

The role of 3-dimethylaminopropylamine and amidoamine in contact allergy to cocamidopropylbetaine

CATERINA FOTI¹, DOMENICO BONAMONTE¹, GIUSEPPE MASCOLO², ANGELA CORCELLI³, SIMONA LOBASSO⁴, LUIGI RIGANO⁵
AND GIANNI ANGELINI¹

¹Department of Internal Medicine, Immunology and Infectious Diseases, Unit of Dermatology, University of Bari,

²National Research Council (CNR), Institute of Research of Waters, ³Department of General and Environmental Physiology, University of Bari, ⁴Chemistry Department, University of Bari, Bari, ⁵Institute of Skin and Products Evaluation (ISPE), Milan, Italy

Since it has been found that all subjects with contact allergy to cocamidopropylbetaine (CAPB) have positive reactions to 3-dimethylaminopropylamine (DMAPA), and reports have appeared in literature of the sensitizing action of amidoamine in products containing CAPB, we aimed to verify the possibility that pure amidoamine may have a sensitizing role in subjects with positive reactions to CAPB. To this end, in 10 patients with contact allergy to a commercial CAPB, we tested DMAPA 1% aq. and a pure amidoamine in concentrations ranging from 0.5% aq. to 0.1% aq. The study showed that all patients with positive reactions to DMAPA reacted to amidoamine at 0.5% and 0.25% aq., while 4 of the 10 also had positive reactions to amidoamine at 0.1% aq. We consider that simultaneous allergic reaction to DMAPA and amidoamine represents cross-reactivity and hypothesize that DMAPA is in fact the true sensitizing substance, while amidoamine, which may in any case release DMAPA *in vivo* as a result of enzymatic hydrolysis, may favour the transepidermal penetration of the sensitizing agent. In addition, we advise that testing of CAPB be suspended, because, as suggested by chemico-structural analyses and demonstrated *in vivo*, when thoroughly purified, it no longer has a sensitizing action.

Key words: 3-dimethylaminopropylamine; amidoamine; cocamidopropylbetaine; contact allergy; cosmetics; structure–activity relationships; surfactants; tensioactives. © Blackwell Munksgaard, 2003.

Accepted for publication 28 February 2003

Shampoo, bath gel, liquid soap and intimate hygiene products have been considered a rare cause of contact dermatitis because they are rinse-off products. The allergens they contain are not, in normal conditions of use, able to induce sensitization, and in any case, they are well tolerated even by patients already sensitized to such substances, as has been observed for methylchloroisothiazolinone + methylisothiazolinone (1) and methyldibromoglutaronitrile (2). One exception to this rule seems to be allergy to certain commercial surfactants, such as cocamidopropylbetaine (CAPB) (3, 4), amphoacetates (5) and sodium lauryl ether sulfate (6, 7), which can cause allergic reactions to rinse-off products and occupational contact dermatitis in hairdressers (3, 8).

The present study is focused on contact allergy to CAPB. This is an amphoteric (betainic) surfactant that has enjoyed increasing popularity in the cosmetic industry, because of its low potential for irritating the skin, together with its thickening power. Cocamidopropylbetaine is prepared by reacting fatty acids (obtained from coconut oil) or coconut oil itself with 3-dimethylaminopropylamine (DMAPA), yielding cocamidopropyl dimethylamine, which is subsequently allowed to react with sodium monochloroacetate to give the end-product CAPB. Depending on the quality of the commercial material, CAPB may contain varying amounts of the reactants and intermediates involved in its synthesis. Major known impurities, together with sodium chloride, monochloroacetic and glycolic acids, are unreacted DMAPA (in very low quantities) and amidoamine.

In preliminary studies, we tested 30 patients with clearly non-irritant allergic reactions to CAPB 1% aq. (FIRMA, Florence, Italy) with the chemicals used in its synthesis and with a sample of CAPB declared by the supplier (Tego Italiana, Milan, Italy) to possess greater purity (4). All patients reacted to DMAPA 1% aq., whereas only 16 (53%) reacted to the CAPB of purer grade. We suggested that DMAPA might be an important allergen in CAPB contact allergy. In later studies, we also showed that purified CAPB did not elicit allergic reactions, supporting the hypothesis that allergy to CAPB could be imputed to impurities present rather than to the tensioactive molecule itself (9).

