Acta Derm Venereol (Stockh) 1987; 67: 315–320

Contact Allergy to the Active Ingredients of Kathon® CG in the Guinea Pig

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Bruze M, Fregert S, Gruvberger B, Persson K. Contact allergy to the active ingredients of Kathon® CG in the guinea pig. Acta Derm Venereol (Stockh) 1987; 67: 315–320.

Preservative Kathon® CG (K-CG) is a commercial preparation, consisting of the two active ingredients (a.i.), 2-methyl-4-isothiazolin-3-one (243-K-CG) and 5-chloro-2-methyl-4-isothiazolin-3-one (5243-K-CG) and also of other components. Both a.i. are known contact sensitizers in humans. In this study guinea pig maximization tests were performed with the a.i. in order to assess and compare the degrees of the sensitizing capacities. The animals were also rechallenged with the sensitizer and 4 chemically related compounds, all being preservatives or known ingredients in preservatives, in order to study the cross-reaction patterns. 5243-K-CG was demonstrated to be a strong sensitizer and 243-K-CG a weak sensitizer. With 5243-K-CG as the sensitizer, 4,5-dichloro-2-methyl-4-isothiazolin-3-one was a possible cross-reacting compound. Possible cross-reactivity was indicated between the a.i. when 243-K-CG was the sensitizer. *Key words: 5-Chloro-2-methyl-4-isothiazolin-3-one; Delayed hypersensibility; Guinea pig maximization test; High performance liquid chromatography; 2-Methyl-4-isothiazolin-3-one.* (Received August 26, 1986.)

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In recent years the preservative Kathon® CG (K-CG) has attracted much attention from a dermatological point of view. Although being a new preservative it is already widely used in cosmetics and toiletries. Its sensitizing capacity in humans has been established (1–6). In a previous paper, one of two active ingredients (a.i.) in K-CG, 5-chloro-2-methyl-4-isothiazolin-3-one (5243-K-CG), was demonstrated to be the main sensitizer in humans (7). This ingredient (5243-K-CG[1]) is, however, present in K-CG at 3 times higher concentration than the other a.i., 2-methyl-4-isothiazolin-3-one (243-K-CG[2]). The main purposes of this study were, therefore, to determine the sensitizing capacities of the a.i. and also to compare the sensitizing capacities and investigate the cross-reaction patterns by using guinea pigs for the sensitization.

MATERIAL AND METHODS

Substances

Induction was performed with 5243-K-CG and 243-K-CG. These compounds were obtained from a commercial preparation of K-CG (1.5% a.i.) furnished by the manufacturer, Rohm and Haas Company, USA. This preparation was separated into fractions by a chromatographic technique, which is described in detail elsewhere (7). Challenges were performed with these 2 compounds and also with 4,5-dichloro-2-methyl-4-isothiazolin-3-one (45243-K-CG) (obtained by the same chromatographic technique as 5243-K-CG and 243-K-CG; data concerning the identification will be published

[1] The abbreviation 5243-K-CG refers to the positions of the substituents in the molecule of 5-chloro-2-methyl-4-isothiazolin-3-one.

[2] The abbreviation 243-K-CG refers to the positions of the substituents in the molecule of 2-methyl-4-isothiazolin-3-one.

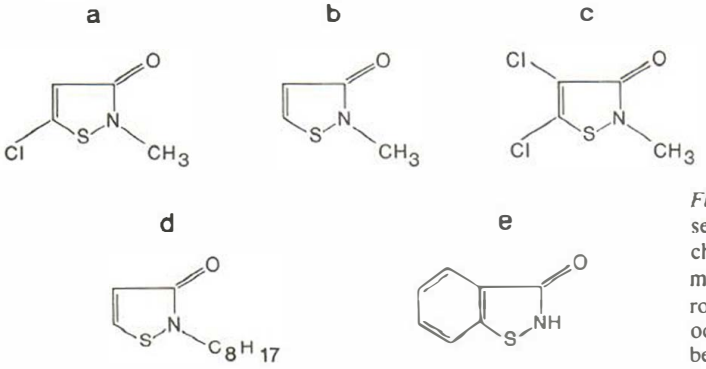

Fig. 1. Structures of compounds used for sensitizations and rechallenges: (*a*) 5-chloro-2-methyl-4-isothiazolin-3-one, (*b*) 2-methyl-4-isothiazolin-3-one, (*c*) 4.5-dichloro-2-methyl-4-isothiazolin-3-one, (*d*) 2-n-octyl-4-isothiazolin-3-one, and (*e*) 1.2-benzisothiazolin-3-one.

