Gibraltar Biological Laboratories, Inc.
23 JUST ROAD, FAIRFIELD, NEW JERSEY
07004
TELEPHONE (201) 227-6882
REPRINTS

Comparative guinea pig assays for contact hypersensitivity

By Herbert N. Prince, PhD
Gibraltar Biological Laboratories, Inc., Fairfield, NJ
and
Theodore G. Prince, MS
Rochelle Du Couer, Inc., Linden, NJ

There are numerous methods for detecting allergic contact dermatitis in guinea pigs.[1-6] Recently there has been an attempt to compare different methods in collateral tests in a single laboratory.[7] Although it is generally agreed that results in the guinea pig can predict results in man, we still face uncertainty as to which test is best. In our collateral study we found that two tests must be performed to identify a formulation as a weak or strong allergen. One of these is the Maguire test and the other is the Landsteiner-Draize test. The former employs Freund's complete adjuvant (FCA),* and the latter test is performed without the use of immunostimulants.

The current guinea pig test methods vary with respect to (a) administration of sensitizing regimens (topical or intradermal), (b) penetration through the stratum corneum (sodium lauryl sulfate irritation or excoriation with sandpaper), (c) stimulation of the immune system (by intradermal FCA or foot pad injection), and (d) number of sensitizing doses prior to challenge. Each toxicology laboratory has a favorate method and few laboratories perform them all. But since all laboratories perform the same eye and skin irritation tests, a standardized test for topical allergenicity should likewise be a goal in experimental dermatology.

In an attempt to simplify the choice of assay and to show clear differences between "weak" and "strong" sensitizers, three known allergens were tested in five experimental models, with and without maximization. For the purposes of our discussion, "maximization" is defined as any traumatic or chemical treatment designed to increase the immune response to the sensitizing agent.

Traumatic maximization, such as excoriation or irritation of the skin, is designed to maximize passage of allergens through the skin in the presence of serous fluid. *Chemical maximization,* such as injection of FCA, is designed to increase acquisition of delayed hypersensitivity by stimulating clonalization of immunocommitted lymphocytes (T-cells) in the regional nodes.[2]

Material and methods

Animals: Hartley strain male guinea pig weighing 350 to 500 grams were employed. The animals were fed and watered ad libitum. Diets were supplemented with fresh greens weekly. Water bottles were routinely sterilized. Animals were cultured from the external nares and found to be negative for beta hemolytic streptococci and gram negative rods.

Allergens: A fragrance, a topical antimicrobial, and a topical anesthetic were tested. Benzocaine crystals were ground and triturated in petrolatum to a concentration of 5% w/w. Sulfamylon cream 5% (obtained commercially) and cinnamic aldehyde 2% in petrolatum† were also used.

Sensitization: Maximization was accomplished as described by Magnusson and Kligman[1] and Maguire.[2] In the modified Maguire test, maximization was accomplished by shaving the skin in the shoulder area and excoriating with sandpaper an area 2 x 2 cm to produce a deep erythema just short of bleeding. Occluded patches were not used. FCA was injected (0.1 ml x 2) by the intradermal route on opposite sides of the sensitized site at day five of the experiment. A total of four doses of allergen were applied topically to the same excoriated site during the one week period of sensitization. When an occluded patch was employed, the site was covered with filter paper 2 x 2 cm and then taped in place.** In the Landsteiner-Draize test, 0.2 ml was rubbed onto the intact skin (epicutaneous administration) every other day for a total of 10 treatments. Intradermal injection of allergen was avoided,[1] since our main interest was in studying the occurrence of

* Freund's Complete Adjuvant (DIFCO), a killed suspension of Mycobacterium tuberculosis H37Ra in oil, stimulates the immune response.

† Kindly supplied by Dr. Donald Opdyka, Research Institute for Fragrance Materials.

