Relative Elicitation Potencies of Seven Chemical Allergens in the Guinea Pig Maximization Test

Tetsuo Yamano,* Mitsuru Shimizu, and Tsutomu Noda

Osaka City Institute of Public Health and Environmental Sciences, 8–34 Tojo-cho, Tennoji-ku, Osaka 543–0026, Japan

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Dose–response profiles for both induction and elicitation phases were evaluated with seven chemical allergens using the guinea pig maximization test. Considering the risk assessment of contact sensitization in its practical sense, the profile of elicitation would be more critical than that of induction, and it should be evaluated in a maximally induced human population or experimental animals. When mean skin reaction scores in a group of maximally sensitized animals with each allergen were plotted against log challenge concentrations, linear regression lines with good fitness were adapted to all allergens. An elicitation threshold calculated from the linear regression line of each allergen could be used as an index of the relative elicitation potency of a chemical. However, unlike other cases of risk assessment, maximum acceptable concentrations for allergens in consumer products cannot be obtained simply by dividing an elicitation threshold by a fixed safety factor. The value of a safety factor for each allergen should be set on a case by case basis. As a practical matter, consumers are at a risk of exposure to allergens at concentrations greater than elicitation thresholds. Thus a dose–response profile for elicitation in maximally sensitized animals should be included in the evaluation of the risk. We propose to use the area under the linear regression line between the threshold and 1% of the elicitation concentration as a relative elicitation potency index of each allergen, because it reflects the integrated degree of skin reaction that would emerge among a maximally sensitized population exposed to an allergen.

Key words —— risk assessment, chemical allergen, induction threshold, elicitation threshold, relative elicitation potency

INTRODUCTION

The process of contact sensitization consists of two phases, induction and elicitation. A topically applied allergen penetrates the skin and induces proliferation of allergen-specific T lymphocytes in a local lymph node. Subsequent exposure to the allergen will elicit cutaneous inflammation that is mediated by activated allergen-specific T lymphocytes. The existence of thresholds for both induction and elicitation phases has been reported in theoretical and practical terms.[1,2] Threshold values are essential bases for risk assessment of contact allergens. Profiles of both phases can be examined by varying induction and elicitation doses using a modified protocol of the guinea pig maximization test (GPMT) proposed by Nakamura *et al*.[3] Our laboratory has reported threshold values of both phases with five allergens so far using this method.[4–7]

*To whom correspondence should be addressed: Osaka City Institute of Public Health and Environmental Sciences, 8–34 Tojo-cho, Tennoji-ku, Osaka 543–0026, Japan. Tel.: +81-6-6771-3186; Fax: +81-6-6772-0676; E-mail: yamanot@aol.com

In general, the risk of a chemical is assessed based on the toxicological threshold values obtained from experimental animal studies. For example, in the case of risk assessment of a chemical in food, acceptable daily intake is determined by dividing the no-observed adverse effect level in rodents by a safety factor of 100, a value assigned in consideration of inter- and intra-species differences. Maximum residue limits of the chemical in foodstuffs are then calculated based on the daily intake of each foodstuff. Thus, exposure to a chemical in food is regulated so that it does not exceed the human threshold. Contrary to this, however, risk assessment of chemical allergens is usually limited to hazard identification, and generally acceptable concentrations of allergens in a product are not determined. This is largely due to the difficulty of establishing an appropriate safety factor to extrapolate experimental data to a human situation. Various factors, such as concentrations of allergens in and leaching from products, duration and frequency of exposure and condition of skin, should be included to calculate the value of a safety factor. With cases of allergic

contact dermatitis being reported constantly in many countries, the need for precise criteria should be clear. Thus, with regard to risk assessment of contact allergens, a dose-response profile especially for the elicitation phase, *i.e.* information on what extent of skin reaction would occur at what challenge dose, should be incorporated in addition to threshold values.

The protocol for an induction phase in the GPMT is far from that of an actual situation in that it uses an adjuvant as a vehicle and the step for a compound to penetrate skin is avoided by intradermal injection. On the plus side, however, compared to various other experimental methods such as the Buehler test and murine local lymph node assay, the GPMT holds an advantage in that it can induce sensitization maximally even with weak allergens.[8,9] As risk ought to be evaluated on the basis of a worst-case scenario, this method seems to be ideal to prepare an optimally induced animal group to evaluate elicitation profiles. With previously reported data for five allergens together with some new data, we propose an index of relative elicitation potencies of chemical allergens in the GPMT.

