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RESEARCH ARTICLE

The local lymph node assay compared with the human maximization test as an indicator of allergic potency in humans using patch test clinic populations

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Abstract

The human maximization test (HMT) is a method to evaluate potency in humans, while the local lymph node assay (LLNA) is a test method that allows for the measuring of the allergic potency of a substance in a rodent. It has been proposed that an EC3 value (the value obtained by the LLNA test, ie, the concentration of an allergen leading to a 3-fold increase of baseline proliferation rate) would be a reliable indicator for a compound's allergic potency in humans. This paper compares the correlation between the EC3 value of a compound and its allergic occurrence in the general population with the correlation between the HMT of the compound and its allergic occurrence in the general population, to determine the relationship to potency. The correlation values when outliers were removed from the sample were -0.56 and -0.71 for LLNA and HMT, respectively, suggesting that there is a possible 20% error margin in LLNA's ability to predict potency. The data also suggest that other factors (such as exposure) could play up to a 30% role in the determination of allergic occurrence in the general population. The potency assays might be made more clinically relevant for predicting allergic frequencies by including a frequency factor and other factors in its dermatotoxicological interpretation.

Keywords: LLNA; HMT; allergic potency; patch test

Introduction

A substance's ability to cause an allergic contact dermatitis reaction depends on the substance's potency as well as on other factors, including the methods of exposure. While potency can be measured by various potency assays such the local lymph node assay (LLNA) and the human maximization test (HMT), a reliable method to measure other factors has yet to be produced.

LLNA is a method to numerically measure the skin-sensitizing potency of a potential allergen in a rodent. In this method, a mouse ear is exposed to a potential allergen and proliferation of nearby located lymph node cells is measured to quantify the effect of the allergen on the system. The concentration of an allergen leading to a 3-fold increase of baseline proliferation rate (EC3) is

considered a threshold for sensitization; thus, the lower the EC3 value, the stronger the allergen. It has been suggested that a compound's EC3 value can be used as a reliable indicator of a compound's skin-sensitizing potency in humans (1).

The human maximization test (HMT), developed by A. Kligman, built on knowledge of enhancing sensitization rates in the Magnusson-Kligman guinea pig assay. The human variant utilizes high test-substance concentration and, for low-irritating compounds, pretreatment of the skin site with sodium lauryl sulfate. It involves the repeated occlusive treatment of a moderately irritated skin site with a high concentration of a test chemical.

This paper compares the ability of various potency assays to predict potency as well as quantitatively evaluates the role that other factors play in allergic reactions.

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Methods

Data regarding frequency of an allergen in the human population were obtained by using published data generated through multicenter studies by the North American Contact Dermatitis Group (NACDG) (2) and the European Surveillance System on Contact Allergies (ESSCA) (3) regarding patch positivity results. A weighted positivity percentage was calculated comprising both studies and taking into account the sample of people included in each study. Note that the human data utilize only patch test-positive results and not confirmed clinical cases.

Published LLNA EC3 values for these compounds were taken from various references (see Table 1). These EC3 values (concentrations, in %) were converted to dose per skin area values (DSAEC3, in $\mu\text{g}/\text{cm}^2$) using the dose volume of 25 μL according to the standard LLNA protocol and an estimated application area of 1 cm^2 for the mouse ear.

HMT data was obtained from Kligman (4,5). The lowest effective concentration applied during the induction phase of the study for each chemical was converted to a dose per unit area ($\mu\text{g}/\text{cm}^2$) using the information on substance concentration, application volume, and area of application given in the publication. Using the information on sensitization incidences given in the

publications, a dose per skin area leading to a sensitization incidence of 5% (DSA05) was derived by linear interpolation. This low but existent effect level was assumed to be comparable to the EC3 effect level in the LLNA.

Eight compounds (nickel, cobalt chloride, neomycin, potassium dichromate, formaldehyde, p-phenylenediamine benzocaine, and mercapto-benzothiazole) were present in both the NACDG and ESSCA studies that also had LLNA and HMT data.

