

Characteristics of patch test reactions to common preservatives incorporated in petrolatum and water, respectively

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Summary

Background. The irritant properties of some preservatives and the use of water as the patch test vehicle for some of them call for a critical evaluation of patch test reactions to preservatives.

Objectives. To examine the association between test vehicle (petrolatum versus water) or the patients' age and history of atopic dermatitis, respectively, and certain patterns of reaction to preservatives.

Patients/methods. Data of 34 631 patients tested in 34 centres with 11 common preservatives were retrospectively analysed. The dynamic reaction patterns, reaction indices (RIs) and positivity ratios (PRs) were statistically evaluated.

Results. All preservatives yielded more crescendo reactions in older than in younger patients. For 10 of 11 preservatives, the percentage of crescendo reactions was slightly higher in patients without a history of atopic dermatitis, and for 10 of 11 agents the RI was higher in patients with a positive history of atopic dermatitis. No consistent vehicle-related effects on reaction characteristics were found. Chlorhexidine digluconate 0.5% in water and sodium benzoate 5% in petrolatum had the lowest RIs, highest PRs, and lowest proportions of crescendo reactions.

Conclusions. Water as a vehicle is unlikely to affect the reaction patterns of preservatives. The generally used patch test preparations of chlorhexidine digluconate and sodium benzoate need improvement.

Key words: age, atopic dermatitis, dynamic reaction pattern, methods, patch test, positivity ratio, reaction index, vehicle, water.

Preservatives are added to a broad variety of common materials in order to prevent their microbial degradation. Several substances are found both in industrial or occupational areas and in skin care products and

cosmetics. Thus, human skin is frequently exposed to preservatives, and this is of dermatological importance, as many preservatives can cause contact allergy (1). Accordingly, preservatives have been routinely applied in diagnostic patch testing (2–4). However, patch testing with preservatives has still remained a complex issue. One problem is that some preservatives have an intrinsic irritant potential that contributes to non-specific reactions (5). One may therefore speculate that some individuals are more likely to respond with non-specific, that is, irritant, patch test reactions to preservatives than others. Moreover, several preservatives cannot be incorporated in petrolatum. Such preservatives are commonly

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dissolved in water for patch testing, and this may cause additional problems. The concentration of an aqueous solution may change in the course of time, owing to instability or to evaporation of water when the container is repeatedly opened. Additionally, because of the liquid nature of the aqueous test preparation, it may be more difficult to precisely apply the predefined amount of allergen in terms of dose per area than with an allergen dissolved in pet. unless particular care is taken.

In an attempt to further elucidate the latter aspect of the role of the patch test vehicle (water versus pet.), we have analysed patch test reactions to important preservatives, some dissolved in water, and some incorporated in pet.. We have compared the characteristics of the test reactions to common preservatives in order to identify relevant differences. Additional attention was paid to possible influences of population characteristics (age and history of atopic dermatitis).

Patients and Methods

This is a retrospective analysis of patch test data filed by the Information Network of Departments of Dermatology (6). The contributing centres and investigators are listed in Table 1. For the purpose of this study, the time period from 1995 to 2008 was chosen. Only tests of those patients who had been tested both with the baseline series of the German Contact Dermatitis Research Group (DKG) and the preservative series of the DKG were included. The only preservatives considered were those that had been tested by all centres in unchanged concentrations and vehicles from 1995 until 2008 and that had elicited positive reactions in at least 0.4% of all evaluable tests, thus providing a sufficient number of reactions (Table 2). Only patients patch tested with all allergens considered were eligible for analysis, to avoid bias resulting from specific omission of certain allergens ($n = 34\,631$). All tests evaluated had been performed with 8-mm Finn Chambers® applied for 2 days on the backs of the patients. Complete documentation of test readings was mandatory for evaluation; if patient data (sex, age, and history of atopic dermatitis) were not complete (noted in a small fraction of cases), these cases were excluded from analyses that included such information. Atopic dermatitis was diagnosed on the basis of the criteria of Hanifin and Rajka (7). All tests had been performed following the guidelines of the International Contact Dermatitis Research Group and DKG (8, 9). Test allergens had been obtained from Trolab-Hermal/Almirall (Reinbek, Germany). The test concentrations used were in accordance with common recommendations (10–12). In accordance with established criteria (8, 9), reactions were read as negative,

Table 1. Contributing test centres and investigators with the number of patients included in the present analysis