elsewhere), Kathon® 893 containing 45 % of the a.i. 2-n-octyl-4-isothiazolin-3-one (K-893) (Rohm and Haas Company), 1,2-benzisothiazolin-3-one (BIT) (ICI, USA) and ethanol 99.5 %. The structures are shown in Fig. 1. Induction and challenge were also performed with 2-methylol phenol (2-MP) (Merck, West Germany).

High Performance Liquid Chromatography (HPLC)

The highest possible contaminations of 5243-K-CG and 243-K-CG in the compounds used for challenges and rechallenges were examined by HPLC and with conditions described in detail elsewhere (7).

Guinea Pig Maximization Test (GPMT)

The GPMT was performed in accordance with the original descriptions (8, 9) 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 (10). 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 one of the 3 sensitization series (1 series with 5243-K-CG and 2 series with 243-K-CG) 42 animals were used. 36 animals participated in the actual sensitization study on 5243-K-CG or 243-K-CG, 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 2-MP and used as a "positive" control group to eliminate the possible influence of expectations on the evaluation of test reactions resulting in underestimation (10). The animals included in these procedures were not engaged in tests for topical irritancy.

Topical irritancy

The topical irritancy of the substances used for inductions and challenges was studied by a 48-hour closed patch test in 4–8 animals for each compound (10). On each animal the compound was applied on 3 patches on the flank; one near the back, one near the abdomen and one between these. The induction substances were also applied on the neck. The testing was performed one week after pretreatment with Freund's complete adjuvant (FCA) (Difco Lab., USA).

Induction procedure

5243-K-CG and 243-K-CG were used for sensitization. For intradermal sensitization 3 injections were given in a row, on each side of the shoulder. (I) 0.1 ml of FCA in water 40% w/v (corresponds to FCA/water 50/50 v/v). (II) 0.1 ml of 5243-K-CG or 243-K-CG in propylene glycol. The concentrations used were equimolar (6.7×10^{-3} mole $\times l^{-1}$) and 0.100 % w/v for 5243-K-CG and 0.076 % for 243-K-CG. (III) 0.1 ml of the preparation consisting of the potential sensitizer (5243-K-CG, 243-K-CG)/FCA-/propylene glycol w/w/v. The figures for the concentrations were the same as for (I) and (II).

24 h before the topical sensitization all the animals were treated with 200 µl of a preparation consisting of sodium lauryl sulphate (SLS) 10% w/v in dimethyl acetamide/acetone/ethanol 99.5% 4/3/3 v/v/v (DAE 433). 200 µl of the suspected sensitizer in ethanol 99.5 %, at a concentration of 0.050 % w/v for 5243-K-CG and 0.038 % for 243-K-CG, was transferred to a 2×4 cm patch of

Whatman 3MM filter paper. The concentrations were equimolar (3.3×10^{-3} mole $\times l^{-1}$). The patch was covered with overlapping, impermeable plastic adhesive tape (Leukofix, Beiersdorf AG, West Germany). This in turn was firmly secured by an adhesive bandage (Acrylastic, Beiersdorf AG). The dressing was left in place for 48 h.

Challenge procedure

Two weeks after the second stage of sensitization a 24-hour occluded patch test (Al-test on Leukofix and firmly secured by Acrylastic) was performed on the right flank with 30 µl of the test solution on each of 2 patches near the back. In each sensitization study 12 test animals received the suspected sensitizer in ethanol 99.5% on both patches. Half the number of animals (six) received the suspected sensitizer on only one of the patches, while the vehicle alone was applied to the other patch. The same number of animals received the suspected sensitizer and the vehicle in the reverse way. The test solution and the vehicle were patch tested in the same way in the control animals (not the animals in the positive control group) but the figure for each application way was halved. The concentrations used were equimolar (1.3×10^{-3} mole $\times l^{-1}$); 0.020% w/v for 5243-K-CG and 0.015% for 243-K-CG.