Values from LLNA and HMT were compared with the patch test positivity of an allergen, and correlation values were obtained. Since both LLNA and HMT were being compared, the analysis was controlled for environmental exposure and allowed for a comparison of potency-predicting abilities. To quantitatively assess the role other factors play in clinical frequency of an allergen, we took the best potency correlation value obtained and subtracted it from 1, since allergic frequency is based on both potency and other factors.

Results

While a negative correlation was obtained for the substances used in this analysis, as would be expected

Table 1. EC3 values and clinical frequencies as reported by the North American Contact Dermatitis Group and the European Surveillance System on Contact Allergies for the 8 compounds analyzed.

Compound	NACDG and ESSCA weighted frequency (%)	LLNA (DSA _{EC3}) ($\mu\text{g}/\text{cm}^2$)	HMT (DSA _{05-HMT}) ($\mu\text{g}/\text{cm}^2$)	References ^a
Nickel ^b	17.1	350	28	Basketter et al. 1994 (9); Basketter and Scholes 1992 (10)
Cobalt chloride	7.8	50	313	Gerberick et al. 1992 (11); Ikarashi et al. 1992 (12,13)
Neomycin	5.7	1,500	2,028	Boussiquet-Leroux et al. 1995 (14); Gerberick et al. 2000 (15)
Potassium dichromate ^c	5	116	111	Basketter et al. 1999 (16); Basketter and Scholes 1992 (10); Ikarashi et al. 1992 and 1993 (13,17); Kimber et al. 1991 (18); Mandervelt et al. 1997 (19)
Formaldehyde ^d	4.2	102	89	Basketter et al. 2001 (20); Basketter and Scholes 1992 (10); Hilton et al. 1998 (21); Sailstad et al. 1995 (22)
p-Phenylene-diamine ^d	3.7	23	16.4	Basketter and Kimber 2001 (23); Basketter and Scholes 1992 (10); Montelius et al. 1994 (24); Warbrick et al. 1999 (25)
Benzocaine	1.2	3,338	3,902	Basketter et al. 1993 and 1995 (26,27); Kimber et al. 1989 (28); Montelius et al. 1994 (24); van Och et al. 2000 (29)
Mercapto-benzothiazole	0.9	1,214	2,269	Basketter et al. 1993 (26); De Jong et al. 2002 (30); Montelius et al. 1994 (24); Scholes et al. 1992 (31)
	Correlation (with outliers)	-0.41	-0.53	
	Correlation (without outliers)	-0.56	-0.71	

^aFor complete reference citations, see the reference list.

^bNickel concentration varied between studies: NACDG, 2.5%, ESSCA, 5.0%.

^cPotassium dichromate concentration varied between studies: NACDG, 0.25%, ESSCA, 0.5%.

^dThe 2 outliers.

DSAEC3 = dose per skin area value for EC3; DSA05-HMT = dose per skin area value for XXX; EC3 = concentration of an allergen leading to a 3-fold increase of baseline proliferation rate; ESSCA = European Surveillance System on Contact Allergies; HMT = human maximization test; LLNA = local lymph node assay; NACDG = North American Contact Dermatitis Group.

because of the negative association between potency value and strength of allergen, the correlation values were only -0.41 for LLNA and -0.53 for HMT (see Table 1, Figure 1, and Figure 2). When 2 outliers (formaldehyde and p-phenylenediamine) whose data points were farthest away from the rest of the sample's trend were removed from the sample, the correlation values improved to -0.56 and -0.71 for LLNA and HMT, respectively, still not reflective of strong correlation. The fact that HMT was the superior predictor of potency was to be expected since it is done in human tissue and, thus, is much more reflective of the human experience. However, in this analysis we quantitatively showed that LLNA is only 80% as accurate as HMT in predicting potency, as the correlation values of LLNA were only approximately 80% of the correlation values of HMT, both before and after 2 outliers were excluded from the sample. Assuming that HMT is not 100% accurate at predicting potency, this would suggest that the LLNA has a possible error margin of over 20% in predicting a substance's allergic potency, at least for the allergens used in our sample.

