

Structure-activity relationships in allergic contact dermatitis induced by methacrylates

Studies of the influence of side-chain length of methacrylates

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Some methacrylates are known to be strong contact sensitizers. To determine the effect of side-chain length on sensitizing potential, we examined 11 derivatives with linear side chains from C₁ to C₁₈ in an experimental sensitization test in guinea pigs. The results showed an increase in the sensitizing potential with increasing length of the alkyl side chain from C₁ to C₁₂. The C₁₂ derivative, lauryl methacrylate, showed the strongest sensitizing potential. Further elongation of the alkyl side chain of methacrylates resulted in a decrease in the potential. With respect to the side-chain-length-dependent sensitizing potential, the present results correspond well with the findings obtained with other compounds like primin, catechols, phenols, hydroquinones, and gallates.

Key words: methacrylates; side-chain length; guinea pig maximization test; allergic contact dermatitis; chemical basis. © Munksgaard, 1999.

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Methacrylic resins have come into widespread use in industrial, medical, and cosmetic products such as Plexiglas, dental plates, surgical prostheses, contact lenses, and artificial finger nails, and as coatings for textile fibers, glass, and rubber products. The most commonly used industrial acrylic monomers are the methyl, ethyl, and n-butyl esters of methacrylates (MA). These compounds are readily transformed into polymeric resins by light, heat, and oxygen.

The widespread use of these MA esters and their resins for medical and dental prostheses and cosmetic purposes has sometimes caused allergic contact dermatitis or stomatitis among industrial workers (1), dentists, dental technicians (2, 3), and patients (4), as well as persons who have used these resin products for cosmetic and household purposes (5). One of the most commonly used MAs as an initial material is the methyl methacrylate monomer. Therefore, allergic contact dermatitis that may occur after the use of methacrylic resins seems to be caused by the monomer itself or possibly by unreacted and trapped oligomers that are released from the resin after long-term use.

Thus, the allergic potentials of methyl MA (3–6) and some other MAs such as 2-hydroxyethyl

MA (7) and ethyl MA (8) have been reported in humans. However, allergic reactions due to other MAs remains to be determined. The allergic potentials of primins (9) and catechols (10) in respect of the effect of alkyl side-chain length have previously been reported. However, there are no data concerning the side-chain-length-dependent sensitizing potential of MAs.

The purpose of this study was therefore to determine the effect of side-chain length on the sensitizing potential of 13 MAs.

Materials and Methods

Chemicals

13 MAs were used in this study. The chemical names and abbreviations of the substances used, as well as their suppliers, are shown in Table 1, and their chemical structures in Fig. 1. All chemicals purchased were high purity products so they were used without further purification. The vehicles employed were purchased from the following producers: Wako Pure Chemical Industries, Ltd., Japan (acetone) and Kozakai Seiyaku Co., Ltd., Japan (olive oil). Freund's complete adjuvant (FCA) was purchased from Difco Laboratories, USA.

Table 1. MAs examined in the present study

Chemical	Abbrev.	Supplier ^{a)}	Molecular formula
methyl methacrylate	C ₁ MA	TCI	CH ₂ :C(CH ₃)COOCH ₃
ethyl methacrylate	C ₂ MA	TCI	CH ₂ :C(CH ₃)COOCH ₂ CH ₃
butyl methacrylate	C ₄ MA	TCI	CH ₂ :C(CH ₃)COO(CH ₂) ₃ CH ₃
hexyl methacrylate	C ₆ MA	TCI	CH ₂ :C(CH ₃)COO(CH ₂) ₅ CH ₃
heptyl methacrylate	C ₇ MA	PL	CH ₂ :C(CH ₃)COO(CH ₂) ₆ CH ₃
octyl methacrylate	C ₈ MA	MPL	CH ₂ :C(CH ₃)COO(CH ₂) ₇ CH ₃
nonyl methacrylate	C ₉ MA	DL	CH ₂ :C(CH ₃)COO((CH ₂) ₈ CH ₃)
lauryl methacrylate	C ₁₂ MA	TCI	CH ₂ :C(CH ₃)COO(CH ₂) ₁₁ CH ₃
tridecyl methacrylate	C ₁₃ MA	MR	CH ₂ :C(CH ₃)COO(CH ₂) ₁₂ CH ₃
hexadecyl methacrylate	C ₁₆ MA	PL	CH ₂ :C(CH ₃)COO(CH ₂) ₁₅ CH ₃
stearyl methacrylate	C ₁₈ MA	TCI	CH ₂ :C(CH ₃)COO(CH ₂) ₁₇ CH ₃
cyclohexyl methacrylate	Cy ₆ MA	TCI	CH ₂ :C(CH ₃)COOC ₆ H ₁₁
2-ethylhexyl methacrylate	EHMA	TCI	CH ₂ :C(CH ₃)COO(C ₂ H ₄)(CH ₂) ₅ CH ₃