Centre	Number of patients	Investigators
Aachen	806	C. Schröder, H. Dickel, J. M. Baron, S. Erdmann
Augsburg	979	A. Ludwig
Berlin Charité	1466	B. Laubstein, M. Worm, T. Zuberbier
Bern, Switzerland	564	D. Simon
Bielefeld	3	I. Effendy
Bochum	1279	Ch. Szliska, H. Dickel, M. Straube
Dermatologikum Hamburg	640	K. Reich, V. Martin
Dortmund	2168	B. Pilz, C. Pirker, K. Kügler, P. J. Frosch, R. Herbst
Erlangen	2276	K.-P. Peters, M. Fartasch, M. Hertl, T. L. Diepgen, V. Mahler
Freudenberg	702	Ch. Szliska
Gera	276	J. Meyer
Göttingen	2204	J. Geier, Th. Fuchs
Graz, Austria	3108	B. Kränke, W. Aberer
Hamburg	399	D. Vieluf, E. Coors, M. Kiehn, R. Weßbecher
Hamburg BUK	58	C. Schröder-Kraft, K. Breuer, U. Seemann
Hannover	1230	T. Schaefer, Th. Werfel
Heidelberg	2736	A. Schulze-Dirks, M. Hartmann, U. Jappe
Heidelberg AKS	441	E. Weisshaar, H. Dickel, T. L. Diepgen
Heilbronn	15	H. Löffler
Jena	1612	A. Bauer, M. Gebhardt, M. Kaatz, S. Schliemann, W. Wigger-Alberti
Krefeld	116	A. Wallerand, M. Lilie, S. Wassilew
Leipzig	341	R. Treudler
Lippe Detmold	71	S. Nestoris
Lübeck	918	J. Grabbe, J. Kreusch
Mainz	253	D. Becker
Marburg	1674	H. Löffler, I. Effendy, M. Hertl
München LMU	428	B. Przybilla, P. Thomas, R. Eben, T. Oppel, T. Schuh
München Schwabing	2085	K. Ramrath, M. Agathos, M. Georgi
München TU	2605	J. Rakoski, U. Darsow
Münster	848	B. Hellweg, R. Brehler
Rostock	61	Ch. Schmitz, H. Heise, J. Trcka, M.A. Ebisch
Tübingen	869	G. Lischka, M. Röcken, T. Biedermann
Würzburg	169	A. Trautmann, J. Arnold
Zürich	1231	B. Ballmer-Weber

weakly positive (+), moderately positive (++), strongly positive (+++), irritant or doubtful, including follicular (f) and questionable (?) reactions.

The readings on days 2 and 3 were used for our analyses, as these are performed regularly, whereas later readings (day 4 and beyond) are mostly employed in

Table 2. Preservatives with overall results in 34 631 patients tested with all preservatives considered

	% reactions (95% CI)			RI (95% CI)	PR (95% CI)
	Positive	?/ir/f	Crescendo		
Allergen in water					
Chlorhexidine digluconate 0.5%	0.40 (0.34–0.47)	0.70 (0.61–0.79)	36.3 (32.8–39.9)	–0.270 (–0.373 to –0.168)	96.4 (91.8–98.8)
MCI/MI 100 p.p.m.	2.42 (2.26–2.59)	0.79 (0.70–0.89)	56.9 (54.2–59.5)	0.506 (0.456–0.556)	68.4 (65.2–71.6)
DMDM hydantoin 2%	0.57 (0.49–0.65)	0.55 (0.47–0.63)	46.5 (42.8–50.3)	0.017 (–0.082 to 0.117)	89.7 (84.7–93.5)
Formaldehyde 1%	1.64 (1.51–1.77)	1.03 (0.93–1.14)	59.9 (57.1–62.7)	0.228 (0.165–0.290)	76.9 (73.3–80.3)
Allergen in pet.					
2-Bromo-2-nitropropane-1,3-diol 0.5%	1.36 (1.24–1.48)	1.08 (0.98–1.20)	49.7 (46.8–52.6)	0.112 (0.045–0.180)	75.1 (70.9–79.0)
Chloroacetamide 0.2%	0.83 (0.74–0.93)	0.47 (0.40–0.55)	47.1 (43.1–51.2)	0.273 (0.184–0.362)	62.0 (56.1–67.7)
Diazolidinyl urea 2%	0.76 (0.67–0.85)	0.49 (0.42–0.57)	52.8 (48.8–56.8)	0.213 (0.120–0.306)	79.0 (73.6–83.8)
Imidazolidinyl urea 2%	0.66 (0.57–0.75)	0.36 (0.30–0.43)	46.3 (42.2–50.5)	0.293 (0.193–0.394)	80.2 (74.4–85.2)
Sodium benzoate 5%	0.80 (0.71–0.90)	1.29 (1.17–1.42)	42.5 (39.6–45.4)	–0.233 (–0.306 to –0.160)	91.7 (87.8–94.7)
Sorbic acid 2%	0.61 (0.53–0.70)	0.74 (0.66–0.84)	47.0 (43.2–50.9)	–0.100 (–0.193 to –0.007)	87.7 (82.5–91.8)
Quaternium-15 1%	0.68 (0.60–0.77)	0.35 (0.29–0.42)	51.3 (46.9–55.6)	0.322 (0.224–0.420)	71.2 (65.0–76.9)

