	2.67
Residual regressions	11	174.7069	34.99	2.10
Pigs/dose	32	4.9933		

[a] Calculated variance ratio.
[b] Tabular variance ratio at 95% confidence level.
[c] One pressure level had only one of the doses and did not enter into the interaction.

TABLE II

Linear Regression Analysis

Treatment No.	No. Obs.	Regression MS[a]	Residual MS	F calcd F(1, n-2)	F tab $\alpha = .05$
Intravenous					
1	8	346.409	9.165	37.8	6.0
2	6	413.919	10.179	40.7	7.7
3	6	86.118	15.632	5.5	7.7
4	8	264.248	40.723	6.5	6.0
5	5	87.725	31.930	2.7	10.1
6	3	130.665	0.001	130,665	161.0
7	8	391.835	10.228	38.3	6.0
8	11	906.034	14.696	61.7	5.1
9	5	38.375	8.765	4.4	10.1
10	NR[b]				
11	NR				
12	NR				
Intradermal					
1	10	229.029	5.621	40.7	5.3
2	8	161.002	6.565	24.5	6.0
3	11	257.762	8.723	29.5	6.1
4	10	397.475	6.158	64.5	5.3
5	10	493.422	2.727	180.0	5.3
6	10	388.609	2.193	177.2	5.3
7	8	237.266	2.617	90.7	6.0
8	10	601.375	6.943	86.6	5.3
9	6	166.926	0.230	725.7	7.7
10	8	117.821	2.498	47.2	6.0
11	6	131.331	5.733	22.9	7.7
12	6	158.382	3.178	49.8	7.7

[a] MS, mean squares.
[b] NR, no response.

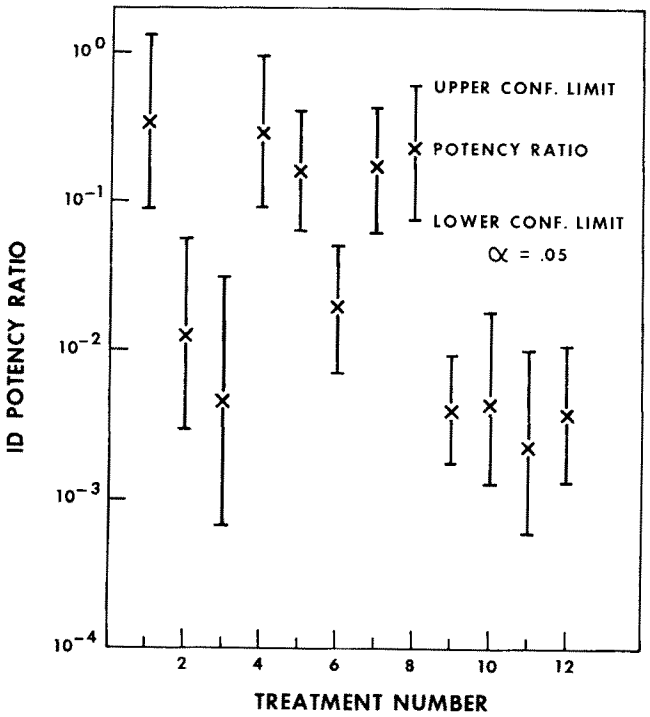

FIG. 1. ID potency ratio.

related the biological response to the weight of the lyophilizate injected. In this series, we used the liquid extract directly as whole extract or with progressive dilution in physiological saline solution. The dose level injected into the guinea pig was calculated in terms of the original meal.

Bioassay Procedures

Intravenous Route

White guinea pigs were used for the test. They were sensitized essentially by the procedure described by Layton et al. (3). The abdominal skin of each test animal was inoculated with 0.05 ml (diluted 1:200) of rabbit anticastor serum. After a waiting period of 2½ to 3 hr, an intravenous injection of Evans blue dye was made into the cephalic vein. This was followed by an injection in the same vein by a solution prepared from a castor meal extract. A positive reaction was indicated by a blueing of the skin at the antibody-antigen sites. The diffusion of the dye at the sites of the antibody-antigen interaction was measured 2½ hr later. In the past, the responses had been graded in terms of a PCA score based on the diameter of the colored spot (5). In the present work we used the average of the length in millimeters of the major and minor axes of the spot as a measure of response.