Recent studies have hypothesized that allergy to CAPB could be due to amidoamine rather than to DMAPA. In 1997, Fowler et al. (10) studied 9 patients with contact allergy to CAPB and tested the possible contaminants: DMAPA (0.1%), amidoamine (0.1% aq.) and monochloroacetic acid (0.1% aq.). No patient reacted to DMAPA or monochloroacetic acid, while 6 patients were positive to amidoamine. McFadden et al. (11) tested 6 patients with allergy to CAPB with varying concentrations of DMAPA ranging from 10 000 p.p.m. to 10 p.p.m., and only when DMAPA was conveyed in sodium lauryl sulfate 0.2%, did they obtain positive reactions, in 2 subjects at a concentration of 10 000 p.p.m. and in 1 at concentrations up to 100 p.p.m.; in 3 of the 6 patients, patch tests with amidoamine 0.1% aq. and 0.01% aq. yielded positive reactions only at the highest concentration. In both studies, the authors' conclusions underlined the principal role played by amidoamine in comparison with DMAPA in CAPB contact allergy. However, it should be borne in mind that the authors used non-purified amidoamine containing non-quantified traces of DMAPA (10), so this conclusion may be challenged. The aim of the present study was to study the role of fully purified amidoamine in CAPB contact allergy.

Patients and Methods

Patch testing

10 patients with contact allergy to CAPB 1% aq. (FIRMA) were selected for this study. All were tested with CAPB 1% aq. (Chemotechnique Diagnostics, Malmö, Sweden), DMAPA at 1% aq. (Chemotechnique Diagnostics) and purified (see below) amidoamine at concentrations of 0.5%, 0.25% and 0.1% aq. The patch tests were performed using Al-Test[®] (Imeco AB, Södertälje, Sweden) products as support, attached with Scanpor[®] tape (Norgesplaster, Venne-sla, Norway). Readings were taken on days 2, 4 and 7.

Amidoamine purification

The amidoamine solution (trade name Tego-Amid[®] D-5040, Tego Italiana) was solubilized in 1% (w/v) ethanol. In order to obtain pure amidoamine (free from DMAPA), we carried out purification of the commercial product by thin-layer chromatography (TLC). Preparatory TLC of the amidoamine was carried out on silica gel 60A Plates 20 cm × 20 cm × 0.5 mm thickness (Merck, Darmstadt, Germany) in solvent containing chloroform/methanol/90% acetic acid (65:4:35, v/v). TLC plates were washed 2× with chloroform/methanol (1:1, v/v) and activated at 120°C before use. Lipids were detected with a specific spray reagent for amides (12) and by exposure to iodine vapour; only the lipids corresponding to the broad blue band stained by the specific reagent for the amide group were recovered from the silica gel. Briefly, the silica containing the amidoamine was scraped from the plate and lipids were then extracted with chloroform/methanol/water (1:2:0.8, v/v) (3×). After centrifugation, the supernatants were combined and by addition of the appropriate amount of chloroform/water (1:1, v/v), 2 phases were obtained. The chloroform layer was collected, diluted with benzene and kept under a stream of nitrogen until dry. The final material was dissolved in water at a concentration of 0.5% and stored at 0°C until use.

Determination of DMAPA in purified amidoamine

The residual concentration of DMAPA in purified amidoamine was monitored by liquid chromatography-mass spectrometry, according to a published derivatization procedure (13) in which methanol was used as the reagent solvent instead of acetonitrile. The LC system was a 1050-Ti pump (Agilent Technologies, Palo Alto, CA, USA) equipped with a ChromolithTM 100 × 4 mm column (Merck). The MS system was an API 165 single quadrupole mass spectrometer (Applied Biosystems/MSD Sciex, Foster City, CA, USA) equipped with a turboionspray interface. Derivatized samples, injected by a Gilson 234 auto-sampler (Gilson, Middleton, WI, USA) equipped with a 9010 Rheodyne valve and a 40-μl loop, were eluted at 1 ml/min according to the following elution program: from methanol/water/ammonium acetate 50 mM in methanol, 40:55:5 (held for 3 min) to 95:0:5 in 7 min (held for 3 min). The flow from the LC system was split to allow 200 μl/min to enter the turboionspray interface, whose experimental conditions (positive ions) were as follows: nebulizer gas (air) = 1.5 l/min; curtain gas (nitrogen) = 1.4 l/min; turboionspray gas

(nitrogen at 300°C) = 61/min; needle voltage = + 3000 V; orifice voltage = + 15 V; and ring voltage = + 50 V. In order to measure the concentration of DMAPA standard purchased from Fluka (Buchs, Switzerland), the MS quadrupole was set to monitor the $[M + H]^+$ ion at 207.3 Da (dwelling time = 0.5 s), and a calibration plot, that turned out to be linear, was built using at least 4 DMAPA standard solutions of different concentrations. The DMAPA detection limit was found to be 0.5 p.p.m.

