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STUDIES ON THE CUTANEOUS SENSITIZATION REACTION OF GUINEA PIGS TO PURIFIED AROMATIC CHEMICALS

Masanori Senma,[1] Noboru Fujiwara,[1] Soichiro Sasaki,[1]
Mosei Toyama,[2] Keiichi Sakaguchi[2] and Ikuo Takaoka[3]

[1]*Department of Dermatology, Hyogo College of Medicine, Nishinomiya, Hyogo,*
[2]*Shiono Koryo Kaisha, Ltd. and* [3]*Momotani Juntenkan, Ltd., Osaka, Japan*

Abstract. The sensitization reactions to purified cinnamic aldehyde and its chemically related substances were investigated using the maximization test on guinea pigs. Mutual cross-sensitivities were also examined. Cinnamic aldehyde was found to be the most potent sensitizer in this series, and a strong cross-sensitizer. From the chemical structures of these substances, the following speculations were made. There was a tendency that as the number of hydrocarbons of alkyl groups replacing the α-hydrogen in the molecule increased, the rate of sensitization reaction declined. The conjugated system of double bonds was suspected to be a determinant factor in relation to the sensitization ability of the substances in this series.

Key words: Sensitization; Maximization test; Cinnamic aldehyde and related substances; Molecular structure; Chemical bond

It is well known that cinnamic aldehyde is a chemical substance used as flavouring material and perfume compound. As it has often been suspected to be a sensitizer causing allergic contact dermatitis (1, 2, 3, 11), much attention has been paid to this chemical (9, 10, 11).

The present study deals with the maximization test (8) in order to establish, in terms of molecular structure and hypersensitivity, the relationship between cinnamic aldehyde and related substances, all chemically purified.

MATERIALS AND METHODS

Cinnamic aldehyde (CALD) and the chemically related substances α-methyl-cinnamic aldehyde (AMCALD), α-amyl-cinnamic aldehyde (AACALD), β-methyl-cinnamic aldehyde (BMCALD), dihydrocinnamic aldehyde (DHCALD), cinnamic alcohol (CALC) and styrene (STYRN) were used in the present investigation. The chemical structure of each substance is illustrated in Fig. 1. All these chemicals were estimated for purity by gas chromatography before use.

Ten male albino guinea pigs, weighing 350–400 g, were used for each group of the test substances. The maximization test was used according to Magnusson & Kligman (8).

The concentration of each chemical for induction by both the intradermal injection and topical patch applica-

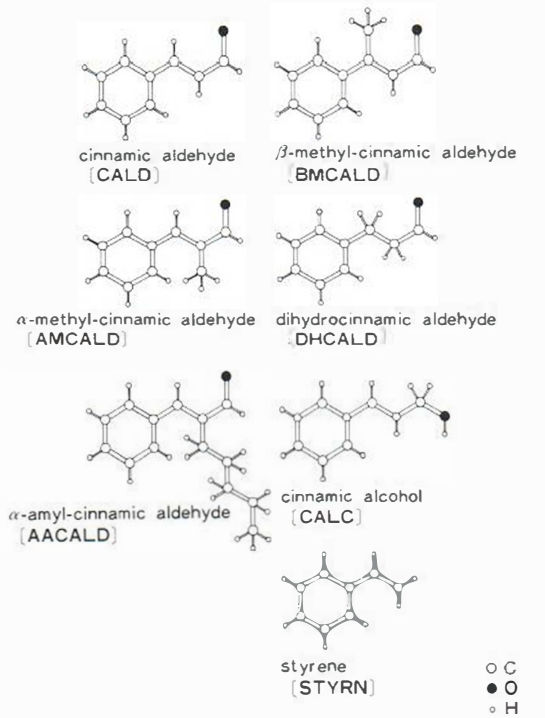

Fig. 1. Molecular structures of cinnamic aldehyde and related substances.

Table 1. *Results of the experiment*

A formula $x*y$ indicates the product of score (x) on the 4-point scale (8) and the number of animals (y); for example, 3*9 and 2*1 mean that the 3-point evaluated reaction was observed in 9 animals, and the 2-point evaluated reaction in 1 animal, respectively. nd=not done

Induction	Challenge							Sensitization rate (%)
	CALC	CALD	AMCALD	AACALD	BMCALD	DHCALD	STYRN	
CALC	0*10	0*2	0*2	0*2	0*2	0*2	nd	0
CALD	1*2	3*9 2*1	1*2	0*2	1*2	0*2	nd	96.7
AMCALD	0*2	2*2	2*3 1*6 0*1	0*2	0*2	0*2	nd	40.0
AACALD	0*2	0*2	0*2	2*1 1*1 0*7	0*2	0*1	nd	11.1
BMCALD	0*2	1*1 0*1	0*2	0*2	2*3 1*5 0*1	0*1	nd	40.7
DHCALD	0*2	0*2	0*2	0*2	0*2	0*10	nd	0
STYRN	nd	0*2	nd	nd	nd	nd	1*3 0*6	11.1

tion was 5% (v/w). To enhance the sensitization, sodium lauryl sulfate (SLS) 10% in petrolatum was massaged into the skin 24 hours before the patch-induction. Two weeks after the topical induction, the animals were challenged by the same test substance at 1% concentration on one flank. The contralateral flank was challenged by a related agent of the series in order to examine the cross-sensitization reaction. The evaluation of the challenged sites was done 24 hours after the removal of the patch, by scoring within the 4-point scale (8).