TABLE III
Equivalent Response Doses[a]

Treatment No.	Dose, μg	
	IV	ID
Untreated meal	10.3	3.5
1	306.0	11.6
2	3140.0	307.0
3	6230.0	848.0
4	99.6	13.3
5	265.0	24.6
6	33100.0	198.0
7	1740.0	23.2
8	768.0	17.5
9	301000.0	971.0
10	NR	794.0
11	NR	1590.0
12	NR	1040.0

[a] Quantities are from linear regression calculated at 12.5 mm response level.

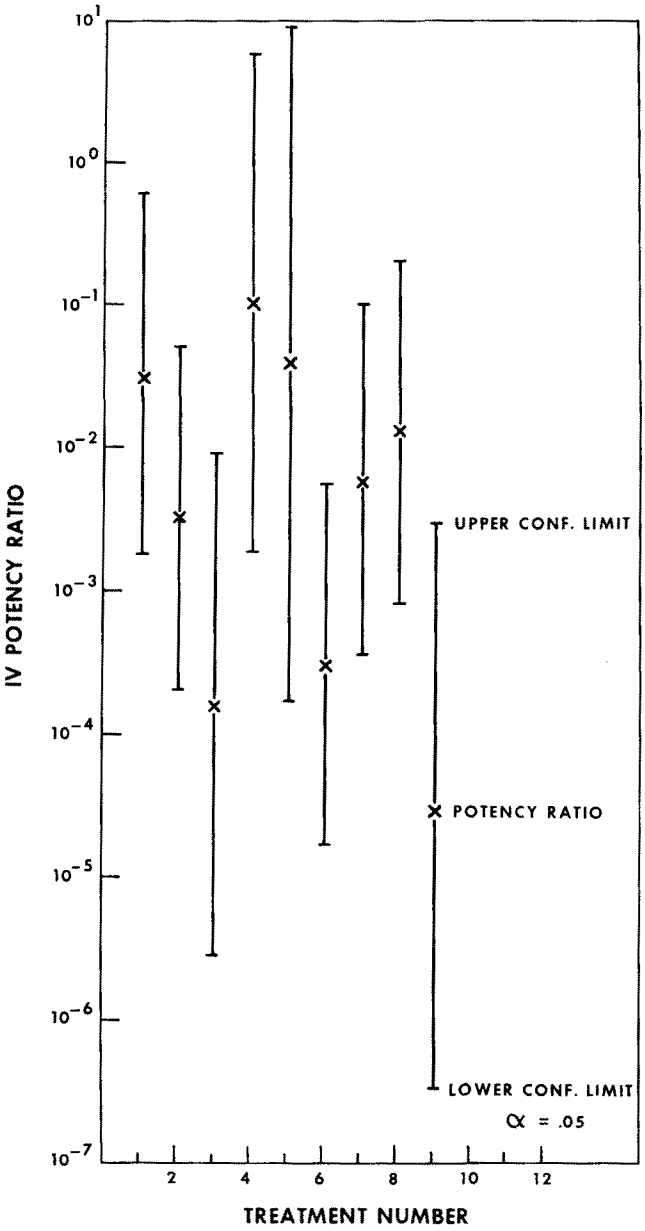

FIG. 2. IV potency ratio.

Intradermal Route

The potency of the castor meal can also be determined by an intradermal injection of the antigen at the antibody site. The Evans blue dye was injected in the cephalic vein as before but the antigen challenge was intradermally injected at the antibody injection site. The response was measured and scored as before.

Two sites on each animal were injected with a highly potent castor extract referred to as CBWU (also referred to as CB-1A-SRI) (6). A response less than 20 mm using 3.7 μg CBWU was cause for rejection of the data from that test animal. The remaining site was injected with 0.0023 μg CBWU. This produced a low level response of about 9 mm. Each animal then served as its own control.

