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INVESTIGATIVE REPORT

Sensitizing Capacity of Two Monomeric Aldehyde Components in p-*tert*-Butylphenol-Formaldehyde Resin

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Contact allergy to p-*tert*-butylphenol-formaldehyde resin is not rare. This resin consists of a large number of substances, most of which are unknown. For diagnostic and preventive reasons, the chemical identity of the sensitizers should be known as well as their sensitizing capacities, cross-reaction patterns and presence in the environment. The aim of this study was to investigate the sensitizing capacities and cross-reaction patterns for 5-*tert*-butyl-2-hydroxy-3-hydroxymethyl-benzaldehyde and 5-*tert*-butyl-2-hydroxy-benzaldehyde in the guinea pig maximization test. 2,6-Dimethylol p-*tert*-butylphenol, p-*tert*-butylcatechol, 2-methylol p-*tert*-butylphenol, p-*tert*-butylphenol, 4-*tert*-butyl-2-(5-*tert*-butyl-2-hydroxy-3-hydroxymethyl-benzyloxymethyl)-6-hydroxymethyl-phenol and 4-*tert*-butyl-2-(5-*tert*-butyl-2-hydroxy-benzyloxymethyl)-phenol were used as potential cross-reacting substances. 5-*tert*-Butyl-2-hydroxy-3-hydroxymethyl-benzaldehyde was shown to be a sensitizer ($p = 0.041$). In animals induced with this compound no cross-reactions to the putative cross-reacting substances were seen. In contrast, 5-*tert*-butyl-2-hydroxy-benzaldehyde failed to induce sensitization and no cross-reactions were detected. *Key words: Guinea pig maximization test; 5-tert-butyl-2-hydroxy-3-hydroxymethyl-benzaldehyde; 5-tert-butyl-2-hydroxy-benzaldehyde.*

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Contact allergy to p-*tert*-butylphenol-formaldehyde resin (PTBP-F-R), a resin used as a binder in many adhesive formulations, is not rare (1–4). Included in the standard patch test series in most countries, the resin consists of a large number of substances, the majority of which are not chemically defined. PTBP-F-R has been used since around 1950, but only a few allergens are known. For diagnostic and preventive reasons the chemical identity of the sensitizers should be known as well as their sensitizing capacities, cross-reaction patterns and presence in the environment. Knowledge of the identity of the sensitizers in PTBP-F-R is a prerequisite for the development of sensitive analytical methods that can make it possible to trace the resin in the environment and therefore help our patients hypersensitive to PTBP-F-R.

The PTBP-F-R substances used in this article are shown in Fig. 1 designated with roman numerals, which are the same as those used in most of our previous articles on allergens in PTBP-F-R. The numbers indicate the order in which the substances elute in a high-pressure liquid chromatography (HPLC) system equipped with a C_{18} column, which means that a higher number indicates a more fat-soluble (non-polar) compound.

The raw materials for the resin are p-*tert*-butylphenol (compound VII) and formaldehyde. Monomeric constituents of the resin come from the reaction of formaldehyde with compound VII. Each molecule of formaldehyde, which reacts with compound VII, forms a hydroxymethyl (methylol) group. In this way, the 2 monomers 2-methylol p-*tert*-butylphenol (IV) and

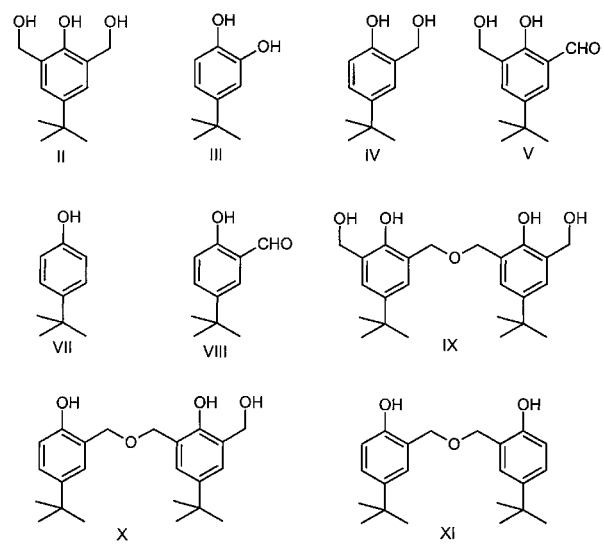

Fig. 1. Chemical structures of substances used in this investigation: 2,6-dimethylol p-*tert*-butylphenol (compound II), p-*tert*-butylcatechol (III), 2-methylol p-*tert*-butylphenol (IV), 5-*tert*-butyl-2-hydroxy-3-hydroxymethyl-benzaldehyde (V), p-*tert*-butylphenol (VII), 5-*tert*-butyl-2-hydroxy-benzaldehyde (VIII), 4-*tert*-butyl-2-(5-*tert*-butyl-2-hydroxy-3-hydroxymethyl-benzyloxymethyl)-6-hydroxymethyl-phenol (IX), 4-*tert*-butyl-2-(5-*tert*-butyl-2-hydroxy-benzyloxymethyl)-6-hydroxymethyl-phenol (X) and 4-*tert*-butyl-2-(5-*tert*-butyl-2-hydroxy-benzyloxymethyl)-phenol (XI).