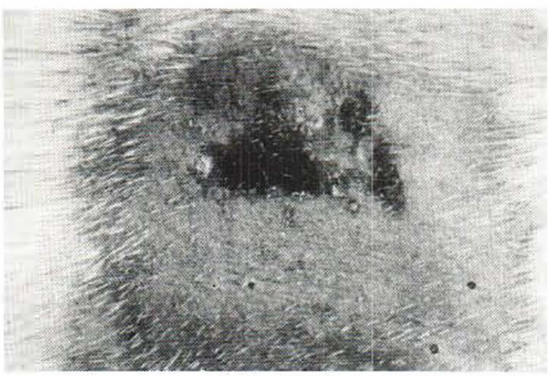

Fig. 2. Reaction observed at the challenged site of the guinea pig: induction; CALD, challenge; CALD.

RESULTS

The results of the sensitization reactions appear in Table 1. The two cross-sensitization reactions between CALD and AMCALD, and between CALD and BMCALD were recorded. Whereas animals induced by CALD reacted to the challenge of CALC, those induced by CALC without sensitization failed to respond to CALD challenge.

It was recognized that CALD was the most potent sensitizer, and a strong cross-sensitizer in this series. Occasionally, as shown in Fig. 2, animals sensitized by this chemical showed intense redness and even erosion in the challenged area. Fig. 3 shows the histological findings from one of the animals sensitized by CALD.

Figs. 4, 5 and 6 demonstrate the sensitization reaction induced by BMCALD and the cross-sensitization reactions between CALD and BMCALD, and between AMCALD and CALD, respectively.

Among the cinnamic aldehyde derivatives, the degree of sensitization was observed with the following sequence: CALD>AMCALD=BMCALD>AACALD.

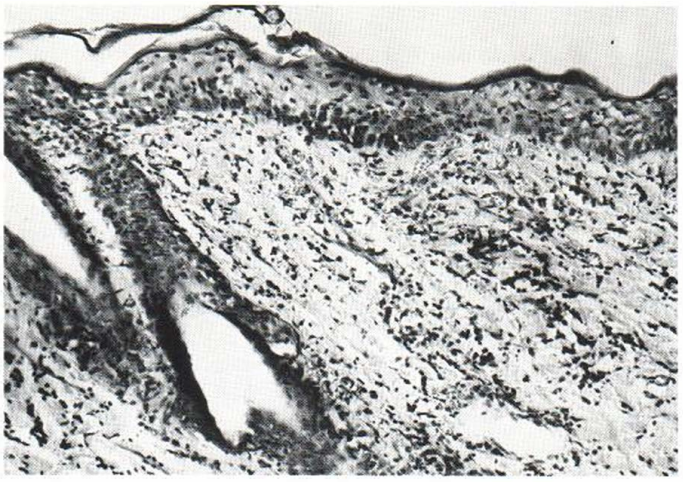

Fig. 3. Dermatopathological changes challenged by CALD in CALD-induced guinea pig (HE, original magnification ×75). Slight intra- and intercellular edema in the epidermis and migration of inflammatory cells through the epidermis and the follicular wall. Marked dilatation of capillaries and moderate perivascular infiltration of small round cells in the dermis.

DISCUSSION

The relationship between molecular structure and hypersensitivity is an interesting topic in the study of allergic reactions. Pioneering work has been performed by Landsteiner and his co-workers (4, 5, 6, 7), who put forward several significant ideas in this field.

Some speculations on the mechanism of cinnamic aldehyde sensitization and quenching effects have been made regarding its molecular structure (9, 10, 11), but conclusive explanation has never been established.

Cinnamic aldehyde (CALD) was considered to have three different kinds of chemically characteristic parts to the molecule: the carbonyl group (aldehyde group), the conjugated double bond, and the benzene nucleus. They are all included in a system of elongated conjugated double bonds.

All substances tested in this series have a benzene ring as common structure. Therefore, it was assumed that the different degrees of sensitization might be attributable to the form of chemical bond attached to the benzene ring.

Dihydrocinnamic aldehyde (DHCALD) and cinnamic alcohol (CALC), both having such functional groups at the terminal of the main chains as a carbonyl group and a hydroxyl group, showed no sensitization reaction, while styrene (STYRN), which had no functional group in the molecule, elicited mild sensitization.