^{a)}TCI: Tokyo Chemical Industry Co., Ltd. PL: Polysciences, Inc. MPL: Monomer-Polymer Laboratories, Inc. DL: Dajac Laboratories, Inc. MR: Mitsubishi Rayon.

Animals and housing conditions

4-week-old female Hartley guinea pigs (clean) were purchased from Japan SLC Inc. and acclimatized for 1 week. The animals were housed in plastic FRP cages with a metal-mesh floor (260 W×382 D×200 H mm) in a room with the temperature maintained at 23±2°C and the humidity at 50 to 60%. Room air was exchanged approximately 15× per h, and a 12-h light/12-h dark cycle (lighting from 7:00 to 19:00) was used. Animals were housed 2 per cage and fed pelleted feed (RC4, Oriental Yeast Co., Ltd.) together with drinking water (tap water) ad libitum.

Guinea pig maximization test (GPMT)

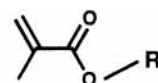
GPMT was performed in accordance with the original procedure of Magnusson & Kligman (11) with some modifications. An appropriate number (5 to 20) of animals were used in each experimental group. Each group of animals was sensitized by injection of given concentrations of the chemicals. For both the intradermal and the topical induction phases, all chemicals used were diluted in the same vehicle and applied at the same concentrations.

The final concentrations for intradermal and topical induction phases are shown in Table 2. We chose appropriate vehicles as follows: olive oil for the induction phase and acetone for the challenge phase, because of their good abilities to solubilize all of the chemicals tested.

21 days after the initial intradermal injection, 0.1 ml aliquots of test chemicals were applied to the shaved area of the flank of each animal for the challenge. The concentrations used in the challenge phase are also shown in Table 2. All concentrations adopted were lower than the irritant concentrations. In all tests, animals were challenged by

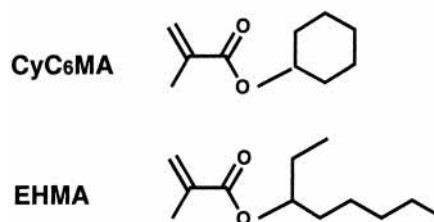
the closed patch test method. The chemicals were held in place for 24 h.

Dermal response (erythema and edema) of each challenge site was evaluated 48 h after the challenge application. The numbers of positive sites for each challenge concentration in the same experimental group were divided by the number of animals of the group to give the sensitization rate (SR). Minimum induction concentration (MIC) was defined as that concentration that could induce a positive response in at least 1 animal in the group tested.



R = -CH₃ to -C₁₈H₃₇

(1) MAs with linear chains



(2) MAs with cyclic or branched side chain

Fig. 1. Chemical structures of MAs. (1) MAs with linear chains. (2) MAs with cyclic or branched side chain.