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2,6-dimethylol p-*tert*-butylphenol (II) are formed. Articles on their allergenic nature have also been published (5–7).

However, in recent studies we have isolated other types of monomers than hydroxymethyl phenols from the resin. These monomeric components showed catechol, benzoquinone and aldehyde structures ((8) and unpublished results, MB) – all possible oxidation products from compound VII and the other monomers, compound II and compound IV. One of the isolated and identified components was compound V. We also identified compound VIII in PTBP-F-R (unpublished results). The latter substance has earlier been identified as a component in PTBP-F-R by Hagdrup et al. (7). Compounds V and VIII are aromatic aldehydes with a phenolic _OH group and are also derivatives of salicylaldehyde.

When eight patients hypersensitive to PTBP-F-R were patch-tested with serial dilutions of compound V, all of them reacted positively (unpublished results, MB). The three most sensitive patients reacted down to a concentration of 0.00017% w/v in acetone. Preliminary results from patch testing with compound VIII indicate that the substance is a contact allergen, weaker, however, than V. Hagdrup et al. patch-tested compound VIII in one patient hypersensitive to PTBP-F-R with negative result (7).

The purpose of this study was to determine the sensitizing capacities of compounds V and VIII, and to investigate the cross-reaction patterns using the guinea pig for sensitization.

MATERIAL AND METHODS

Substances

The following substances were used: methanol and acetone, *pro analysi* manufactured by Lab-Scan (Ireland); compounds III, VII and 2-methylol phenol (2-MP) were manufactured by Acros Organics (Belgium); compounds II and IV were synthesized at our department, according to a method described by Agatha & Schubert (5) but with some modifications. Compounds V and VIII were synthesized by Synthelec AB (Lund, Sweden) and nuclear magnetic resonance spectrometry was used to confirm their identity. Compound IX (in earlier papers (9, 10) called: 5,5′-di-*tert*-butyl-2,2′-dihydroxy-3,3′-dihydroxymethyl-dibenzyl ether), compound X (in earlier papers (9, 10) called 5,5′-di-*tert*-butyl-2,2′-dihydroxy-3-hydroxymethyl-dibenzyl ether) and compound XI (in earlier papers (9, 10) called 5,5′-di-*tert*-butyl-2,2′-dihydroxy-dibenzyl ether) were synthesized at our department as described elsewhere (9).

Analytical HPLC

Analytical HPLC was performed using a Varian 5000 (Varian, USA) pump system equipped with a Rheodyne 7010 injector (Rheodyne) with a 20 μl loop and a column (4.6 mm i. d. × 250 mm) packed with 5 μm Nucleosil C_{18} (Macherey-Nagel & Co.). The built in UV-100 detector (Varian) was operated at a wavelength of 280 nm. Different mixtures of methanol and water were used as mobile phases and the flow rate was 1 ml/min.

Purity of test substances

Analytical HPLC was used to investigate the purity of the test substances, compounds V and VIII.

Guinea pig maximization test (GPMT)

The GPMT was performed in accordance with the original description (11), but with some modifications in order to increase the standardization of the test and also to create conditions for objective evaluation, including statistical calculations of the patch test reactions and the inclusion of a positive control group (12, 13). The test and control animals, also the animals in the positive control group, were randomly distributed to the cages.

Animals

Albino female guinea pigs of the Dunkin-Hartley strain (J. A. Sahlin, Sweden) weighing 300–400 g were used. For each of the sensitization series, 42 animals were used; 36 animals participated in the actual sensitization study, 12 in the control group and 24 in the test group, while the remaining 6 animals comprised an additional control group. These 6 guinea pigs were sensitized to and challenged with the known sensitizer 2-MP and used as a positive control group. Before each sensitization series, an additional 4–8 animals were used to study the topical irritancy of each substance used for induction and challenge.

The Malmö/Lund Ethics Committee on Animal Experiments approved the study.

