

Patch test dose–response study: polysensitized individuals do not express lower elicitation thresholds than single/double-sensitized individuals

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Summary

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Accepted for publication

28 August 2008

Key words

elicitation dose–response relationship, methyldibromo glutaronitrile, nickel sulphate, p-phenylenediamine, polysensitization, susceptibility

Conflicts of interest

None declared.

DOI 10.1111/j.1365-2133.2008.08921.x

Background It is not known if reduced elicitation thresholds are evident among polysensitized individuals when using allergens to which the patients are already sensitized. Reduced elicitation thresholds may be an expression of increased reactivity in this patient group.

Objectives To examine and compare elicitation dose–response curves and elicitation thresholds in a polysensitized vs. a single/double-sensitized group for allergens to which the test subjects were already sensitized.

Patients/methods Fifty-one patients (13 polysensitized and 38 single/double-sensitized) were patch tested with nickel sulphate, methyldibromo glutaronitrile (MDBGN) and p-phenylenediamine (PPD) in dilution series. The ratio between the doses eliciting a response in 50% of patients in the two groups was used as the measure for relative sensitivity.

Results The dose–response curves of the polysensitized group for MDBGN and PPD were shifted to the right, and for nickel sulphate shifted to the left, compared with the single/double-sensitized group. The relative sensitivity for each of the three allergens and a combined relative sensitivity for all three allergens were not significantly different when comparing the polysensitized and single/double-sensitized groups.

Conclusion No increased sensitivity, in the form of distinct elicitation thresholds, could be demonstrated in polysensitized individuals compared with individuals with one or two contact allergies.

Polysensitized patients appear more frequently than would be expected by chance,^{1,2} are more easily sensitized and show greater elicitation responses when experimentally exposed to dinitrochlorobenzene (DNCB).² These findings suggest that polysensitized individuals are particularly sensitive to sensitization and immunologically reactive.

It is not known if reduced elicitation thresholds are evident among polysensitized individuals when using allergens to which the patients are already sensitized. Reduced elicitation thresholds may be an expression of increased reactivity in this patient group which may result in severe and long-lasting disease.

The aim of this study was to examine and compare the elicitation threshold and elicitation dose–response curves in polysensitized patients vs. a reference group of single/double-sensitized patients for the allergens nickel sulphate, methyldibromo glutaronitrile (MDBGN) and p-phenylenediamine (PPD).

Materials and methods

Data were compiled from three previous investigations and reanalysed with the specific purpose as described. The three original studies were all approved by the local ethics committee (Copenhagen County) and all study subjects gave their written informed consent before enrolment.

Twenty nickel-allergic test subjects were patch tested with a nickel sulphate dilution series of 19 different concentrations ranging from 0.0000228% to 3% and one ethanol/water control. Eighteen MDBGN-allergic test subjects were patch tested with a MDBGN dilution series of 19 different concentrations ranging from 0.0000077% to 0.5% and one ethanol/water control. Fifteen PPD-allergic test subjects were patch tested with a PPD dilution series of eight different concentrations ranging from 0.0001% to 1% and one white petrolatum control.

For all three allergens, the patch tests were performed on the back using Finn Chambers and Scanpor tape. A 48-h occlusion was used. The readings were carried out on day 2, day 3/4 and day 7 and the reading on day 3 was used for the statistical calculations. Materials and methods are thoroughly described in the previous studies.^{3–5} None of the 51 test subjects had active eczema at the time of patch testing with the dilution series.

The threshold concentration was defined as the weakest concentration giving a positive response on day 3 in a continuous line of patch-test reactions starting from the highest concentration. A doubtful reaction (+?) was considered positive in this setting as the test subjects were verified as sensitized and the +? reaction was registered in succession with 1+/2+/3+ reactions.

All 51 test subjects were patch tested with the European baseline series as part of ordinary diagnostics and treatment. If relevant, the test subjects were also tested with additional series. Routine patch-testing methods and materials at the Department of Dermatology and Allergology, Copenhagen University Hospital Gentofte, Denmark have been described previously.⁶ Positive reactions to allergens in the European baseline series or supplementary series were counted as additional allergies. A definition of three or more than three contact allergies was chosen for polysensitization, as suggested in two recent reviews.^{7,8} The individual combinations of allergens were not chemically/structurally related.