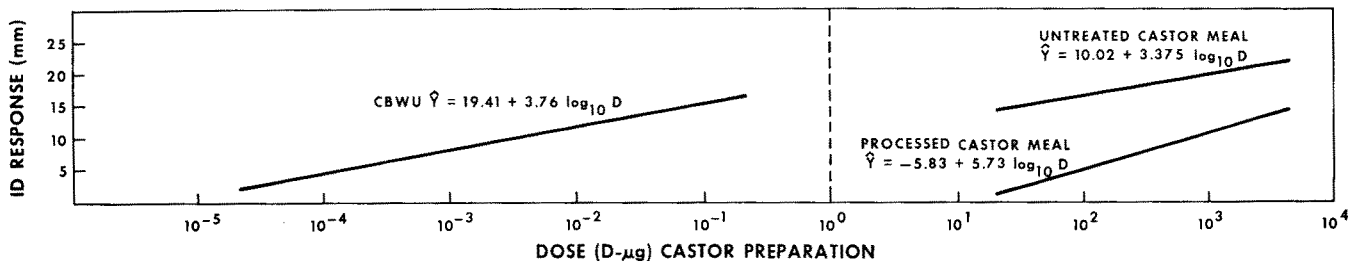

FIG. 3. Castor meal antigen log dose response.

Results

The analysis of variance of the experimental data is shown in Table I. The treatment X log dose interaction is significant. Accordingly, simple linear log dose response regressions of extracts obtained from two different treatment combinations may not be parallel. A potency comparison, therefore, is meaningful only if a particular response level is specified (7). For our purposes, the potency has been defined as the ratio of the dose of the untreated castor meal extract to that which has been processed.

Table II shows the individual linear regression analyses for each process treatment. It is apparent that the ID assay shows a consistently strong linear log dose response relationship while the IV method shows inconsistency. Each of the regressions was solved for the equivalent dose required to elicit a 12.5 mm response. These values are shown in Table III. The ID potency ratios and the confidence limits ($\alpha = .05$) for each of the processes are shown in Figure 1; the IV ratios in Figure 2 (8). A comparison between these Figures illustrates the greater precision to be expected using the ID assay technique.

The log dose response for the untreated meal, a typical processed meal and the CBWU control are shown in Figure 3.

There are distinct advantages for adopting the ID technique to assay residual antigen in castor meal. In terms of equivalent response areas (Table III), the ID test is more sensitive than the IV. Processes 10, 11 and 12 of this series failed to elicit a PCA response using the IV method but did show a response using the ID method. Because of the increase in sensitivity, we are able to detect differences at lower levels of antigenicity. This is an important consideration, since we are trying to estimate small residual amounts of undestroyed allergen.

The estimate of guinea pigs per dose variance (Table I) is significantly smaller for the ID than the IV test; calculated F value = 1.92, tabular $F_{.05} = 1.68$. The response variability between pigs within dose levels is the most important single factor contributing to error in estimating response for a given dose. Since the ID route results in less variability between pigs, there is more precision in the dose-response estimate. This is an important consideration, since fewer animals will be required to obtain the same precision when comparing potency ratios.

When using the IV method, the antigen challenge is made through the cephalic vein. The entire animal, therefore, has been challenged by a single dose. With the ID technique one guinea may be challenged with as many as eight concentrations of antigen extract. The intradermal antigen challenge allows more flexibility in experimental designs. Fewer test animals would be required to bracket a dose level to obtain a specified response level.

Using the ID test with a single dose per animal, reliable statistical significance can be obtained with as little as three test animals. The time required to prepare and condition the animal for test does not allow for immediate decisions as to lot disposition. For this reason, it is not a completely satisfactory method for production assays. Ideally, a more rapid test would be more suitable. An in vitro test is currently being developed by this laboratory. Until it is completed and tested, the ID technique is our best alternative.

Since we have adopted the ID test, we have evidence ($\alpha = .05$) that the antigen content of the untreated pomace varies. For this reason, CBWU is currently being considered as a standard reference.

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