The data also suggest that other factors play up to a 30% role in the determination of the frequency of an allergen in the general population, as the best correlation

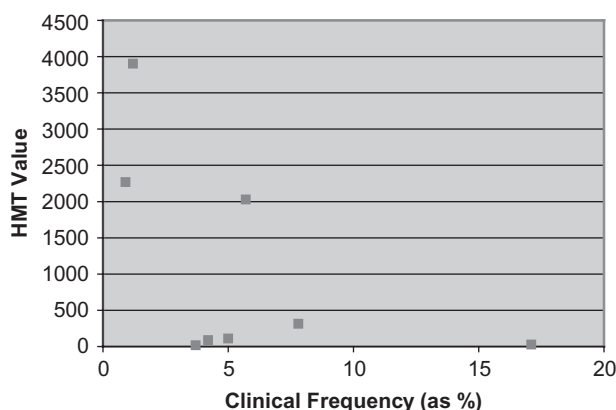


Figure 1. Graph of the human maximization test (HMT) EC3 (concentration of allergen leading to a 3-fold increase of baseline proliferation rate) value versus clinical frequency.

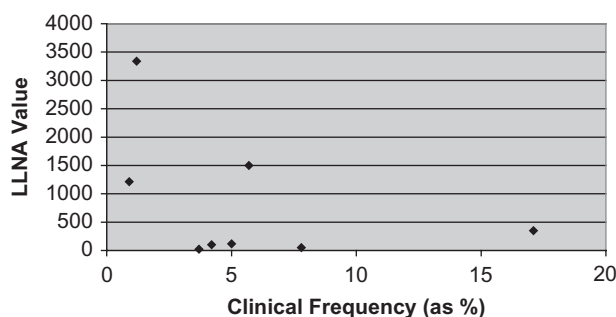


Figure 2. Graph of local lymph node assay (LLNA) value versus clinical frequency.

value for a potency assay was only -0.71 . The effects of such factors on clinical frequency can best be seen by comparing individual substances with each other. For example, nickel has an HMT value of 28, while p-phenylene-diamine has an HMT value of 16.4, suggesting the p-phenylene-diamine is about a 50% stronger allergen. However, nickel has over a 4-fold higher clinical frequency of 17.1% compared with p-phenylene-diamine's 3.7%, the opposite of what would have been expected based on potency data alone. Nickel is handled much more frequently by the general public, which allows for a greater chance of first exposure and, thus, a higher rate of allergic reaction than p-phenylene-diamine.

Discussion

The error margin in LLNA's ability to predict potency can best be explained by the way in which the LLNA data are gathered—through exposure to mouse tissue. Obviously, one cannot fully mimic the human response to an allergen by using a nonhuman model.

The age of the patch test patients could have affected our results, as a disproportionate number of patch patients are elderly, and elderly patients are more likely to have a positive patch test. Furthermore, patch test data may not be representative of the general population (6).

While the LLNA and HMT are presumably adequate predictors of the allergic potency of a substance, there has yet to be created a model that accurately reflects the human experience as regards exposure to an allergen. This could be changed by adding an exposure frequency factor to the LLNA or HMT calculation or creating a new weighted value that takes into account various environmental factors. This could be done by measuring exposure rates of certain substances and then weighting them with their current LLNA or HMT value to create a new number. Such a number would be more relevant in predicting actual allergic frequencies as well as how a change in the demographics of exposure could lead to changes in allergic frequency rates.

We note the numerous limitations of this exercise. Data from different laboratories, investigators, locations, and years were pooled and the limited test chemical data set can or did produce inaccuracies. The end points vary: 1) Patch test positivity was uncorrected for chemical interpretation (contact allergy rather than clinical allergic contact dermatitis); 2) the guinea pig data may have often shown poor reproducibility in different laboratories; and 3) the LLNA offers potency information rather than the elicitation data present in the other 2 systems. Proposals in 2005 by the LLNA teams (7) offer potential methods to move the LLNA data (induction and hazard identifiers) into a clinically relevant realm.