MATERIALS AND METHODS

Chemicals —— The names and abbreviations of the seven allergens employed are listed in Table 1. BIT was obtained from Zeneca Co., Ltd. (Japan), CPIP from Nagase Kasei Co., Ltd. (Japan), IPBC from Nippon Ohrin Co., Ltd. (Japan), TMBCDPB from Inui Co., Ltd. (Japan), MBP from Ouchi Shinko Co., Ltd. (Japan), PTBPS from Shipuro Kasei Co., Ltd. (Japan), and Tinuvin P was from Ciba-Geigy Co., Ltd. (Switzerland).

Guinea Pig Maximization Test —— Five to six week old female Hartley guinea pigs from SLC (Shizuoka, Japan) were used. Essentially, the procedure described by Nakamura *et al.*[3] was followed. Ten or 17 animals were used for each sensitization group. The concentrations for the first (intradermal) induction are shown in Table 2. Maximum doses for induction were set at 5% or the highest non-necrotic concentrations, as was described by Magnusson and Kligman.[10] Olive oil and Freund's complete adjuvant emulsified with an equal volume of distilled water were used as vehicles. Seven days after the first induction, 25% (W/W) test compound in 200 mg white petrolatum was applied occlusively for 48 hr over the injection site.

Two weeks after the second induction, 0.1 ml aliquots of various concentrations of test compound in acetone were applied all at once to the shaved area of the flank for challenge. Three to five concentrations were set in a logarithmic scale. Maximum doses for challenge were set at 5% or more for non-irritants (BIT, TMBCDPB, MBP, PTBPS, and Tinuvin P), and at the highest sub-irritating concentrations for irritants (CPIP and IPBC). Forty-eight hr after challenge, each site was scored for erythema (0 to 4) and edema (0 to 3) according to the criteria of Sato *et al.*[11] Total scores (erythema plus edema) with the same challenge concentration in a group were summed and divided by the number of animals in the group to give a mean response (MR) value, an index for skin reaction to a given concentration of test compound for challenge.

RESULTS

Dose-Response Profiles for the Induction Phase of Seven Allergens

When the animals were intradermally injected at the lowest dose with each test compound, no sensitization was observed for any of the chemicals, even though 25% of each allergen was applied for 48 hr at the injection site as a second induction protocol (Table 2). It is obvious that the intradermal injection, not topical application, was essential for

Table 1. List of Allergens Evaluated in the Study

Abbreviation	Chemical name	Use
BIT	1,2-benzisothiazolin-3-one	Antibiocide
CPIP	*p*-chlorophenyl-3-iodopropargylformyl	Antibiocide
IPBC	3-iodo-2-propynyl butylcarbamate	Antibiocide
TMBCDPB	4,4′-tetramethylene-bis(4-carbamoyl-1-decylpyridinium bromide)	Antibiocide
MBP	α-methylbenzylphenol	Rubber antioxidant
PTBPS	*p* -*t* -butylphenylsalicylate	Ultraviolet stabilizer
Tinuvin P	2-(2′-hydroxy-5′-methylphenyl)benzotriazole	Ultraviolet stabilizer

Table 2. Results of Modified GPMT for Seven Allergens

Allergen	1st Induction (ppm)	n	Maximum elicitation[a] (ppm)	Skin reaction[b]	Reference
BIT	5	10	50000	N	(6)
	50	10		P	
	500	10		P	
	5000	10		P	
CPIP	0.5	10	100	N	(7)
	5	10		P	
	50	10		P	
	500	10		P	
IPBC	0.5	10	500	N	(7)
	5	10		P	
	50	10		P	
	500	10		P	
TMBCDPB	10	10	50000	N	
	100	10		P	
	1000	10		P	
	10000	10		P	
MBP	500	10	100000	N	
	5000	10		P	
	50000	10		P	
PTBPS	50	10	50000	N	(5)
	500	10		P	
	5000	10		P	
	50000	10		P	
Tinuvin P	50	10	250000	N	(4)
	500	10		P	
	5000	17		P	
	50000	17		P	

a) Animals were challenged at 5% or more for non-irritants, and at sub-irritating concentrations for irritants. *b*) Skin reaction was evaluated 48 hr after challenge. N: no animal in the group exhibited skin reaction. P: At least one animal in the group exhibited skin reaction.

these allergens to sensitize the animals. Degree of sensitization for the induction phase was evaluated using the mean reaction (MR) score, erythema plus edema, for each intradermal injection dose group 48 hr after challenge with the highest dose of corresponding chemicals. An increase in the MR scores against log intradermal induction doses was observed for three allergens (Fig. 1A). With other allergens, an overload effect or a constantly weak degree of sensitization was observed with respect to the increasing induction doses (Fig. 1B, C).