?, questionable; ir, irritant; f, follicular; CI, confidence interval; MCI/MI, methylchloroisothiazolinone/methylisothiazolinone; PR, positivity ratio; RI, reaction index.

Point estimates supplemented with 95% CIs.

special situations only, such as testing with 'late-reacting' allergens, or doubtful reactions until day 3, or other reasons. As an additional criterion, the dynamic reaction pattern was considered (13). A crescendo reaction was defined by a stronger reaction on day 3 than on day 2; a decrescendo reaction had to be stronger on day 2 than on day 3; and a plateau reaction was defined by readings of the same category on both days. Any test with a +, ++ or +++ reading on day 3 was classified as positive. The reaction index (RI) and positivity ratio (PR) were calculated on the basis of the readings on day 3, to further characterize the reaction profile of the different allergens. The RI is the number of positive reactions minus the number of other reactions (doubtful and irritant), divided by the total number of non-negative reactions (14–16). The PR is the fraction of + reactions among all positive reactions, expressed as a percentage (16, 17). Statistical analyses were performed with the statistical software package SAS (version 9.2; SAS Institute, Cary, NC, USA) and based on published guidelines (18). An asymptotic confidence interval (CI) to the RI was calculated following a recent suggestion (19). The PR was supplied with an exact CI based on the binomial distribution (18).

Results

Data of 34 test centres were eligible for this analysis, including one centre from Switzerland and one from Austria; the others were located all over Germany (Table 1). Eleven preservatives met the inclusion criteria for evaluation (Table 2). Of a total of 34 631 patients tested with

all 11 preservatives considered, 37.7% were male, 50.7% were ≤ 49 years of age, and 23.6% suffered from previous or current atopic dermatitis. In 40.5% of all cases, the tests had been performed because allergic contact dermatitis was suspected, in 41.8% of cases this diagnosis had been considered to be less likely, and the rest of the patients had been tested for various other reasons. As was to be expected, the several preservatives yielded different percentages of positive and questionable/irritant reactions (Table 2). In accordance with such variations, the RIs and PRs calculated for the various preservatives were also quite different (Table 2). Moreover, the percentages of crescendo reactions related to all non-negative reactions on day 3 with the allergen in question proved to be different (Table 2).

The patients' age and, much less so, a negative history of atopic dermatitis were found to be relevant factors for the likelihood of crescendo-type positive reactions when these figures were compared for the different preservatives (Table 3). All preservatives yielded more crescendo reactions in older patients than in younger ones, and this difference was statistically significant for 5 of 11 allergens (Table 3). Similarly, for all but 1 of the 11 preservatives, the percentage of crescendo reactions was slightly higher in patients without a history of atopic dermatitis than in individuals with a positive history of atopic dermatitis (Table 3). On the other hand, in patients with a positive history of atopic dermatitis, the RI was higher for 10 of 11 allergens (Table 3). There was no apparent correlation between the patients' age or history of atopic dermatitis and the PR (data not shown).