Results

The patch test results are summarized in Table 1. All subjects sensitized to CAPB from FIRMA had positive reactions to DMAPA and purified amidoamine at 0.5% and 0.25% aq. Only 4 of them also had positive reactions to amidoamine 0.1% aq. Moreover, no patient had positive reactions to the CAPB from Chemotechnique Diagnostics, which was probably of greater purity. Control patch tests with purified amidoamine at 0.5% aq. performed in 20 healthy subjects gave negative results.

Discussion

Contact allergy to impurities in detergents is a growing problem that has given rise to considerable debate. In preliminary studies, in patients allergic to CAPB, we tested the reagents and intermediate products used in its synthesis. In view of the results we obtained, we suggested that DMAPA might have an important role in contact allergy to CAPB. Non-purified amidoamine, instead, tested at a concentration of 0.05% aq. (because higher concentrations provoked clearly irritant reactions) gave negative results, as did monochloroacetic acid (4). Since then, we have consecutively tested 13 642 subjects with

eczematous dermatitis of various types and found that allergy to DMAPA is a frequent complaint, with a constant frequency over the years, that affected 3.4% of the population studied. In over 70% of this population, the positive results of the patch tests were relevant. In particular, patients reported intolerance to detergents and presented with eczematous lesions of the face (especially eyelids), with frequent involvement of the axillae and genitals (data not shown).

Recent reports of allergy to amidoamine (10, 11) caused us to reconsider its role in contact allergy to CAPB. For this purpose, we used a pure amidoamine, and our results led us to conclude that allergic reactions to CAPB are to be attributed to both DMAPA and amidoamine, present in varying quantities in commercial CAPB.

A simultaneous positive reaction to DMAPA and amidoamine could be due to cross-reactivity. In fact, at the skin level, amidoamine (an amphiphilic substance with an affinity for keratin) undergoes enzymatic hydrolysis of the amide bond, releasing DMAPA (Fig. 1). The latter may be the true sensitizing molecule, bearing in mind the following considerations on the chemical and structural nature of the 2 substances.

As regards the structure-activity relationship, amidoamine seems to have a low biological skin sensitization power: in fact, the amide group alone does not possess sensitizing characteristics and is unlikely to be an enhancer for the associated amino group (4, 14). On the contrary, it should reduce by mutual attraction the electron donor ability of the neighbouring amino group, being slightly acid in character. Moreover, in previous works, we have demonstrated that the primary amino group, when alone in primary amines with the same alkyl chain as DMAPA, such as in *n*-propylamine (never yet reported as a sensitizing agent), does not have sensitizing effects in patients allergic to DMAPA (15).

Table 1. Sensitization to DMAPA and amidoamine in patients with contact allergy to cocamidopropylbetaine

Patient no.	Cocamidopropylbetaine (1% aq.*)	Cocamidopropylbetaine (1% aq.†)	3-Dimethylaminopropylamine (1% aq.)	Pure amidoamine (0.5% aq.)	Pure amidoamine (0.25% aq.)	Pure amidoamine (0.1% aq.)
1	++	—	++	++	++	++
2	++	—	++	++	++	—
3	++	—	++	++	++	—
4	++	—	++	++	++	—
5	++	—	++	++	+	—
6	++	—	++	++	++	++
7	++	—	++	++	++	++
8	++	—	++	++	+	—
9	++	—	++	++	+	—
10	++	—	++	++	++	++

*FIRMA, Florence, Italy.

†Chemotechnique Diagnostics, Malmö, Sweden.

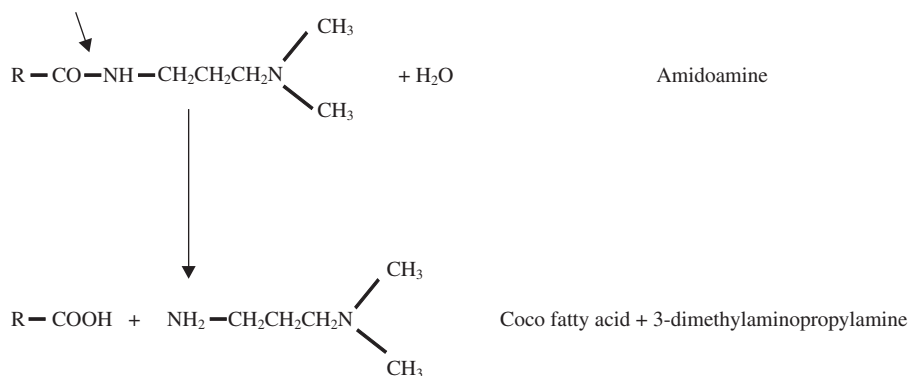


Fig. 1. Enzymatic hydrolysis of amidoamine and release of 3-dimethylaminopropylamine.