Dose–Response Profiles and Relative Potencies for the Elicitation Phase of Seven Allergens

Elicitation dose-response was evaluated with the maximally sensitized animal group for each allergen based on the induction profiles evaluated in Fig. 1. As shown in Fig. 2, when MR scores were plotted against log elicitation concentrations of each allergen, linear regression lines were fitted for all chemicals throughout the dose ranges employed. Table 3 summarizes elicitation-related factors of linear regression lines for each allergen. Using concentrations of x-intercepts as elicitation threshold would be better than using experimentally obtained maximum no-effect levels, because the latter are dependent on the spacing of the challenge doses. The area under the linear regression line between the threshold and a specific elicitation concentration reflects the integrated degree of skin reaction and would make a good index for relative elicitation potency of allergens. The values of the area for each

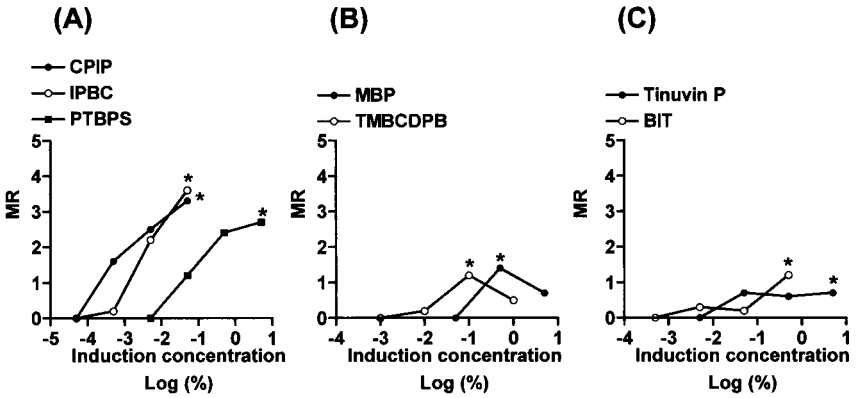

Fig. 1. Relationship between Induction Concentration and Maximum Skin Reaction Score for Seven Allergens

Dose-related skin reaction for the first induction concentration was evaluated with seven allergens. With each allergen, three to four doses were employed with respect to the first induction procedure (see Table 2). Each symbol represents mean response score of the group 48 hr after challenge with maximum concentration of each compound (see Table 2). (A): Linear relation was observed within the induction dose range used. (B): Overload effect of skin reaction was observed within the induction dose range used. (C): Skin reaction was constantly weak within the induction dose range used. *: The first induction dose at which a group of animals was maximally sensitized with each allergen.

allergen from threshold to 1% (the area of a triangle enclosed by the x-axis, a dotted vertical line, and a linear regression line for each allergen in Fig. 2 are listed in Table 3 as relative elicitation potencies.

DISCUSSION

To be induced with an allergen, or having allergen-specific T lymphocytes per se, is not necessarily a disease state, as was discussed by Jayjock and Lewis.[12] In the case of risk assessment of contact allergens, it is the elicitation phase around which regulation should be focused. For this goal, the threshold and the dose response-profile of the elicitation phase in an appropriate experimental model are essential factors. In the GPMT, in addition to using an adjuvant as a vehicle, an intradermal injection bypasses the skin penetration step of a chemical, so that the sensitizing capability of a chemical of specific physicochemical property will be augmented. Nakamura *et al.*[8] reported that the ratio of the induction concentrations inducing a 50% positive response for maleic anhydride using the GPMT to Buehler test, a guinea pig test without an adjuvant and an intradermal injection, was 300000. Accordingly, dose–response profiles of tested compounds in this study of the induction phase reflect their inherent induction potency but are not linked to risk evaluation in real use situations.