Table 3. Characteristics of reactions to preservatives in relation to patients' age and history of atopic dermatitis (AD)

Allergen	Median age of patients (years) with:		% crescendo reactions (related to all positive reactions) in patients:		% crescendo reactions (related to all positive reactions) in patients with:		RI in patients with:	
	Positive reactions	?/ir/f reactions	≤49 years old	>49 years old	Positive history of AD	Negative history of AD	Positive history of AD	Negative history of AD
Allergen in water								
Chlorhexidine digluconate 0.5%	58	51	28.4 (23.8–33.3)	45.0 (39.7–50.3)	36.0 (28.6–43.8)	36.8 (32.5–41.4)	–0.160 (–0.384 to 0.063)	–0.307 (–0.433 to –0.181)
MCI/MI 100 ppm	50	54	49.3 (45.5–53.2)	64.6 (60.9–68.1)	52.6 (47.0–58.1)	59.5 (56.1–62.8)	0.544 (0.444–0.645)	0.479 (0.415–0.544)
DMDM hydantoin 2%	48	49	41.5 (36.4–46.7)	51.8 (46.3–57.5)	41.4 (34.0–49.1)	48.7 (43.9–53.6)	0.055 (–0.152 to 0.262)	–0.020 (–0.146 to 0.107)
Formaldehyde 1%	50	50	55.4 (51.3–59.3)	65.5 (61.4–69.3)	58.6 (52.8–64.2)	60.0 (56.4–63.5)	0.262 (0.137–0.386)	0.199 (0.118–0.279)
Allergen in pet.								
2-Bromo-2-nitropropane-1,3-diol 0.5%	56	54	44.1 (39.7–48.6)	54.2 (50.3–58.1)	48.0 (41.8–54.4)	49.9 (46.2–53.5)	0.056 (–0.094 to 0.206)	0.147 (0.063–0.230)
Chloroacetamide 0.2%	60	57	41.2 (34.7–47.9)	50.8 (45.6–56.0)	42.6 (33.1–52.5)	50.0 (45.1–54.9)	0.333 (0.112–0.555)	0.259 (0.155–0.363)
Diazolidinyl urea 2%	51	53	49.8 (44.1–55.6)	56.1 (50.4–61.6)	54.4 (45.7–62.9)	51.9 (46.8–57.0)	0.354 (0.170–0.537)	0.183 (0.063–0.303)
Imidazolidinyl urea 2%	49	54	41.2 (35.6–47.0)	52.4 (46.3–58.4)	47.2 (38.9–55.7)	47.3 (41.9–52.7)	0.371 (0.179–0.563)	0.238 (0.106–0.370)
Sodium benzoate 5%	40	41	41.5 (38.0–45.1)	45.1 (40.0–50.3)	41.4 (36.2–46.8)	42.9 (39.0–46.8)	–0.190 (–0.0321 to –0.059)	–0.271 (–0.369 to –0.173)
Sorbic acid 2%	56	56	39.7 (34.0–45.5)	53.0 (47.8–58.2)	47.4 (37.9–56.9)	47.6 (42.9–52.4)	0.000 (–0.232 to 0.232)	–0.162 (–0.276 to –0.048)
Quaternium-15 1%	45	47	48.7 (42.9–54.5)	54.8 (48.0–61.6)	48.8 (39.9–57.8)	51.6 (45.9–57.2)	0.422 (0.234–0.609)	0.309 (0.183–0.435)

?, questionable; ir, irritant; f, follicular; CI, confidence interval; MCI/MI, methylchloroisothiazolinone/methylisothiazolinone; RI, reaction index.

Statistically significant differences are in bold ($p < 0.05$, 95% CIs do not overlap).

Point estimates supplemented with 95% CIs.

A comparison between the preservatives tested in pet. and those tested in water did not reveal any consistent difference between these two vehicle-defined groups with regard to the RIs and PRs, or to the frequencies of crescendo-type positive reactions.

Methylchloroisothiazolinone (MCI)/methylisothiazolinone (MI) and formaldehyde (both tested in water), as well as chloroacetamide and quaternium-15 (both tested in pet.), were shown to be good test preparations, characterized by favourable values of their RIs and PRs and rather high percentages of positive crescendo reactions. At the other end of the spectrum, chlorhexidine digluconate in water and sodium benzoate in pet. were found to have very low RIs, high PRs, and low proportions of positive crescendo reactions.

Discussion

In this study, 11 common preservatives were compared with regard to the basic characteristics of the reactions that they had elicited in a large patch test population under standardized conditions. We worked on the basis that questionable and irritant reactions should not be neglected for the purposes of patch test standardization (20), and we also considered the time-dependent development of positive reactions. Moreover, a certain preservative may possibly yield different findings when tested with different allergen preparations. Previous studies, for example, showed that MCI/MI yielded more positive reactions when tested in water than when tested in pet. (21), whereas DMDM hydantoin gave more positive reactions when tested in pet. than when tested in water (22, 23). Therefore, our findings do not reflect the allergenic potential of an agent as such, but should be strictly interpreted in the context of the particular test conditions, including test concentrations and test techniques.