** Blenderm, 3M Company

TABLE 1

Protocols for the five Experimental Models

Method	Day 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	34
GPM[a]	Ⓐ Ⓑ Ⓒ						SLS	[A]	[A]											[D]	←	Read	→		
Maguire[b]	[A]*		[A]		[A] Ⓒ		[A]													[D]	←	Read	→		
Maguire modified[c]	A*		A*		A* Ⓒ		A*													[D]	←	Read	→		
L-D[d]	A		A		A		A		A		A		A		A		A		A						D
L-D[e] modified	A Ⓒ		A		A		A		A		A		A		A		A		A						D

* excoriation of clipped skin (razor and dry ice or sandpaper) in the modified test.

a. Magnusson and Kligman (1970) : intradermal allergen emulsified in adjuvant + 10% sodium lauryl sulfate.
b. Maguire (1973) : split-adjuvant technique (CFA, 0.1 ml x 2, intradermal and allergen topically).
c. Split-adjuvant technique without occlusive dressings for induction, challenge by closed 2 x 2 cm filter patch for 24 hours.
d. Landsteiner-Draize (Draize et al 1944 ; Draize 1959) = topical induction without irritation or adjuvant (0.2 ml E.O.D.)
e. Landsteiner-Draize : topical induction without irritation, with adjuvant (foot pad CFA injection, Chung, et al, 1970)

LEGENDS

Ⓐ = allergen alone, intradermal
[A] = allergen alone, occlusive patch, 0.2 ml 4 times to same site in 1 week.
A = allergen alone open (no patch), 0.2 ml to same site without dressing to simulate normal application
⊖ = allergen emulsified in adjuvant, intradermal
○ = adjuvant alone, intradermal, 0.1 ml ID on 2 separate sides of excoriated sensitizing site
Ⓓ = challenge, closed patch, 2 x 2 cm filter paper patch under tape
D = challenge open (no patch)
CFA = Complete Freund's adjuvant, DIFCO (H37 Ra emulsified in mineral oil)

delayed hypersensitivity in an experimental setting that simulated the use of topical products. The Landsteiner-Draize modified test (LDM) included intradermal injection of FCA, as described by Chung and coworkers.

Challenge: All challenge sites were distal to the sites of sensitization. In most cases, 0.1 ml of ointment was applied to a 2 x 2 cm patch. The patch was then taped for 24 hours onto the clipped skin of the posterior dorso-lateral flank. The patch was removed and the reading immediately recorded as the 24 hour score. Additional readings were taken at 48 and 72 hours to detect ascending or descending reactions. Immunological competence of the test animals in the FCA-treated groups was determined at the end of the experiment by rubbing 0.1 ml of FCA onto the contralateral posterior flank. Readings were then made at 24, 48 and 72 hours for erythema and induration of the tuberculin type. Other details of the various methods are collated in Table 1.

Discussion of tests

GPM Test. This is the guinea pig maximization test of Magnusson and Kligman. In this test the allergen is inoculated by the intradermal route in the shoulder area; allergen is then emulsified with FCA and again injected by the intradermal route in the same area. On the seventh day the skin is irritated with 10% sodium lauryl sulfate. On the eighth and ninth days the allergen is applied to the maximized site with occlusive patches. Challenge is accomplished on the twentieth day at a distal site upon application of occluded patches containing allergen.

Maguire Test. This is the split-adjuvant technique in which the allergen and adjuvant are given separately (not combined as in the GPM test). Allergen is applied under occluded patches to excoriated skin in the shoulder every other day (not injected as in the GPM test) to a total of four treatments. At the time of the fourth application of allergen, FCA is injected intradermally at the site of repeat sensitization. Challenge is accomplished on day 20 by application of an occluded patch at a distal site.

Maguire Modified Test (MM). This is the Maguire split-adjuvant technique modified to eliminate the use of occluded patches during application of allergen. Four sensitizations are carried out by rubbing 0.2 ml of substance or formulation on to the clipped skin of the shoulder area. The skin is reexcoriated with sandpaper just short of bleeding before each dose of allergen. The MM test more closely simulates use conditions since topical application is accomplished without the use of occlusive dressings.

Landsteiner-Draize Test (LD). In this classical technique the allergen is applied topically every other day for a total of ten treatments; neither adjuvants nor dressings are employed. After a rest period of 14 days to allow synthesis of antibodies), a distal posterior site is challenged by rubbing onto the clipped skin 0.2 ml of test substance. A patch is not employed. Readings are then taken for three consecutive days to determine presence or absence of dermatitis.