There seems to exist a wide ranging difference in susceptibility to contact allergens in the human population. Skin reactions are most liable to occur in individuals most highly sensitized. In an experimental model, therefore, the group maximally induced with an allergen should be used to evaluate

Table 3. Summary of Elicitation-Related Factors of Seven Allergens

Allergen	Slope	r^2	Elicitation threshold[a] log(%)	MR at 1%[b]	Elicitation potency[c]
MBP	1.0	0.97	−0.45	0.4	0.1
TinuvinP	0.3	0.99	−2.34	0.6	0.7
TMBCDPB	0.5	0.99	−1.60	0.9	0.7
BIT	0.4	0.91	−2.33	1.0	1.1
PTBPS	1.2	0.98	−1.58	1.8	1.4
IPBC	4.6	0.98	−2.06	9.5	9.8
CPIP	4.0	1.00	−2.83	11.3	15.9

Variables of linear regression lines for each allergen in Fig. 2. *a*, *b*) Calculated values from linear regression lines for each allergen in Fig. 2. *c*) -Log (elicitation threshold (%)) × MR value at 1% × 0.5

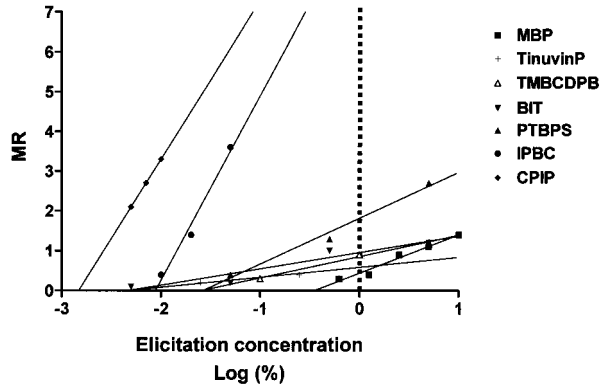

Fig. 2. Relationship between Challenge Concentration and Skin Reaction Score for Seven Allergens

Dose-related skin reaction for challenge concentration was evaluated with seven allergens. For evaluating of the elicitation profile, the maximally sensitized group of animals with each allergen was used (the group with an asterisk in Fig. 1). With each allergen, three to five concentrations of the compound in acetone were applied all at once for challenge. Each symbol represents mean response score of the group 48 hr after challenge with each compound.

an elicitation profile. As shown in Fig. 1, injection of an allergen at the highest non-necrotic concentration, a recommended protocol in the original GPMT,[10] did not always result in the highest sensitization. A similar bell-shaped profile of a dose-response curve for the induction phase using the GPMT was reported by Andersen *et al.*[13] for formaldehyde. Evaluating dose-response for the induction phase seems useful to accurately select the optimal induction dose for subsequent evaluation of elicitation potency of an allergen.

Traditionally, the number of animals in a group that show a positive skin reaction has been used as an index of sensitization potency of a tested compound. Moreover, the intensity of skin reaction at each challenged site was ignored in the evaluation. In the present study, using a MR score that ranges from 0 to 7 as an index of skin reaction, good linearity was kept between sensitization potency and log challenge concentrations throughout the dose ranges employed for all tested allergens. An elicitation threshold value calculated from a regression line would be an index of the relative elicitation potency of an allergen. However, the broad diversity of the slope values among regression lines means that the order of the extent of skin reactions for allergens varies depending on challenge doses. For example, Tinuvin P is a more potent sensitizer than IPBC in that the former has a lower elicitation threshold, 46 ppm, compared to the latter, at 88 ppm. However, when sensitized animals are challenged with these chemicals at doses of more than 100 ppm, IPBC would elicit a far more potent skin reaction than Tinuvin P. Thus, the dose–response profile of elicitation should also be taken into consideration to evaluate the risk of a chemical allergen. The area of a triangle enclosed by the x-axis, a dotted vertical line and a linear regression line for each allergen in Fig. 2 reflects the integrated degree of skin reaction that would occur when a sensitized individual is exposed to an allergen. This value includes both the threshold and the dose–response profile, slope of the regression line, in its mathematical expression.

A maximum challenge dose of 1% was selected to compare relative elicitation potencies of all allergens at one time. Although 1% is a critical value adopted by EC[14] on whether to label the existence of a sensitizer in a product or not, it is rather arbitrary and not definitive. Concentrations of chemicals such as those examined in this study vary widely in commercial products.[3,15] The range of challenge doses upon which relative elicitation potency is evaluated should be selected on a case by case basis, provided reliable data on the concentration is available.

Use of a sensitizer in a product is not regulated under the concept of maximum acceptable concentrations calculated based on an experimentally obtained threshold. Consumers who are exposed daily to allergens at concentrations greater than their elicitation thresholds are at risk and some of them are suffering from contact dermatitis. The area under the linear regression line between the threshold and a specific elicitation concentration would be a good index to compare relative elicitation potencies of chemical allergens.

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