Our results confirm that patch test preparations of particular preservatives are quite heterogeneous with regard to the frequencies of the positive and other types of reactions that they elicit. This is revealed by their different percentages of positive, questionable (?/f) and irritant reactions, their different RIs and PRs, and their different frequencies of positive crescendo reactions.

Our first question was whether the time course of positive reactions and the RI of a preservative are related to some characteristics of the tested population or are mainly attributable to the particular preservative. For example, one might speculate that in older patients, or in patients with a positive history of atopic dermatitis, one might find time courses of patch test reactions or RIs different from those in other patients. For 10 of 11 allergens, the RI was, in fact, higher in patients with a positive history

of atopic dermatitis. However, we cannot explain this finding, and no trend towards a higher RI in patients with a positive history of atopic dermatitis was observed in a previous analysis of test reactions to contact allergens of a standard series (15). For the moment, therefore, it remains to be verified whether such a trend is, in fact, specifically associated with test reactions to preservatives. All preservatives studied yielded more crescendo reactions in older patients than in younger ones, and this difference was statistically significant for 5 of 11 allergens (Table 3). Furthermore, for 10 of the 11 preservatives, the percentage of crescendo reactions was slightly higher in patients without a history of atopic dermatitis than in individuals with a positive history of atopic dermatitis (Table 3). In a previous study with contact allergens of a standard series, a trend towards more crescendo reactions was not found to be related to the patients' age or a positive history of atopic dermatitis (13, 24). However, a relationship between crescendo reactions and clinical relevance was described (13). Therefore, one could speculate that, in our study, the higher percentages of crescendo reactions to preservatives in older patients and, perhaps, in patients with a negative history of atopic dermatitis could indicate a higher percentage of relevant reactions in these groups of patients. In any case, these possible associations should be assessed by further studies.

Our main question was whether the test vehicle, water versus pet., was relevant for the characteristics of the reactions. As compared with pet., water as a solvent for allergens may pose problems related to lower stability of aqueous solutions, evaporation, skin irritation, and difficulties with exact dosing. However, when the RIs, PRs and rates of crescendo reactions of water-dissolved agents are compared with the corresponding figures for pet.-dissolved ones, no consistent differences are found. This also applies when subgroups of patients defined by age or history of atopic dermatitis are considered. We can therefore conclude that the test vehicle seems not to be a decisive methodological factor for the reaction

patterns obtained with the preparations of the preservatives evaluated. An important practical consequence of this finding is that water as a vehicle for preservatives is unlikely to impair patch test quality, at least with regard to the preparations of preservatives, reaction characteristics, and the test techniques applied in the present study. This is reassuring, as otherwise patch testing with preservatives would become complicated.

Our data clearly show that some test preparations of preservatives are not optimal for their purpose. A patch test preparation that yields considerably more questionable and/or irritant reactions than positive ones makes reading and interpretation of reactions to this preparation problematical. According to their low RIs, of the 11 preparations evaluated in this study, chlorhexidine digluconate 0.5% in water and sodium benzoate 5% in pet. are the most obvious candidates for improvement. As revealed by their high PRs, nearly all of their positive reactions are only weak (+) ones. Interestingly, these same agents also have the lowest percentages of positive reactions with a crescendo course (Tables 2 and 3). This means that a considerable part of their positive reactions developed with a time course possibly influenced by irritation during the early stages. Such reactions are, in fact, very difficult to discriminate from non-allergic ones. It follows that reactions to the current preparations of chlorhexidine digluconate and sodium benzoate are particularly difficult to assess. Therefore, it would be interesting to check whether an increase in their patch test concentrations could yield a shift towards more unambiguous reactions – either irritant ones or allergic ones. For chlorhexidine digluconate, a test concentration of 1% was preferred by some investigators, and further study was suggested for optimization (12, 25). For sodium benzoate, we have not found similar clues in the literature. A modification of the test concentrations should be evaluated in terms of gains and losses in sensitivity and specificity, respectively, against a gold standard such as a repeated open application test plus specific information from the history.

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