Attempts to decrease contact dermatitis in humans date back to the 1943 Draize publications. However, we have not yet prevented new allergies from reaching humans, nor have we always identified safe concentrations of allergens for which adequate alternatives are not available (8). Hopefully, the expanding tools listed here and their sophisticated interpretation will lead in the next decades to decreasing frequency of contact dermatitis.

Acknowledgments

Declaration of interest: The authors report no conflicts of interest.

References

1. Schneider K, Akkan Z. Quantitative relationship between local lymph node assay and human skin sensitization assays. *Regul Toxicol Pharmacol*. 2004; 39:245–255.
2. Pratt MD, Belsito DV, DeLeo VA, Fowler JF Jr, Fransway A.F, Maibach HI, Marks JG, Toby Mathias CG, Rietschel RL, Sasseville D, Sheretz EF, Storrs FJ, Taylor JS, Zug K. North American Contact Dermatitis Group Patch—Test Results, 2001–2002 Study Period. *Dermatitis*. 2004; 15(4):176–183.
3. Uter W, Hegewald J, Aberer W, Ayala F, Bircher AJ, Brasch J, Coenraads PJ, Schuttelaar MLA, Elsner P, Fartasch M, Mahler V, Belloni Fortina A, Frosch PJ, Fuchs T, Johansen JD, Menne T, Jolanki R, Krecisz B, Kiec-Swierczynska M, Larese F, Orton D, Preserico A, Rantanen T, Schnuch A. The European standard series in 9 European countries, 2002/2003—first results of the European Surveillance System on Contact Allergies. *Contact Dermatitis*. 2005; 53:136–145.
4. Kligman AM. The identification of contact allergens by human assay. III. The maximization test: a procedure for screening and rating contact sensitizers. *J Invest Dermatol*. 1966; 47:393–409.
5. Kligman AM. The identification of contact allergens by human assay. II. Factors influencing the induction and measurement of allergic contact dermatitis. *J Invest Dermatol*. 1966; 47:375–392.
6. Mirshahpanah P, Maibach HI. Relationship of patch test positivity in a general versus an eczema population. *Contact Dermatitis*. 2007; 56:125–30.
7. Basketter DA, Andersen KE, Liden C, Van Loveren H, Boman A, Kimber I, Alanko K, Berggren E. Evaluation of the skin sensitizing potency of chemicals by using the existing methods and considerations of relevance for elicitation. *Contact Dermatitis*. 2005; 52:39–43.
8. Nguyen SH, Dang TP, MacPherson C, Maibach HI. Prevalence of patch test results from 1970 to 2002 in a multi-centre population in North America (NACDG). *Contact Dermatitis*. 2008; 58(2):101–106.
9. Basketter DA, Scholes EW, Kimber I. The performance of the local lymph node assay with chemicals identified as contact allergens in the human maximization test. *Food Chem Toxicol*. 1994; 32:543–547.
10. Basketter DA, Scholes EW. Comparison of the local lymph node assay with the guinea-pig maximization test for the detection of a range of contact allergens. *Food Chem Toxicol*. 1992; 30:65–69.
11. Gerberick GF, House RV, Fletcher ER, Ryan CA. Examination of the local lymph node assay for use in contact sensitization risk assessment. *Fundam Appl Toxicol*. 1992; 19:438–445.
12. Ikarashi Y, Tsuchiya T, Nakamura A. Detection of contact sensitivity of metal salts using the murine local lymph node assay. *Toxicol Lett*. 1992; 62:53–61.
13. Ikarashi Y, Ohno K, Tsuchiya T, Nakamura A. Differences of draining lymph node cell proliferation among mice, rats and guinea pigs following exposure to metal allergens. *Toxicology*. 1992; 76:283–292.
14. Boussiquet-Leroux C, Durand-Cavagna G, Herlin K, Holder D. Evaluation of lymphocyte proliferation by immunohistochemistry in the local lymph node assay. *J Appl Toxicol*. 1995; 15:465–475.