The mean age of the patients was 45.8 years (SD $\pm$ 10.4) for the polysensitized group ($n = 13$) and 43.6 years (SD $\pm$ 13.4) for the single/double-sensitized group ($n = 38$). An independent samples test showed no difference in mean age between the two groups ($P = 0.603$). In the polysensitized group 11 (85%) were female and in the single/double-sensitized group 34 (90%) were female. No difference in sex distribution between the two groups was seen (Fisher's exact test, $P = 0.638$).

Statistics

A logistic dose-response model equivalent to the distribution of the threshold doses⁹ was estimated from the observed threshold dose-response data by means of asymptotic maximum likelihood methods using specially developed statistical software written in APL (Manugistics Inc., Rockville, MD, U.S.A.). The statistical analysis comprised likelihood ratio tests (χ^2) of goodness of fit, estimation of pairs of parallel logistic threshold dose-response curves, tests of parallelism and calculation of relative sensitivity with 95% confidence intervals (CI). Parallel response vs. $\log_{\text{dose}}$ relationships are required for expressing the relative sensitivity or potency as a single number,⁹ i.e. the ratio between doses that elicit positive responses in the same fraction of the subjects, e.g. ED₅₀ (dose eliciting a reaction in 50% of the subjects). Statistical tests were regarded as significant if $P \leq 0.05$.

Independent samples t-test was used to compare age means. The observations were independent and assumption of normality and for equal variances was met.

Results

Nickel sulphate

Twenty nickel-allergic patients were patch tested with a nickel sulphate dilution series. One person did not react to any of the nickel sulphate solutions and was excluded from further analysis. Of the remaining 19 test subjects, 10 had no additional contact allergies, six had one additional contact allergy and three had two additional contact allergies. The lowest threshold concentration observed in the polysensitized group was 0.02%, and in the single/double-sensitized group it was 0.01%. None of the nickel-allergic patients reacted to the ethanol/water control. The parallel dose-response curves for nickel sulphate are shown in Figure 1.

Methyldibromo glutaronitrile

Eighteen MDBGN-allergic subjects were patch tested with a MDBGN dilution series. Of these, four individuals had no additional contact allergies, five had one additional contact allergy and nine had two or more than two additional contact allergies. The lowest threshold concentration observed in the polysensitized group was 0.002%, and in the single/double-sensitized group it was 0.0001%. One of the MDBGN-allergic patients reacted with a few papules, but no erythema and no infiltration, to the ethanol/water control. The parallel dose-response curves for MDBGN are shown in Figure 2.

p-Phenylenediamine

Fifteen PPD-allergic patients were patch tested with a PPD dilution series. One person did not react to any of the PPD solutions and was excluded from further analysis. Of the remaining 14 test subjects, 10 had no additional contact allergies, three had one additional contact allergy and one had three additional contact allergies. The lowest threshold concentration observed in the polysensitized group was 0.05%,

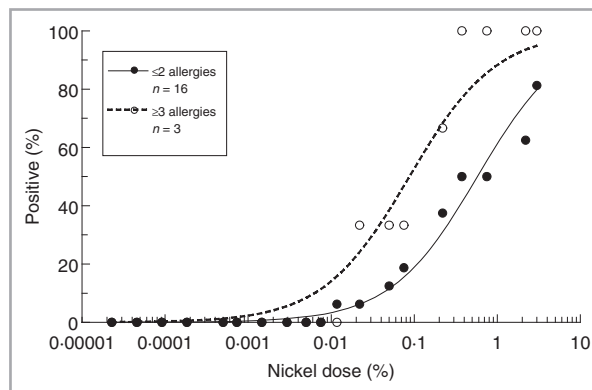


Fig 1. Parallel dose-response curves for nickel sulphate for a polysensitized group and a single/double-sensitized group. The dots represent the percentage of test subjects reacting at a given concentration.