Landsteiner-Draize Test, Modified (LDM). This test was performed exactly as the LD test described above except that immunostimulation was employed. FCA was injected into the foot pad on day one, along with initial topical application of allergen.

Results

The data in Tables 2-4 indicate the great disparity between the classical Landsteiner-Draize test

and the maximization tests. In no case was the GPM test superior to the Maguire method. In fact, the Maguire test detected reactions at rates of 85%, 80% and 100% vs GPM rates of 30%, 45% and 80% for benzocaine, sulfamylon, and cinnamic aldehyde respectively.

It should be pointed out that guinea pig mortality was not uncommon in both the GPM and Maguire tests (5 to 10%). The modified Maguire (MM) test produced fewer deaths. At the same time it compared well with the GPM test (55% vs 30%, 40% vs 45%, and 100% vs 80%). Thus, the modified Maguire test was in our hands a sensitive and economical experimental model and is recommended for "weak" sensitizers. With respect to the modified LD test (nonoccluded topical induction plus FCA), mortality was not seen and low reactivity rates were noted (15%, 10%, 20%). The epicutaneous LD test without adjuvant was the least sensitive, detecting only the strongest allergen.

TABLE 2

5% Benzocaine – Hypersensitivity Assay in 5 Guinea Pig Models
(Petrolatum base)

Animal No.	Clinical Intensity of Contact Dermatitis (a)				
	Topical L-D (b)	Topical L-D w/adjuvant	M-K GPM(c)	Maguire split-adjuvant	Maguire modified (d)
1	0	••	•	••	•
2	0	0	0	••	0
3	0	0	••	0	•
4	0	0	0	•••	•
5	0	••	0	•	•
6	0	0	•	•	••
7	0	0	•	0	0
8	0	0	0	••	••
9	0	0	0	••	0
10	0	0	0	•••	0
11	0	0	0	0	•••
12	0	0	0	•	0
13	0	0	0	••	0
14	0	0	0	••	•
15	0	0	0	•	••
16	0	••	••	•••	0
17	0	0	0	••	•
18	0	0	0	•	0
19	0	0	••	•	••
20	0	0	0	•	0
Total %	0/20 0	3/20 15%	6/20 30%	17/20 85%	11/20 55%

a. 0 = negative; • = pink; •• = bright pink; ••• = bright pink with induration; •••• = red with induration.
b. Landsteiner-Draize (topical to shaved non-stressed sites, alone or with Freund's adjuvant in foot pads).
c. Magnusson-Kligman Guinea Pig Maximization test
d. Open induction to excoriated skin under use-conditions without occlusive dressing

TABLE 3

5% Sulfamylon Cream – Hypersensitivity Assay in 5 Guinea Pig Models
(Emulsion base)

Animal No.	Clinical Intensity of Contact Dermatitis (a)				
	Topical L-D (b)	Topical L-D w/adjuvant	M-K GPM(c)	Maguire split-adjuvant	Maguire modified (d)
1	0	0	0	•	•
2	0	0	0	•	0
3	0	•	•	••	0
4	0	0	•	••	•
5	0	0	0	•	0
6	0	0	•	••	0
7	0	0	0	0	••
8	0	±	•	•••	0
9	0	0	0	•	0
10	0	±	••	•	•
11	0	•	0	••	0
12	0	0	0	0	•
13	0	0	•	•	0
14	0	0	•	0	•••
15	0	0	•	••	0
16	0	±	0	•	0
17	0	0	0	••	•
18	0	±	••	0	0
19	0	0	0	•	••
20	0	0	0	••	0
Total %	0/20 0	2/20 10%	9/20 45%	16/20 80%	8/20 40%

a. 0 = negative; • = pink; •• = bright pink; ••• = bright pink with induration; •••• = red with induration.
b. Landsteiner-Draize (topical to shaved non-stressed sites, alone or with Freund's adjuvant in foot pads).
c. Magnusson-Kligman Guinea Pig Maximization test
d. Open induction to excoriated skin under use-conditions without occlusive dressing

TABLE 4

2% Cinnamic Aldehyde – Hypersensitivity Assay in 5 Guinea Pig Models
(Petrolatum base)