Rechallenge was performed one week after the challenge according to the technique previously described (11). The animals in the first series with 243-K-CG were not rechallenged. 0.1 ml of the solutions described in (II) the section of "induction procedure" was injected intradermally in the neck two days after the first challenge application. Five days later (one week after the first challenge application) the animals were rechallenged with the sensitizer and 4 chemically related substances and also the vehicle, which were applied to the left, non-tested flank. The same positions as for the challenge were used and also two positions near the abdomen and the remaining two positions between the back and the abdomen. A distribution pattern, based on a Latin square table, was used for the rechallenge. The sensitizer and each one of the 4 chemically related substances and also the vehicle were applied twice in each position on the control animals and the corresponding figure for the test animals was 4. The animals were rechallenged with all substances in ethanol 99.5% at equimolar concentrations (1.3×10^{-3} mole $\times l^{-1}$); 5243-K-CG 0.020% w/v, 243-K-CG 0.015%, 45243-K-CG 0.025%, K-893 0.029% a.i. and BIT 0.020%.

Controls

The animals in each control group were treated in the same way, concerning the induction and challenge procedures, as the corresponding animals in the test group except that the suspected sensitizer was not administered during the induction and in the booster dose before rechallenge. The 6 animals in the particular "positive" control group were sensitized to and challenged with 2-MP according to procedures described in detail elsewhere (10).

Evaluation

The reactions were evaluated blind 24 h after the 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 number of positive animals in the corresponding control group and also to the number of positive animals tested with the vehicle alone. The assessment of whether an animal was positive or not was based on the result for only one patch chosen in advance for those animals which had obtained the test solution on both patches (6 control animals and 12 test animals). When both comparisons yielded significant values the compound was considered to be a contact sensitizer. The significance levels (the lowest level was chosen when not identical) $p<0.05$; $p<0.01$; $p<0.001$ were used to designate a week, moderate and strong sensitizer respectively. For the rechallenge a comparison was made only between the number of positive animals in the test and control groups for each substance.

Statistical calculation

Fisher's exact test for two proportions was used.

RESULTS

The highest possible contaminations of 5243-K-CG and 243-K-CG in the compounds used for challenges and rechallenges, were below 0.5% w/w for all substances.

Table I shows the results of the sensitization to and challenge with 5243-K-CG and 243-K-CG. The difference in the number of positive animals for 5243-K-CG between the test and control groups was statistically significant ($p<0.001$), although one control animal

reacted to the test solution and one test animal reacted to the vehicle. The difference in the number of positive animals for the first series of 243-K-CG was, on the other hand, non-significant. However, when the sensitization was repeated with a new series, 11 test animals were positive and no controls reacted. Nor did the test animals react to the vehicle alone and both comparisons were statistically significant ($p<0.01$). The significant level was lower ($p<0.001$) when the results for the series were added, but higher ($p<0.05$) when the calculations were adjusted to the number of animals in one series.

Table II shows the results of rechallenge with the sensitizer and compounds with similar chemical structures. The number of positive animals in the test group was lower for both 5243-K-CG and 243-K-CG after rechallenges compared to challenges. The decrease was not due to fewer positive test reactions in the additional test positions.

No significant differences were noted between the number of positive test and control animals for those animals that were tested for cross-reactivity to 5243-K-CG and 243-K-CG. Possible cross-reactivity, however, was indicated to 45243-K-CG with 5243-K-CG as the sensitizer and to 5243-K-CG with 243-K-CG as the sensitizer.