15. Gerberick GF, Ryan CA, Kimber I, Dearman RJ, Lea LJ, Basketter DA. Local lymph node assay: validation assessment for regulatory purposes. *Am J Contact Dermatitis*. 2000; 11:3–18.
16. Basketter DA, Lea LJ, Dickens A, Briggs D, Pate I, Dearman RJ, Kimber I. A comparison of statistical approaches to the derivation of EC3 values from local lymph node assay dose responses. *J Appl Toxicol*. 1999; 19:261–266.
17. Ikarashi Y, Tsukamoto Y, Tsuchiya T, Nakamura A. Influence of irritants on lymph node cell proliferation and the detection of contact sensitivity to metal salts in the murine local lymph node assay. *Contact Dermatitis*. 1993; 29:128–132.
18. Kimber I, Weisenberger C. Anamnestic responses to contact allergens: applications in the murine local lymph node assay. *J Appl Toxicol*. 1991; 11:129–133.
19. Mandervelt C, Clottens FL, Demedts M, Nemery B. Assessment of the sensitization potential of five metal salts in the murine local lymph node assay. *Toxicology*. 1997; 120:65–73.
20. Basketter DA, Wright ZM, Warbrick EV, Dearman RJ, Kimber I, Ryan CA, Gerberick GF, White IR. Human potency predictions for aldehydes using the local lymph node assay. *Contact Dermatitis*. 2001; 45:89–94.
21. Hilton J, Dearman RJ, Harvey P, Evans P, Basketter DA, Kimber I. Estimation of relative skin sensitizing potency using the local lymph node assay: a comparison of formaldehyde and glutaraldehyde. *Am J Contact Dermatitis*. 1998; 9:29–33.
22. Sailstad DM, Krishnan SD, Tepper JS, Doerfler DL, Selgrade MK. Dietary vitamin A enhances sensitivity of the local lymph node assay. *Toxicology*. 1995; 96:157–163.
23. Basketter DA, Kimber I. Predictive testing in contact allergy: facts and future. *Allergy*. 2001; 56:937–943.
24. Montelius J, Wahlkvist H, Boman A, Fernström P, Grabergs L, Wahlberg JE. Experience with the murine local lymph node assay: inability to discriminate between allergens and irritants. *Acta Derm Venereol*. 1994; 74:22–27.
25. Warbrick EV, Dearman RJ, Lea LJ, Basketter DA, Kimber I. Local lymph node assay responses to paraphenylenediamine: intra- and inter-laboratory evaluations. *J Appl Toxicol*. 1999; 19:255–260.
26. Basketter DA, Selbie E, Scholes EW, Lees D, Kimber I, Botham PA. Results with OECD recommended positive control sensitizers in the maximization, Buehler and local lymph node assays. *Food Chem Toxicol*. 1993; 31:63–67.
27. Basketter DA, Scholes EW, Wahlkvist H, Montelius J. An evaluation of the suitability of benzocaine as a positive control skin sensitizer. *Contact Dermatitis*. 1995; 33:28–32.
28. Kimber I, Hilton J, Weisenberger C. The murine local lymph node assay for identification of contact allergens: a preliminary evaluation of in situ measurement of lymphocyte proliferation. *Contact Dermatitis*. 1989; 21:215–220.
29. van Och FMM, Slob W, de Jong WH, Vandebriel RJ, van Loveren H. A quantitative method for assessing the sensitizing potency of low molecular weight chemicals using a local lymph node assay: employment of a regression method that includes determination of the uncertainty margins. *Toxicology*. 2000; 146:49–59.
30. De Jong WH, Van Och FMM, Den Hartog Jager CF, Spiekstra SW, Slob W, Vandebriel RJ, Van Loveren H. Ranking of allergenic potency of rubber chemicals in a modified local lymph node assay. *Toxicol Sci*. 2002; 66:226–232.
31. Scholes EW, Basketter DA, Sarll AE, Kimber I, Evans CD, Miller K, Robbins MC, Harrison PT, Waite SJ. The local lymph node assay: results of a final interlaboratory validation under field conditions. *J Appl Toxicol*. 1992; 12:217–222.