Induction and challenge procedures

Intradermal induction with the test substance was done on day 1 and epicutaneous induction on day 7 and day 8. The animals rested from day 11 until day 21. Challenge 1 and challenge 2 were performed on day 22 and the test results were read on day 24. Challenge 1 was aimed at sensitizing the capacity of the induction substance, while challenge 2 was used to detect cross-reactions. Induction and challenge were performed with non-irritant solutions for all substances according to results from toxicity testing. The induction and challenge procedures are described in detail elsewhere (9).

The preferred concentrations of the test substances, for both induction and challenge, were equimolar to the ones used when the monomers or the dimers of P-F-R were tested in the GPMT (14, 15). The concentrations for induction and challenge with the monomers of P-F-R were 2.0 and 1.2 $mol \times l^{-1}$, respectively. The concentrations for topical induction and challenge with dimers of P-F-R were 99 and 79 $mmol \times l^{-1}$, respectively. However, compound V gave a yellow staining in the test area in a concentration equimolar to the monomers in P-F-R and this could interfere with the blind reading of the test results. A test concentration equimolar to the dimers in P-F-R was therefore chosen for this substance. Compound VIII was highly irritant and had to be tested in a comparatively low concentration. Compounds III and VII used for challenge 2 are also irritants and were therefore tested at their highest non-irritating concentration. The concentrations of the test substances for induction and challenge are given in Table I.

Evaluation

The reactions were evaluated blindly 24 h after removal of the patches. The minimum criterion of an allergic (positive) reaction was a confluent erythema. The number of positive animals in each test group was statistically compared to the

Table I. *Concentrations and vehicles used for induction of guinea pigs with compounds V and VIII, and challenge with compounds II, III, IV, V, VII, VIII, IX and XI*

		Procedure and vehicle					
		Intradermal induction (acetone/FCA/pg)		Topical induction (acetone)		Challenge acetone	
Compound	Molecular weight	% w/v	mol × l^{-1}	% w/v	mol × l^{-1}	% w/v	mol × l^{-1}
II	210					25	1.2
III	166					7.5	0.45
IV	180					22	1.2
V	208	0.80	0.038	2.0	0.096	1.6	0.077
VII	150					2.0	0.13
VIII	178	0.030	0.0017	0.15	0.0084	0.090	0.0051
IX	402					3.2	0.079
XI	342					2.7	0.079

pg= propylene glycol; FCA= Freund's complete adjuvant.

number of positive animals in the corresponding control group and also to the number of positive test animals tested with the vehicle alone. When both comparisons yielded significant values, the compound was considered to be a contact sensitizer. For challenge 2 a comparison was made only between the number of positive animals in the test and control groups for each substance. The evaluation is described in more detail elsewhere (9).

Statistical calculation

Fisher's exact test was used.

RESULTS

Investigation of the purity of compound V showed that the highest possible concentration of known sensitizers in PTBP-F-R such as compounds II, III, IX and X was $<$0.03% w/v. However, 0.48% of compound IV was detected and approximately 0.5% of an unknown substance. In compound VIII, no contaminants exceeded the detection limit of 0.03%.

The results of challenge 1 after induction with compounds V and VIII, respectively, are given in Table II. In the group of animals subjected to induction with compound V, seven test animals showed positive test reactions. No control animals reacted positively to compound V. The difference in the numbers of positive animals for compound V in the test and control groups was statistically significant ($p = 0.041$). In the group of animals subjected to induction with compound VIII, only two test and two control animals showed positive test reactions.

Table II. *Challenge 1; test reactions after induction and challenge with compounds V and VIII*

	No. of positive animals			
Induction substance	T/*n*	C/*n*	V/*n*	P/*n*
V	7/24*	0/12	0/12	0/6
VIII	2/24	2/12	0/12	3/7

*$p = 0.041$.

T= test reactions to the suspected sensitizer in test animals; C= test reactions to the suspected sensitizer in control animals; V= test reactions to the vehicle in test animals; P= test reactions in positive control animals after induction and challenge with 2-methylol-phenol; *n*= number of tested animals in the 4 groups C, T, V and P.

Results from challenge 2 after induction with compounds V and VIII, respectively, are given in Table III. In animals subjected to induction with compound V, four test animals and two control animals reacted to compound III, one test animal and no control animal reacted to compound IV. Induction with compound VIII gave no differences between test and control groups that were statistically significant in challenge 2.

DISCUSSION

In our research on allergens in PTBP-F-R, we have in recent years isolated several allergens and there are indications of many more to be isolated and identified. Among all these allergens it becomes important to distinguish between strong, moderate and weak sensitizers and substances that elicit allergic reactions mainly by cross-reaction. The sensitizing capacities and cross-reaction patterns of these allergens can virtually only be investigated in animals.

