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Technical Report

A structure-activity relationship study on the mechanisms of methacrylate-induced toxicity using NMR chemical shift of β-carbon, RP-HPLC log P and semiempirical molecular descriptor

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To clarify the mechanism of methacrylate-induced toxicity, a total of 24 acrylates, methacrylates, and dimethacrylates were chosen for a structure-activity relationship (SAR) study in terms of NMR chemical shifts, semiempirical molecular descriptors, and reverse phase (RP)-HPLC log P. Molecular descriptors as well as bulk, electronic, and energy descriptors were calculated using the PM3/CONFLEX method. A significant multiple linear regression equation for methacrylates in mice was denoted as log $1/LD_{50}$ (which was function $[-(E_{HOMO}+E_{LUMO})/2$, log P]). Besides, significant linear regression equations for methacrylates were denoted as log $1/ED_{50}$ in HeLa S3 and in HGF cells as function [E_{HOMO} and/or log P]. Results showed that the ^{13}C NMR chemical shift of β-carbon for methacrylates was correlated with their E_{HOMO}. Findings of this study thus suggested that it might be possible to predict methacrylate-induced toxicity using physicochemical properties.

Key words: Methacrylates, Structure-activity relationships (SAR), Physicochemical properties

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INTRODUCTION

In dentistry, methacrylate monomers are widely used as materials for denture bases, denture linings, restorative resins, and in bonding agents. In general, monomers do not polymerize completely in the air; therefore, remaining unpolymerized methacrylates in dental materials are an important factor that causes irritation of the oral tissues. Depending on the lipophilicity of the monomers, they predominantly reside in the cell membranes of oral tissues.

On the subject of cytotoxicity/toxicity of monomers which is based on their structure-activity relationships (SARs), it has been investigated by many researchers[1-9]. In particular, Lawrence *et al.* found a significant relationship between Hansch π[10] (the sum of substituent constants) and acute toxicity in methacrylates[1]. In the Hansch model, sigma electron distributions were calculated for the methacrylates as well as the net σ charges on the carbonyl carbon (Q^{σ}) in the regression analyses including the π-term[1].

On the adverse effects of acrylates, it was previously reported that inhaled acrylates caused sulfhydryl depression in the liver, lung, kidney, and blood[11]. In another study, Tanji and Hashimoto reported that acute oral toxicity (50% lethal dose, LD_{50}) in mice for acrylates, but not for methacrylates, was related to their reactivity with glutathione (GSH) in addition to the logarithm of octanol-water partition coefficient, log P[4]. On the 50% cytotoxic concentration (ED_{50}) of various methacrylates for HeLa S3 cells, Yoshii also reported on a correlation between ED_{50} and log P[6].

To clarify the cytotoxicity mechanism of methacrylates, we previously used phosphatidylcholine liposomes as a model for biomembranes to investigate the interaction between liposomes and methacrylates using differential scanning calorimetry (DSC) and nuclear magnetic resonance (NMR) spectroscopy. Our findings suggested that lipid-soluble monomers induced a transition of phospholipid acyl chains in the organized gel state to a randomized liquid crystalline phase, and that the responsible driving force is the hydrophobicity of monomers[7,8]. In addition, when methacrylates were incorporated into the lipid bilayers of phospholipid liposomes — whereby the latter served as a model for biological membranes, the NMR chemical shifts of β-carbon and of the protons attached to the carbon of methacryloyloxy groups shifted to a higher field or underwent a shielding[8,9]. This suggested that the NMR chemical shifts of β-carbon can be used as a SAR parameter to predict biological activities. It is noteworthy that some nucleophiles such as water, hydroxy anion (OH^-), and GSH interact with β-carbon, especially in acrylates. Therefore, the magnitude of β-carbon is a considerable factor to the nucleophilic reactions involving methacrylate monomers — such as copolymerization reaction,

nucleophilic attack, electrostatic binding, and ester hydrolysis — and which result in toxicity. On this premise, the magnitude of NMR chemical shift is correlated with and serves as a useful measure of the reactivity of methacrylate monomers.

Computational chemistry has opened a new field for SAR research in toxicology[11,12]. Many SARs with quantum chemical descriptors have been reported for aquatic species[13], and the SAR of estrogen-like bisphenol A analogs for breast cancer MCF-7 was reported previously using quantum chemical descriptors[14]. Furthermore, using AMPAC, a semiempirical computer program, SARs have been used to accurately predict the mutagenicity of bis-GMA, a monomer commonly used in dentistry[15]. Similarly, conformational and quantum analyses of dental adhesive carboxylic acid and carboxylic acid anhydride monomers have been performed using a semiempirical computer program[16].

On the use of semiempirical quantum mechanical methods, they have been employed to calculate the quantitative relationships between log P — as estimated by reverse-phase high-performance liquid chromatography (RP-HPLC) — and various bulk and electronic properties of methacrylates[17]. Recently, we investigated the SARs of aliphatic and aromatic methacrylates with a focus on their hemolytic activities, and found that the hemolytic activity of aromatic methacrylates was dependent on their electrophilicity[18]. In another study, it was found that chemical hardness played a key role in the cytotoxic activity of methoxyphenolic compounds against human oral tumor cells[19]. However, with regard to the use of computational chemistry for SAR studies of methacrylates[15,17,18], the reports are comparatively scarce.