Table 2. Results of GPMT on MAs

Chemical	Induction	Challenge	N	SR (%)	MIC (M)
	Concentration (M)	Concentration (M)			
methyl methacrylate (C ₁ MA)	1	10 ⁻¹	14	14.3	1
	10 ⁻¹		10	0	
	10 ⁻²		10	0	
ethyl methacrylate (C ₂ MA)	1	1	5	60.0	10 ⁻¹
	10 ⁻¹		5	20.0	
	10 ⁻²		5	0	
butyl methacrylate (C ₄ MA)	10 ⁻¹	10 ⁻¹	5	80.0	10 ⁻¹
	10 ⁻²		5	0	
hexyl methacrylate (C ₆ MA)	10 ⁻¹	10 ⁻¹	15	33.3	10 ⁻¹
	10 ⁻²		9	0	
	10 ⁻³		15	0	
heptyl methacrylate (C ₇ MA)	1	10 ⁻¹	15	46.7	1
	10 ⁻¹		5	0	
octyl methacrylate (C ₈ MA)	10 ⁻¹	1	20	95.0	10 ⁻⁵
	10 ⁻²		5	60.0	
	10 ⁻³		5	60.0	
	10 ⁻⁴		5	80.0	
	10 ⁻⁵		10	20.0	
	10 ⁻⁶		5	0	
nonyl methacrylate (C ₉ MA)	1	10 ⁻¹	5	40.0	1
	10 ⁻¹		5	0	
lauryl methacrylate (C ₁₂ MA)	10 ⁻⁴	10 ⁻¹	5	100.0	10 ⁻⁷
	10 ⁻⁵		5	100.0	
	10 ⁻⁶		10	30.0	
	10 ⁻⁷		10	30.0	
	10 ⁻⁸		5	0	
tridecyl methacrylate (C ₁₃ MA)	10 ⁻³	10 ⁻¹	10	20.0	10 ⁻³
	10 ⁻⁴		5	0	
hexadecyl methacrylate (C ₁₆ MA)	10 ⁻³	10 ⁻¹	10	30.0	10 ⁻⁴
	10 ⁻⁴		5	20.0	
	10 ⁻⁵		5	0	
stearyl methacrylate (C ₁₈ MA)	10 ⁻³	10 ⁻¹	5	20.0	10 ⁻³
	10 ⁻⁴		5	0	
ethylhexyl methacrylate (EHMA)	10 ⁻¹	10 ⁻¹	10	40.0	10 ⁻¹
	10 ⁻²		5	0	
cyclohexyl methacrylate (Cy ₆ MA)	1	10 ⁻¹	5	40.0	10 ⁻¹
	10 ⁻¹		5	20.0	
	10 ⁻²		5	0	

SR: Sensitization rate; MIC: Minimum induction concentration.

Results

The experimental results are summarized in Table 2. The 13 methacrylates were divided into 3 groups: (A) methacrylates having an aliphatic side chain with an even number of carbons, (B) those with a side chain having an odd number of carbons, and (C) others. In groups A and B, all pairs of MIC and carbon numbers of the side chain are plotted in Fig. 2.

As shown in Table 2, each MA was capable of inducing allergic contact dermatitis in the treated animals. C₁, C₂, C₄, C₆, C₇, and C₉ MAs proved to be moderate sensitizers, whereas C₈, C₁₂, C₁₃,

C₁₆, and C₁₈ MAs were found to be much stronger ones. Within the group of methacrylates tested, lauryl (C₁₂) MA was clearly the strongest sensitizer. A characteristic interdependence of sensitizing potential and side-chain length of alkyl MAs was observed in either group A or group B. A difference in sensitizing potential was found between groups A and B. Based on analysis using the χ^2 test, this difference was significant at the 95% confidence level. Alkyl MAs with a linear side chain having an even number of carbons were stronger sensitizers than those with one having an odd number of carbons. An optimum reacting size was determined as a length of 12 carbon atoms. In

