Acta Derm Venereol (Stockh) 1992; 72: 264–265

Skin Sensitization to Cinnamic Alcohol: The Role of Skin Metabolism

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Cinnamic alcohol and cinnamic aldehyde are a cause of allergic contact dermatitis in man and give rise to similar rates of positive reactions in routine patch testing. However, data from animal models indicates that the aldehyde is the stronger sensitizer of the two. Circumstantial evidence has pointed to the conversion of alcohol to aldehyde in skin as the cause of cinnamic alcohol sensitization. This report discusses the subject in the light of studies of skin metabolism of cinnamic alcohol. Evidence of limited cross reactivity between cinnamic alcohol and cinnamic aldehyde is supported by data showing conversion of cinnamic alcohol to cinnamic aldehyde by an epidermal enzyme.

(Accepted December 16, 1991.)

Acta Derm Venereol (Stockh) 1992; 72: 264–265.

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Whilst cinnamic aldehyde is recognised as having a strong sensitization potential in the guinea pig which can be expressed in man (reviewed in 1, 2), the alcohol is only a weak sensitizer in the guinea pig model (3, 4). However, in man cinnamic alcohol is almost as frequent a cause of allergic contact dermatitis as is the aldehyde (5, 6), or may even be a more frequent contact allergen (7, 8). These trends must be seen in the light of the industry guidelines which restrict the use of cinnamic alcohol to a maximum of 4% but do not impose any restriction on the use level of cinnamic aldehyde (9). However, these guidelines are not the only determinant of total human exposure. It is possible that cinnamic alcohol (via oxidation) and cinnamic aldehyde give rise to the same allergen in vivo, perhaps via the combination of reactive aldehyde species with skin protein. Data consistent with this hypothesis has recently been presented (4).

In this report, the possibility of oxidation of cinnamic alcohol in skin has been investigated by monitoring alcohol dehydrogenase activity in epidermal homogenate supernatant. Sensitization data which examines cross reactions between cinnamic alcohol and cinnamic aldehyde is also reported.

MATERIALS AND METHODS

Materials

Trans-cinnamic alcohol (tCAlc) and trans-cinnamic aldehyde (both the normal forms of these chemicals) were obtained from Quest International, Ashford, Kent and were stored at 4°C under nitrogen. Cis-cinnamic alcohol (>97% by gas liquid chromatography) was a kind gift from Dr David Roberts, Unilever Research Limited, Port Sunlight. Standard ethanol and alcohol dehydrogenase enzyme kit plus nicotinamide adenine dinucleotide (NAD) were obtained from Sigma, UK.

Guinea pig sensitization test method

All the sensitization tests were conducted using the guinea pig maximization test (GPMT) (10). Briefly, preliminary irritation tests were carried out in groups of four albino Dunkin–Hartley strain guinea pigs to determine concentrations of test substance suitable for induction of sensitization and for challenge. Ten guinea pigs were then treated in the shoulder region with a series of six intradermal injections of test material at slightly irritant concentration in combination with Frend's complete adjuvant to induce sensitization; 6–8 days later sensitization was boosted by an occluded 48-hour patch of test material at a mildly irritating concentration placed over the injection sites. Twelve to fourteen days later the animals were challenged on one clipped and razored flank by an occluded 24-hour patch containing test material at the maximum non-irritant concentration. A group of 4 animals treated as above but without the test material served as controls. Reactions were scored 24 and 48 h after patch removal for edema and erythema on a scale of 0–3.

Preparation of epidermal extract

Sheets of clipped and shaved skin from Dunkin–Hartley guinea pigs weighing 500–600 g were frozen by placing the dermal aspect onto a block of dry ice. The epidermis was scraped off with scalpel. The extent and effectiveness of this process was confirmed by histology (data not shown). Immediately after, 0.5 g frozen epidermal tissue was added to 1 ml of buffer (0.85% NaCl containing 20 mM hydroxyethyl piperazine ethane sulphonate (HEPES), pH 7.2 + 2 mM phenylmethylsulphonyl fluoride) and homogenized on ice in a Duall glass homogenizer. The supernatant was recovered by centrifugation of the homogenate at 11,600 g for 30 s. The resulting epidermal extract was stored on ice and used within 2 h of preparation.

Measurement of alcohol dehydrogenase activity

The NAD-ADH kit with an ethanol standard was used to set up the method and to confirm that trans-cinnamic alcohol could be metabolized by ADH. Subsequently the NAD-glycine buffer from the kit was employed with trans-cinnamic alcohol (1% in dimethyl sulphoxide) with the epidermal extract replacing alcohol dehydrogenase. 20 µl of 1% trans-cinnamic alcohol or DMSO and 50 µl of epidermal extract were used with the final volume made up to 3.0 ml with NAD-glycine buffer. The increasing absorbance at 340 nM caused by the reduction af NAD to NADH was then monitored in a Perkin–Elmer spectrophotometer, with the sample warmed to 33°C to mimic epidermal conditions.