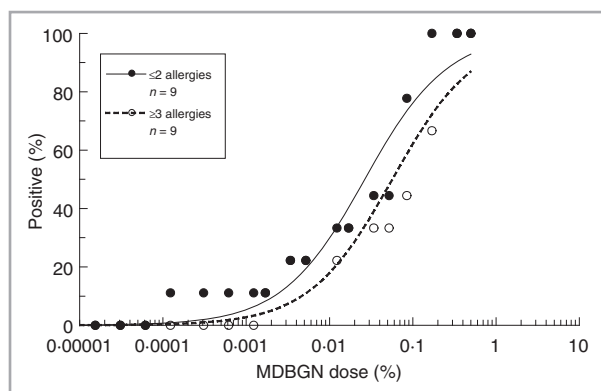


Fig 2. Parallel dose-response curves for methyldibromo glutaronitrile (MDBGN) for a polysensitized group and a single/double-sensitized group. The dots represent the percentage of test subjects reacting at a given concentration.

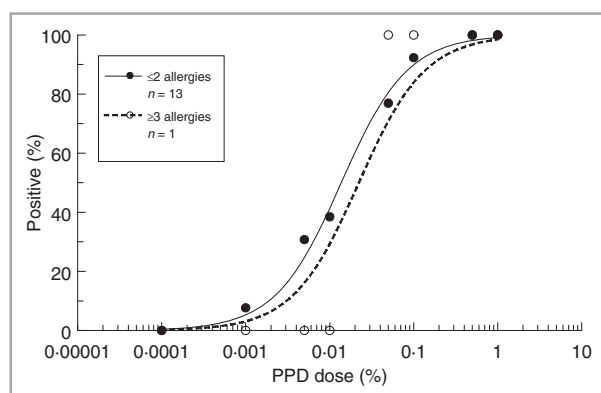


Fig 3. Parallel dose-response curves for p-phenylenediamine (PPD) for a polysensitized group and a single/double-sensitized group. The dots represent the percentage of test subjects reacting at a given concentration.

and in the single/double-sensitized group it was 0.001%. None of the PPD-allergic patients reacted to the petrolatum control. The parallel dose-response curves for PPD are shown in Figure 3.

Relative sensitivity

The relative sensitivity was defined as the ratio between the ED_{50} for the single/double-sensitized group vs. the polysensi-

tized group for each allergen separately and for the allergens combined (total test population size 51). The relative sensitivities with 95% CI are shown in Table 1. The relative sensitivities for the three allergens were identical ($P = 0.46$) which made it possible to summarize the relative sensitivities for each allergen into one combined relative sensitivity. The combined relative sensitivity was 68% (95% CI 19–251) indicating the lowest sensitivity among the polysensitized group; however, there was no significant difference in sensitivity between the single/double-sensitized and polysensitized groups, as the CI included 100%.

Discussion

Under controlled experimental settings using a highly potent allergen (DNCB), polysensitized patients are sensitized by lower induction doses and have greater elicitation responses at every eliciting dose than monosensitized patients and healthy controls.² We examined whether or not the elicitation dose-response curve for polysensitized individuals would be displaced to the left compared with a reference group of individuals with one or two contact allergies for allergens to which the patients were already sensitized and, if so, if elicitation thresholds could be estimated beneath which only highly susceptible patients would react.

We analysed the results from testing 51 subjects with dilution series to one of three common allergens to which they were already sensitized. The dose-response curves for the polysensitized group for the allergens MDBGN and PPD were shifted to the right indicating a lower sensitivity compared with the single/double-sensitized group. The dose-response curve of the polysensitized group for the allergen nickel sulphate was shifted to the left indicating a higher sensitivity compared with the single/double-sensitized group. The differences between the dose-response curves were measured in relative sensitivities based on the assumption of parallelism. No significant difference could be demonstrated between the dose-response curves for the polysensitized group vs. the single/double-sensitized group for each of the allergens separately or combined.