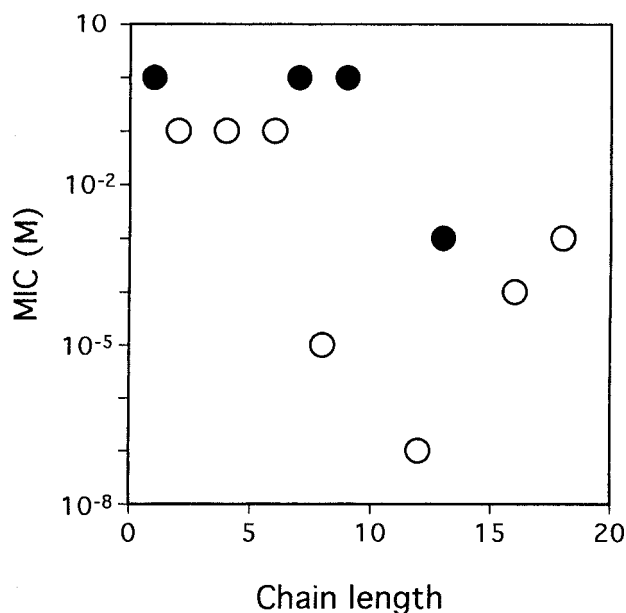


Fig. 2. MICs of the aliphatic methacrylic derivatives with side chains having even (○) and odd (●) numbers of carbons.

group C, ethylhexyl MA with 2 branches of side-chains and cyclohexyl MA with cyclic carbons were weaker sensitizers than those of hexyl and octyl MAs, respectively.

Discussion

This study experimentally examined for the 1st time the sensitizing potential of MAs with the number of carbon atoms increasing from C₁ to C₁₈. Basketter et al. (12) observed an increase in sensitizing capacity with simple bromoalkanes: the maximum was reached at a side chain length of C₁₅/C₁₆, with a decrease in the response by further elongation of the side chain. Roberts et al. (13) showed that alkyl alkanesulfonates are able to sensitize guinea pigs increasingly with an increase in the length of the alkyl chain from C₆ to C₁₂ and that the sensitizing potential decreased from C₁₂ to C₁₆. Baer et al. (10), Kurtz & Dawson (14), and Hausen et al. (9) also found a similar alkyl chain length-related effect of catechols and quinones on allergic contact hypersensitivity.

This study also found a similar correlation between alkyl chain length and sensitization as in the case of bromoalkanes, alkanesulfonates, quinones, phenols and catechols. From these results, we can conclude that an MA with a linear side chain has its maximum sensitizing capacity when the length of the side chain is C₈ to C₁₃.

At present, such MAs having an alkyl chain from C₈ to C₁₃ are not utilized for medical or dental purposes; however, they are being synthesized

and manufactured, and used in various fields. Accordingly, as shown in this study, information about the quantitative structure-activity relationships of various MAs is very important.

In studies on alkanesulfonates, the decline in sensitization response of substances with a side chain longer than C₁₆ was estimated to be more likely due to an overload effect rather than to diminished penetrability (13).

In spite of the increase in their lipophilicity, methacrylic derivatives with a side chain longer than C₁₆ showed a decline in sensitizing potential (data not shown). We assume that higher hydrophobicity might have restricted the penetration of the derivatives from skin.

The reason for the difference in the sensitizing potential between group A and group B is not clear; however, it is possible that the difference might be caused by the nature of the processing of the side chain in antigen-presenting cells or the recognition of the epitope by major histocompatibility antigen complex (MHC) class II molecules. In group C, ethylhexyl MA with 2 branches of side chains and cyclohexyl MA with cyclic carbons were weaker sensitizers rather than hexyl and octyl MAs, respectively. Such differences in the sensitizing potentials of group C might be caused by their lower hydrophobicity than the linear ones.

The relationship between the relative alkylating index (RAI) and the sensitizing potential of various compounds has been investigated in several studies (15–18). However, it is difficult to compare the sensitizing potentials of the compounds with each other, since the RAI value depends on the concentration tested. The sensitizing potentials of MAs and acrylates have been reported by other investigators (16, 19). However, the data presented by us do not coincide with the results reported by other investigators, because of differences in the methods, such as vehicle and/or concentrations employed.

In this study, we performed the GPMT of 13 MAs using the same schedule and obtained MICs of them. The chemical structures, in terms of linear side chain length, showed good correlation with the sensitizing potentials. Based on the present results, we suggest that the evaluation using MICs is highly useful to assess the sensitizing potentials of many kinds of chemicals.

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