RESULTS

Sensitization tests of cinnamic alcohol and aldehyde

Table I contains the GPMT results for cinnamic aldehyde and both trans-cinnamic alcohol and cis-cinnamic alcohol. Study of this last mentioned chemical was based on alcohol dehydrogenase enymes in skin being specific for the trans-isomer (11). Thus cis-cinnamic alcohol should not be metabolized in skin to the aldehyde. Cinnamic aldehyde was a strong sensitizer, whilst trans-cinnamic alcohol produced weak responses in 20% of the test guinea pigs. Cis-cinnamic alcohol exhibited only marginal reactivity. Both cis- and trans-cinnamic alcohol produced some cross challenge reactions in guinea pigs sensitized to cinnamic aldehyde, but these were lower with the cis-isomer. In contrast, cinnamic aldehyde failed to elicit any reactions when cross challenged onto guinea pigs sensitized to either cis- or trans-cinnamic alcohol.

Epidermal metabolism of cinnamic alcohol

The results of studies with commercial alcohol dehydrogenase confirmed that this enzyme could rapidly metabolize trans-

Table I. *Guinea pig sensitization test results*

Test substance	Test concn. (%)		Challenge substance (concn. %)		
	II[1]	IP[1]	CAld[2] (0.75%)	tCAlc[3] (40%)	cCAlc[4] (40%)
Cinnamic aldehyde	0.2	2.5	9/10 (2.0)[5]	4/10 (1.1)	3/10 (1.0)
Cinnamic aldehyde	0.2	2.5	10/10 (2.2)	5/10 (1.0)	2/10 (0.8)
t-Cinnamic alcohol	0.25	100	0/10 (0)	?2/10 (0.4)	Not done
c-Cinnamic alcohol	0.25	100	0/10 (0)	Not done	1/10 (0.8)

1. II = induction injection; IP = induction patch; 2. CAld = cinnamic aldehyde; 3. tCalc = trans-cinnamic alcohol; 4. cCalc = cis-cinnamic alcohol; 5. Results expressed as number positive/test group (mean erythema on positive responders, scale 0–3) A ? indicates an equivocal result.

cinnamic alcohol to cinnamic aldehyde (data not shown). Table II contains the results where the epidermal extract was used in place of the commercial enzyme. The data shows that after 15 min at 33°C a small conversion of alcohol to aldehyde occurred. The level of cinnamic aldehyde produced was calculated according to the method detailed by Sigma (Procedure No. 332-UV). A concentration of 0.036% (in the test cuvette) was achieved.

DISCUSSION

Slight evidence of cross reactivity between trans-cinnamic alcohol and cinnamic aldehyde has been reported in the guinea pig (3). Weibel et al. (4) reported a high degree of cross reactivity, but their cinnamic alcohol contained 1.2% cinnamic aldehyde, and this is likely to have affected their results. In the studies reported here using pure materials, only limited evidence of cross reactivity was found. In addition, cis-cinnamic alcohol which cannot be metabolized to the aldehyde by alcohol dehydrogenase (11) showed a similar degree of slight cross reactivity. Thus, the data from guinea pig studies is not conclusive. Human data from subjects with an allergic sensitivity to either compound cannot really help as there is no way with such commonly used fragrance chemicals of establishing whether the picture is one of cross reactivity or multiple concomitant sensitization.

Studies by Weibel & Hansen (12) showed that whilst cinnamic aldehyde could be converted to the alcohol by human skin, the alcohol was *not* converted to the aldehyde. This data contrasts with the results presented in this report, which show a small, but potentially significant conversion of trans-cinnamic alcohol to cinnamic aldehyde. Further circumstantial support for cinnamic aldehyde as the in vivo allergen comes from the identification of alcohol dehydrogenase in both guinea pig (13) and human skin (14).

In conclusion, there is evidence that epidermal enzymes can, at least to a small extent, metabolize trans-cinnamic alcohol to cinnamic aldehyde, and this is in accord with the weak sensitization potential of cinnamic alcohol and the limited extent of cross challenge seen with cinnamic aldehyde. Human sensitization to cinnamic alcohol and cinnamic aldehyde may occur to a similar degree because of a higher degree of exposure to the more weakly sensitizing alcohol. However, it is also possible to speculate that human epidermis has a higher capacity to metabolize trans-cinnamic alcohol to cinnamic aldehyde and it is this which renders it an effective sensitizer in man.

Table II. *Conversion of cinnamic alcohol to cinnamic aldehyde by epidermal extract*

	OD 340 nm (15 minutes)	Calculated % = cinnamic aldehyde
Epidermal extract	0.024	n/a
DMSO	0.000	n/a
DMSO + epidermal extract	0.020	n/a
tCAlc	0.000	0.000
tCAlc + epidermal extract	0.110	0.036

n/a = not applicable.

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