3-Dimethylaminopropylamine is an aliphatic diamine that contains 1 primary and 1 tertiary amine group with distinct reactivity, which makes it of interest in many chemical synthesis processes for raw materials used in the cosmetic field. 3-Dimethylaminopropylamine is reported as a strong sensitizer in the guinea-pig maximization test (16), a finding later confirmed by studies using the local lymph node assay (17). The simultaneous presence of 2 amino groups, separated by 2 or 3 carbon atoms (3 in the case of DMAPA), is often observed in molecules with sensitizing potential. The electronic configuration of such amines, in fact, is related to a preferential spatial arrangement of the electron pair on the 2 nitrogens and then of the whole molecule (Fig. 2). They tend to stay apart from one another due to electronic repulsion of their orbitals and will tend to establish hydrogen bonds with hydrogens and methyl groups of the other amino group. The molecule is electronically and structurally asymmetrical, and such asymmetry is a common feature of potentially sensitizing substances. 1 of the 2 amino groups of DMAPA is a dimethyl substitute and therefore more reactive than the simple

amine group towards electrophilic groups. Finally, the ability of DMAPA to elicit allergic reactions is generally proportionally increased by the surfactants it is dissolved in (carrier effect), according to the level of skin aggressiveness of the surfactants themselves (18).

Taken together, it is likely that the impurities present in betaine tensioactives may have a reciprocally enhancing effect on the ability of each to cause contact allergy. As it is an amphiphilic molecule, amidoamine is easily absorbed by the skin and probably penetrates to a deep level; besides, it acts as an alkaline surfactant, has irritant potential and may thus favour the transepidermal passage of DMAPA. Enzymes with hydrolytic activity present in the epidermis could break the amide bond, thus releasing DMAPA and fatty acids. The hydrolytic action occurs at the amide group and is fostered by the effect of the tertiary amine group that can approach the amide group without distorting the molecule bonds, as it is 3 carbon atoms away.

In conclusion, using ultrapurified reagents, we have verified that both DMAPA and amidoamine are sensitizing agents. Amidoamine may trigger a cross-reaction to DMAPA via enzymatic hydrolysis. In any case, bearing in mind the points made above, we do not think it is useful to continue testing CAPB, which when purified possesses no sensitizing potential, as borne out by simple structural analysis (19) and verified in studies *in vivo* (9). We therefore suggest that testing be focused on CAPB impurities, in other words DMAPA and amidoamine, preferably in the presence of skin-penetration enhancers. There are a number of reasons for our suggestion that DMAPA and (well purified) amidoamine be tested in the future. Besides their use in alkyl-amidobetaine production, DMAPA and amidoamine are frequent intermediates in the production of many other cosmetic raw materials. There are many different producers of cosmetic raw

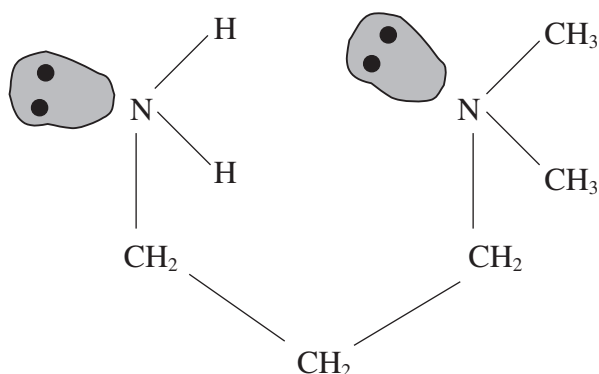


Fig. 2. Preferential arrangement of 3-dimethylaminopropylamine.

materials in the world, who use several different types of purification technology. Even if producers now check DMAPA and amidoamine levels in all such materials more carefully, traces (at least p.p.m.) of the same substances are technically unavoidable, and moreover, no official limits as to their content in commercial products have existed till now. In the light of these considerations, the frequency of exposure to DMAPA and amidoamine is likely to remain high in the future.