The threshold for elicitation is influenced by several exogenous and endogenous factors. Allergen concentration, frequency, duration and occlusiveness of application, body area location of exposure, and simultaneous exposures to other

Table 1 Relative sensitivity^a for each allergen separately and for the allergens combined

Allergen	Number of test subjects (1–2 contact allergies)	Number of test subjects (≥ 3 contact allergies)	Relative sensitivity (%)	95% Confidence intervals
Nickel	16	3	658	57–31 377
MDBGN	9	9	47	4–293
PPD	13	1	62	2–1334
Total	38	13	68	19–251

Test for same sensitivity: $\chi^2(2) = 1.50$, $P = 0.46$. ED_{50} , dose eliciting a reaction in 50% of subjects; MDBGN, methyldibromo glutaronitrile; PPD, p-phenylenediamine. ^a ED_{50} (group with one or two contact allergies)/ ED_{50} (group with three or more than three contact allergies).

allergens and irritants, were all controlled in this study. Active inflammation did not affect the results as none of the test subjects had eczema at the time of patch testing. Even though testing with each allergen was performed during series of up to 6 months, seasonal variations probably did not influence the results as the polysensitized and the single/double-sensitized individuals were tested randomly, and not in groups. There was also no age or sex difference between the polysensitized and single/double-sensitized groups which could affect the results.

The test subjects were included based on a positive patch-test response to the allergen of interest. The test subjects had been referred to our Department of Dermatology and Allergology in need of diagnosis and treatment of a skin disorder. The circumstances surrounding sensitization for the individual test subjects were not standardized and the test subjects consequently expressed heterogeneity regarding real-life conditions of exposures leading to sensitization. The magnitude of the elicitation response is dependent on the induction dose: the greater the induction dose, the greater the elicitation response.¹⁰ Heterogeneity between cases and controls regarding conditions of exposure leading to sensitization may have influenced the strength and threshold of the elicitation response which may explain why no difference could be detected. However, this setting is reflecting the exposure situations in reality.

DNCB sensitization in polysensitized individuals divided according to exposure into a group with high occupational exposure and a group with ubiquitous baseline exposure showed that only the latter group expressed a decreased sensitization threshold.² The polysensitized individuals in our study were not subdivided according to specific conditions leading to polysensitization, a task that would be difficult and not realistic. Only one subgroup of polysensitized individuals expressed an increased sensitivity, and furthermore this was a subgroup without intense exposure as a cause of polysensitization; therefore, heterogeneity, not only between cases and controls regarding conditions of exposures, but also within our used case group, may also be the reason why no difference was detected.

Allergic mechanisms are allergen specific which may also explain the different outcomes when using MDBGN, PPD and nickel sulphate in contrast to DNCB.

The grouping of patients with one or two contact allergies vs. patients with three or more than three contact allergies is based on a previous recommendation. This seemed reasonable when focusing on patients with suspected increased reactivity, as patients with three or more than three contact allergies appear more frequently than would be expected by chance^{1,2} and pairs of contact allergies can often be ascribed to concomitant exposure and cross-reactivity, in contrast to triplets of contact allergies.⁷ Increased reactivity may be a graded

phenomenon where the reactivity increases with increasing number of contact allergies.² The choice to combine individuals with one or two contact allergies in the reference group could, therefore, have obliterated any true but small difference. The statistical calculations were, however, also performed for a monosensitized group vs. a group with two or more than two contact allergies, which also almost equalized the number of test subjects in each study group, but it did not change the results. No significant differences were detected.

No increased sensitivity could be demonstrated in polysensitized subjects compared with individuals with one or two contact allergies. The dose–response relationships were based on data from small test groups which reduces the precision of the calculated relative sensitivities. The polysensitized test group was particularly small and the number of test subjects in each study group diverged. It is possible that a difference in relative sensitivity between the two groups truly exists but it was not strong enough to appear in this experimental setting and with the number of test subjects used.

Acknowledgments

The original nickel sulphate dose–response study was financially supported by NiPERA (Nickel Producers Environmental Research Association) and the original PPD dose–response study was financially supported from Aage Bang's Foundation.

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