From the results obtained, the conjugated system of double bonds or the participation of π-electrons

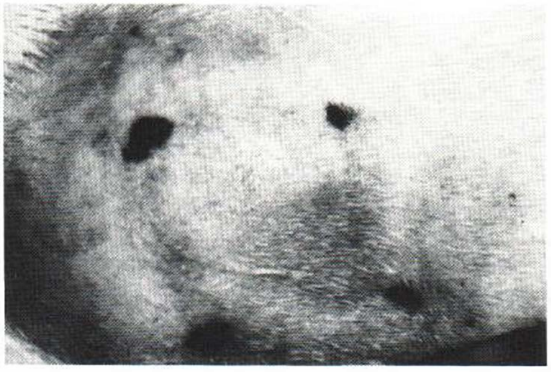

Fig. 4. Reaction observed at the challenged site of the guinea pig: induction; BMCALD, challenge; BMCALD.

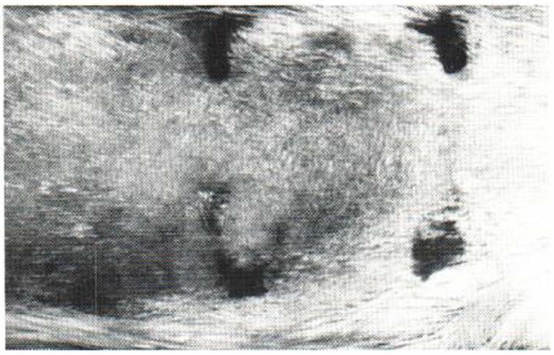

Fig. 5. Cross-reaction observed at the challenged site of the guinea pig: induction; CALD, challenge; BMCALD.

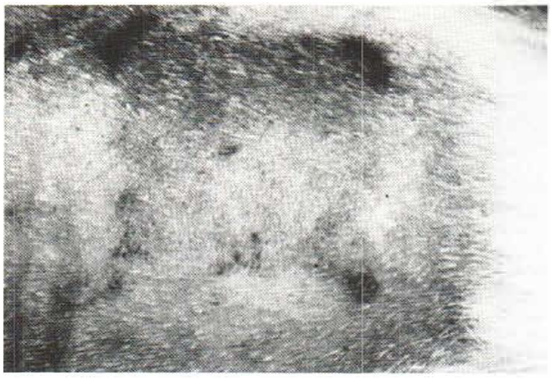

Fig. 6. Cross-reaction observed at the challenged site of the guinea pig: induction; AMCALD, challenge; CALD.

included in this system was considered to be conceivably connected with sensitization.

As to the α-derivatives, the larger was the number of hydrocarbons of alkyl groups replacing the α-hydrogen in the molecule, the more mild was the degree of sensitization reaction.

It is well recognized in the field of physical organic chemistry that the conjugated compounds are so very reactive because they possess delocalized π-electrons. This peculiar fact may be associated with the allergic reactions seen in the present study. The substitute alkyl groups on the α-carbon may play an important role in weakening the response at some stage in the allergic procedure.

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REFERENCES

1. Drake, T. E. & Maibach, H. H.: Allergic contact dermatitis and stomatitis caused by a cinnamic aldehyde-flavored toothpaste. Arch Dermatol *112:* 202, 1976.
2. Fisher, A. A.: Contact Dermatitis. 2nd ed., 372 pp. Lea & Febiger, Philadelphia, 1973.
3. Hjorth, N.: Eczematous allergy to balsam, allied perfumes and flavouring agents. Acta Dermatovener *41:* Suppl. 46, 1961.
4. Landsteiner, K. & Jacobs, J.: Studies on the sensitization of animals with simple chemical compounds. J Exp Med *61:* 643, 1935.
5. — Studies on the sensitization of animals with simple chemical compounds II. J Exp Med *64:* 625, 1936.
6. Landsteiner, K. & Chase, M. W.: Studies on the sensitization of animals with simple chemical compounds. IV. Anaphylaxis induced by picryl chloride and 2 : 4 dinitrochlorobenzene. J Exp Med *66:* 337, 1937.
7. — Studies on the sensitization of animals with simple chemical compounds. IX. Skin sensitization induced by injection of conjugates. J Exp Med *72:* 431, 1940.
8. Magnusson, B. & Kligman, A. M.: The identification of contact allergens by animal assay. The guinea pig maximization test. J Invest Dermatol *52:* 268, 1969.
9. Opdyke, D. L. J.: Inhibition of sensitization reactions induced by certain aldehydes. Fd Cosmet Toxicol *14:* 197, 1976.
10. Saunders, H.: A perfumer views sensitization. Perfumer & Flavorist *1:* 32, 1976.
11. Suskind, R. R. & Majeti, V. A.: Occupational and environmental allergic problems of the skin. J Derm *3:* 3, 1976.

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M. Senma, Ph.D.
Department of Dermatology
Hyogo College of Medicine
1-1 Mukogawa-cho
Nishinomiya
Hyogo
Japan