Table I. *Test reactions after sensitization to and challenge with 5-chloro-2-methyl-4-isothiazolin-3-one (5243-K-CG) and 2-methyl-4-isothiazolin-3-one (243-K-CG)*

C = control animals, T = test animals receiving the suspected sensitizer, V = test animals receiving the vehicle alone on one patch and the suspected sensitizer on the other, P = positive control animals sensitized to and challenged with 2-methylol phenol, *n* = number of tested animals in the 4 groups C, T, V, P

		Number of positive animals			
		C	T	V	P
Animal group	*n* ...	12	24	12	6
5243-K-CG		1	19	1	2
243-K-CG					
Series 1		0	4	0	3
Series 2		0	11	0	4

Table II. *Test reactions after rechallenge with 5-chloro-2-methyl-4-isothiazolin-3-one (5243-K-CG), 2-methyl-4-isothiazolin-3-one (243-K-CG), 4,5-dichloro-2-methyl-4-isothiazolin-3-one (45243-K-CG) Kathon 893 (K-893), 1,2-benzisothiazolin-3-one (45243-k-CG), Kathon 893 (K-893), 1,2-benzisothiazolin-3-one (BIT) and ethanol 99.5% (E)*

T = test group, C = control group

Sensitization substance	Number of animals	Number of positive animals after rechallenge with					
		5243-K-CG	243-K-CG	45243-K-CG	K-893	BIT	E
5243-K-CG							
T	24	12	1	3	0	0	0
C	12	4	0	0	1	0	0
243-K-CG							
T	24	3	8	2	0	1	2
C	12	0	0	0	0	0	0

DISCUSSION

The sensitizing capacity of the preservative K-CG has been established in both humans and guinea pigs (1–6, 12). The guinea pig testing was performed by using a modified Buehler's occluded epicutaneous patch technique (12). Although getting a positive result, the authors drew the conclusion that K-CG was a safe preservative since there was a "no response concentration" (12).

It was recently shown in humans that the main sensitizer in K-CG is 5243-K-CG (7). This finding does not exclude an equally high or higher sensitizing capacity of the other a.i., 243-K-CG, which is present at lower concentration in K-CG (25% of 1.5%) than 5243-K-CG (75% of 1.5%) (13). To the best of our knowledge, sensitization studies with the a.i., 243-K-CG and 5243-K-CG, have not been performed earlier. In this study the GPMT for the two a.i. was carried out in the same way as regards the number of molecules administered (equimolar concentrations), the vehicles and the use of SLS for both compounds. 5243-K-CG was demonstrated to be a strong sensitizer and 243-K-CG a weak sensitizer, and the impression from patch testing in humans (7) was thus confirmed.

In the first series with 243-K-CG there was a non-significant difference in the number of positive animals between the test and the control animals. The corresponding comparisons in the second series, however, were significant. There is no statistically significant difference between the two sensitization studies with 243-K-CG. The comparisons remained significant when the two series were added, and also when the figures for the added series were adjusted to be correspondable to the number of animals in one series.

The sensitizing capacity of the two preservatives K-893 and BIT has been demonstrated in both humans and guinea pigs (3, 14, 15). Cross-reactivity between K-CG and BIT has been discussed (3) but could not be demonstrated in this study.

Sensitization studies in guinea pigs may be preformed to answer/elucidate many questions. The main use and probably the most valuable use of the GPMT is the predictive patch testing. By performing GPMT with new compounds with unknown sensitizing capacities the companies may sometimes be saved invests for sensitizing compounds and what is more important—humans may be saved unnecessary suffering. The demonstration of a compound as a sensitizer in the guinea pig does not imply that it cannot be used in humans, but its introduction on the market should be carefully analysed with regard to the exposure of the compound to human skin. A compound, that is a strong sensitizer in guinea pigs, will be expected to give a high incidence of allergic contact dermatitis in humans, if the compound is used in products of leave-on type and applied to both normal and damaged skin. The high frequency of patients with contact allergy to K-CG is, thus, not surprising.

ACKNOWLEDGEMENTS

The authors are indebted to Ingrid Thulin and Lena Persson for valuable technical assistance. This investigation was supported by grants from The Swedish Work Environment Fund (ASF 85–1079) and Rohm and Haas Company.

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