Animal No.	Clinical Intensity of Contact Dermatitis (a)				
	Topical L-D (b)	Topical L-D w/adjuvant	M-K GPM(c)	Maguire split-adjuvant	Maguire modified (d)
1	0	•	•	••	•
2	0	±	••••	••	••
3	0	0	••	•	••
4	±	0	•	•	•
5	0	0	•	••••	•
6	0	0	••	••	0
7	0	±	••	••	•••
8	±	••	±	•••	••••
9	±	0	•	•••	••
10	0	0	••	••••	0
11	•	0	••	•	••
12	0	0	0	••	•
13	0	0	••	•	•••
14	0	±	0	•	0
15	0	±	••••	••	•
16	0	•	•	••	•
17	±	0	•••	••	0
18	0	•	••	••	••
19	0	0	0	•••	••
20	•	±	•	•	0
Total %	2/20 10%	4/20 20%	16/20 80%	20/20 100%	15/20 75%

a. 0 = negative; • = pink; •• = bright pink; ••• = bright pink with induration; •••• = red with induration.
b. Landsteiner-Draize (topical to shaved non-stressed sites, alone or with Freund's adjuvant in foot pads).
c. Magnusson-Kligman Guinea Pig Maximization test
d. Open induction to excoriated skin under use-conditions without occlusive dressing

An overall evaluation of the results of the five experimental models yielded a pattern of reactivity, in decreasing order of sensitivity, as shown below.

Five Animal Models With Four Degrees of Sensitivity

Grade	Test
I	Maguire (M)
II	Mod. Maguire* (MM) or GPM
III	LD Modified (LDM)
IV	LD*

*2-tier assay system; formulation should be tested in MM & LD

The present data show that the LD test will only detect strong sensitizers like cinnamic aldehyde and that the MM test will detect weaker agents such as sulfamylon. It is recommended that new formulations be assayed in both tests—with and without adjuvants. If the formulation appears experimentally to be a strong sensitizer (for example LD = + and MM = +), then perhaps patch tests on only 50 humans would be necessary. If the formulation appears to be a weak sensitizer (for example, LD = 0 and MM = +) then the traditional 200 subjects would be warranted.

It is our feeling that human subjects should not be tested with a new substance and that the size of the panel should not be decided until the "strong" or "weak" potential is determined. Only collateral assays in 2 guinea pig models can determine this potential (2-tier assay).

Summary

Maximization tests in guinea pigs were clearly superior to the classical Landsteiner-Draize (LD) test, especially with regard to the detection of a weak allergen. Of five experimental models studied, the Maguire test was the most sensitive; the LD test detected the fewest reactions. Both tests should be run in a 2-tier assay, so that formulations or substances can be categorized as "weak" or "strong" with respect to topical sensitizing potential. Test data thus obtained can be extrapolated to man and the predictive nature of preclinical tests enhanced.

Acknowledgment

We wish to thank Drs. Henry Maguire (Institute for Cancer Research) and Frank Marzulli (FDA) for their helpful comments.

References

1. Magnusson, B., and Kligman, A. M., Allergic Contact Dermatitis in the Guinea Pig, Charles C. Thomas, Springfield, IL, 1970
2. Maguire, H. C. Jr., The bioassay of contact allergens in the guinea pig, J. SCC 24, 151-62, 1973.
3. Chung, C. W.; Giles, A. L.; and Carson, T. R., Induction of delayed hypersensitivity in guinea pigs by aflatoxins, other courmarins and furazolium, J. Invest. Dermatol. 55, 396-403, 1970.
4. Draize, J. H.; Woodward, G.; and Calvery, H. O., Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes, J. Pharmacol. Exp. Therap. 82, 377-90, 1944.
5. Draize, J. H., Dermal Toxicity, in Appraisal of the Safety of Foods, Drugs and Cosmetics, (Educ. Comm.) Assoc. Food and Drug Officials of U.S., Texas State Health Dept., 1965.
6. Maiboch, H., ed., Animal Models in Dermatology, Relevance to Human Dermatopharmacology and Dermatotoxicology, Churchill Livingstone, London and New York, 1975.
7. Klecak, G.; Geleick, H.; and Frey, J. R., Screening of fragrance materials for allergenicity in the guinea pig. Comparison of four testing methods, J. SCC 28, 56-64, 1977.