We have recently demonstrated that compound V can elicit contact allergic reactions in humans hypersensitive to PTBP-F-R (unpublished results), and we have preliminary results from patch testing with compound VIII indicating an allergenic potential, but of a lower degree than for compound V. Patients hypersensitive to PTBP-F-R have shown positive patch test reactions to concentration down to around 2 ppm of compound V.

In this study, it is demonstrated that compound V is a sensitizer in the guinea pig, but no cross-reactions to compounds II, III, IV, VIII, IX or XI were demonstrated or indicated. With compound VIII as the sensitizer, no sensitization capacity or cross-reactivity was indicated. However, this investigation does not exclude the possibility that compound VIII is

Table III. *Challenge 2; test reactions in animals after induction with compounds V and VIII, and challenge with II, III, IV, V, VII, VIII, IX and XI*

			No. of positive animals							
Induction substance	Group	*n*	II 25%	III 7.5%	IV 22%	V 1.6%	VII 2.0%	VIII 0.090%	IX 3.2%	XI 2.7%
V	Test	24	0	4	1	NT	NT	0	0	0
	Control	12	0	2	0	NT	NT	0	0	0
VIII	Test	24	0	5	3	2	3	NT	NT	1
	Control	12	0	4	2	1	1	NT	NT	2

n= number of animals; NT= not tested; %= % w/v.

a sensitizer. The necessity for using non-irritating concentrations sets a limit concerning the concentrations that can be used for induction and challenge.

The monomers and dimers of PTBP-F-R are chemically related to the monomers and dimers of P-F-R, respectively, as all of them are methylol phenols (hydroxymethyl phenols). To enable comparisons of the sensitizing capacities of monomers and dimers from the two resins, the concentrations used for induction and challenge should be equimolar for the respective groups (14). In P-F-Rs there are several dimers which can all elicit allergic reactions in humans hypersensitive to this type of resin (16). Three of the dimers in P-F-R were tested with the GPMT and all were demonstrated to be sensitizers (14). The significance levels $p < 0.05$, $p < 0.01$ and $p < 0.001$ were used to designate the sensitizers as weak, moderate or strong, respectively (14). Compared to the dimers of P-F-R, compound IX can be classified as a strong sensitizer and compound X as a moderate sensitizer (13, 14). Solutions of monomers for challenge equimolar to solutions earlier used for challenge with monomers in P-F-R, respectively, were preferred (14, 15). However, in this study we had to use lower concentrations for both compound V and compound VIII.

Owing to yellow staining of the test area after challenge, compound V was tested in a lower concentration than the monomers of P-F-R. A concentration equimolar to the dimers of P-F-R was used instead. Use of this lower concentration means that the sensitizing capacities of compound V and the monomers of P-F-R cannot be compared, whereas those of compound V and the dimers of P-F-R and PTBP-F-R are comparable. If compound V is classified according to the standards that have been used for dimers in P-F-R and PTBP-F-R, it is a weak allergen. However, the fact that no animal in the positive control group reacted positively (Table II) indicates that test results concerning V might have been underestimated. When the P-F-R monomer 2-MP was tested in the GPMT it was classified as a strong sensitizer. However, when this substance was used for induction in the GPMT in concentrations equimolar to the dimers of P-F-R no sensitizing capacity could be detected (13). This indicates that compound V would have shown a higher sensitization capacity if tested at a higher concentration.

Compound VIII showed an unusually high topical irritancy in the guinea pig as compared to the other monomers of PTBP-F-R. The preferred test concentration for compound VIII was 21.5% w/v. However, the highest non-irritating concentration that could be used for challenge with compound VIII was 0.090% w/v. For challenge with the known irritants, compounds III and VII, concentrations of 7.5% and 2.0% w/v, respectively, could be used without irritant reactions. This shows a limitation of the GPMT, as a sensitizing capacity to highly irritating substances can be difficult to show. On the other hand, strongly irritating substances that can sensitize in the GPMT in very low concentrations are for example the chloro-methylisothiazolinones (17). The mechanism of the irritant response might be of importance. An irritant reaction can be due to alkylating properties of a substance. This type of reactivity can also produce haptenated proteins and cell receptors that promote the development of an allergic response. However, substances like acids and bases and some organic solvents can produce an irritant reaction without pronounced antigen production.

Compounds V and VIII are possibly oxidation products formed from compounds II and IV, respectively. Substances known for which oxidation leads to enhanced sensitization capacity are for example limonene (18). Whether or not oxidation of compounds II and IV increases their sensitizing capacity is impossible to say from this investigation. However, oxidation of compound IV into VIII considerably increases the irritancy.

In this study we have shown that compound V has a sensitizing capacity and pointed out some limitations of the GPMT.

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