References

1. Frosch P J, Lahti A, Hannuksela M, Andersen K E, Wilkinson J D, Shaw S, Lachapelle J M. Chloromethylisothiazolone/methylisothiazolone (CMI/MI) use test with a shampoo on patch-test-positive subjects. Results of a multicentre double-blind crossover trial. *Contact Dermatitis* 1995; 32: 210–217.
2. Tosti A, Vincenzi C, Smith K A. Provocative use testing of methylidibromoglutaronitrile in a cosmetic shampoo. *Contact Dermatitis* 2000; 42: 64–67.
3. de Groot A C, van der Walle H B, Weyland J W. Contact allergy to cocamidopropyl betaine. *Contact Dermatitis* 1995; 33: 419–422.
4. Angelini G, Foti C, Rigano L, Vena G A. 3-Dimethylaminopropylamine: a key substance in contact allergy to cocamidopropylbetaine? *Contact Dermatitis* 1995; 32: 96–99.
5. Foti C, Bonamonte D, Mascolo G, Tiravanti G, Rigano L, Angelini G. Aminoethylethanolamine: a new allergen in cosmetics? *Contact Dermatitis* 2001; 45: 129–133.
6. Connor D S, Ritz H L, Ampulski R S. Identification of certain sultones as the sensitizers in an alkyl ethoxy sulfate. *Fette-Seifen-Anstrichmittel* 1975; 77: 25–29.
7. Lindup W E, Nowell P T. Role of sultone contaminants in an outbreak of allergic contact dermatitis caused by alkylethoxy-sulphates: a review. *Food Chem Toxicol* 1978; 16: 59–62.
8. Frosch P J. On behalf of the Deutsche Kontaktallergie-gruppe-Aktuelle Kontaktallergene. *Hautartz* 1990; 41 (Suppl. 16): 129.
9. Angelini G, Rigano L, Foti C, Rossi P, Vena G A. Pure cocamidopropylbetaine is not the allergen in patients with positive reactions to commercial cocamidopropylbetaine. *Contact Dermatitis* 1996; 35: 352–353.
10. Fowler J F, Fowler L M, Hunter J E. Allergy to cocamidopropyl betaine may be due to amidoamine: a patch test and product use test study. *Contact Dermatitis* 1997; 37: 276–281.
11. McFadden J P, Ross J S, White I R, Basketter D A. Clinical allergy to cocamidopropyl betaine: reactivity to cocamidopropylamine and lack of reactivity to 3-dimethylaminopropylamine. *Contact Dermatitis* 2001; 45: 72–74.
12. Bischel M C, Austin J H. Modified benzidine method for the chromatographic detection of sphingolipid and acid polysaccharides. *Biochim Biophys Acta* 1963; 70: 598–600.
13. Prieto-Blanco M C, Lopez-Mahia P, Prada Rodriguez D. Determination of diethylaminopropylamine in alkylaminoamides by high-performance liquid chromatography. *J Chromatogr Sci* 1997; 35: 265–269.
14. Angelini G, Rigano L. The allergen cocamidopropyl betaine. *Contact Dermatitis* 1998; 39: 210–211.
15. Angelini G, Rigano L, Foti C, Vena G A, Grandolfo M. Contact allergy to impurities in surfactants: amount, chemical structure and carrier effect in reaction to 3-dimethylaminopropylamine. *Contact Dermatitis* 1996; 34: 248–252.
16. Guinea pig maximization test of *N,N*-dimethyl-1,3-propanediamine. Air Products and Chem, data on file.
17. Basketter D A, Lea L J, Cooper K, Stocks J, Dickens A, Pate J, Dearman R J, Kimber I. Threshold for classification as a skin sensitizer in the local lymph node assay: a statistical evaluation. *Food Chem Toxicol* 1999; 37: 1167–1174.
18. Angelini G, Rigano L, Foti C, Grandolfo M, Grüning B. Carrier and inhibitory effects of surfactants on allergic contact reaction to 3-dimethylaminopropylamine. *Contact Dermatitis* 1998; 39: 152–153.
19. de Silva O, Basketter D A, Barrat M D et al. Alternative methods for skin sensitisation testing. *The report and recommendations of ECVAM Workshop 19*.

Address:

Prof. Caterina Foti
 Clinica Dermatologica
 Policlinico
 Piazza Giulio Cesare, 1
 70124 Bari, Italy
 e-mail: c.foti@dermatologia.uniba.it