The aim of the present study, therefore, was to re-investigate the mechanism of methacrylate-induced toxicity based on SAR. This study was carried out in light of recent developments using quantum chemical descriptors such as the highest occupied molecular orbital (HOMO), the lowest unoccupied molecular orbital (LUMO), and electronegativity (χ) as calculated by the semiempirical PM3 method — in addition to log P. Besides, this study also investigated the following: (i) the correlation between log P, as estimated by RP-HPLC, and that based on MOPAC (PM3) program; and (ii) the correlation between log P and various bulk properties (van der Waals area, VDW_{area}) and electronic properties (HOMO energy, E_{HOMO}; total dipole moment, μ) as calculated by the PM3 method. All PM3 calculations were performed with a CONFLEX software, as DFT (Density Functional Theory) calculations are time-consuming[18,19]. Finally, the correlations between ^{13}C NMR chemical shifts of β-carbon and E_{HOMO} or toxicity of the methacrylate monomers were also described in this study.

MATERIALS AND METHODS

Monomers and toxicological data

Monomers used in this study are abbreviated as follows: Methyl acrylate (MAA), ethyl acrylate (EAA), *n*-propyl acrylate (nPAA), *n*-butyl acrylate (nBAA), isobutyl acrylate (iBAA), lauryl acrylate (LAA), 2-hydroxyethyl acrylate (HEAA), 2-hydroxypropyl acrylate (HPAA), methyl methacrylate (MMA), ethyl methacrylate (EMA), *n*-propyl methacrylate (nPMA), *n*-butyl methacrylate (nBMA), isobutyl methacrylate (iBMA), lauryl methacrylate (LMA), 2-hydroxyethyl methacrylate (HEMA), 2-hydroxypropyl methacrylate (HPMA), 4-methacryloyloxyethyl trimellitate anhydride (4-META), benzyl methacrylate (BZMA), neopentyl dimethacrylate (NPGDMA), ethylene dimethacrylate (EDMA), diethyleneglycol dimethacrylate (DEGDMA), triethyleneglycol dimethacrylate (TEGDMA), 2,2-bis[4-(2-hydroxy-3-methacryloyloxypropyloxy)phenyl]propane (bis-GMA), and dimethacryloyloxyethoxy (2,2,4-trimethyl-1,6-hexamethylene diurethane) (UDMA).

To determine the acute oral toxicity of test monomers, male dd-Y mice (body weight: 24−27 g) were used. LD_{50} was assayed using four animals per dose level and with four different doses[4]. ED_{50} values of monomers against HeLa S3 cells were obtained from published literature[6]. To test the cytotoxic activities of bis-GMA, TEGDMA, nBMA, and MMA against human gingival fibroblasts (HGF), they were carried out using MTT assay as previously reported[7-9]. A dose-response curve of relative cell viability was plotted to delineate the concentrations of the monomers that depressed MTT-formazan production by 50% (ED_{50}). The values were expressed as the mean of six cultures[7,8].

NMR chemical shifts

The ^{13}C NMR chemical shifts for various methacrylates in chloroform-d ($CDCl_3$) was obtained from published literature[20]. Those of 4-META, EDMA, TEGDMA, and bis-GMA were determined in $CDCl_3$ using a JEOL Alpha-500 NMR spectrometer[9].

RP-HPLC

RP-HPLC data were obtained from published literature[21]. Briefly, the HPLC system was composed of Water Associates components, including a M-6000 pump and a Model U6 K injection valve. A UV-visible spectrophotometer (JASCO UNIDEC-100) and a differential refractometer (Model R401, Water Associates) were used as detectors. Retention time (t_r) values were measured from the chromatographic peaks by a B-381/H recorder (Rigaku Denki). The column was a 30×0.4 cm Corasil C_{18} reverse phase

column (Water Associates), and the mobile phase was a methanol-water mixture. Other conditions employed for RP-HPLC included a flow rate of 1 mL/min and a room temperature of 24±0.2°C.

Each hydrophobicity index was derived from the capacity factor k', which was calculated as follows:

$$k' = (t_r - t_0)/t_0$$

where t_r and t_0 are the retention times of the monomer and unretained benzenesulfonic acid respectively.

Computational calculation

Molecular geometries and reaction energies of monomers were calculated to derive their respective heat of formation values. Electronegativity, χ, is calculated as follows:

$$\chi = -(E_{LUMO}+E_{HOMO})/2$$

where E_{LUMO} and E_{HOMO} are the frontier orbital energy levels.

All calculations were performed using the PM3/CONFLEX method. This method offered twofold benefits: it required less computational time than the Density Functional Theory (DFT) method as well as provided more reliable computations than the PM3 method alone[18,19]. To obtain fine geometry details in the present study, initial geometry optimization was first performed using CONFLEX5 (Conflex, Tokyo, Japan). Following which, calculations by the PM3 method in the MOPAC 2000 program were carried out on a Tektronix CAChe workstation (Fujitsu Ltd., Japan). Each monomer's log P value was also determined using the MOPAC (PM3) 2000 program.

RESULTS

Correlation between NMR chemical shifts and HOMO

Table 1 shows the chemical shifts ($\delta_{C\beta}$, $\delta_{C\alpha}$) and the chemical shift differences between α- and β-carbon, $|\delta_{C\alpha}-\delta_{C\beta}|$, for the acrylates, methacrylates, and dimethacrylates tested in this study. Table 3(A) then shows the SAR results for the physicochemical properties. As seen in Eq. (1), a linear relationship is

Table 1 ^{13}C NMR chemical shifts of α-carbon ($\delta_{C\alpha}$) and β-carbon ($\delta_{C\beta}$), heat of information, HOMO and LUMO for acrylates and methacrylates.

No.	Monomer	NMR chemical-shift (ppm,in $CDCl_3$)* $\delta_{C\beta}$	$\delta_{C\alpha}$	$\lvert\delta_{C\alpha}-\delta_{C\beta}\rvert$	Heat of formation kcal/mol	E_{HOMO} eV	E_{LUMO} EV
1	MAA	130.56	128.15	2.41	−67.387	−11.066	−0.082
2	EAA	130.24	128.59	1.65	−72.173	−11.040	−0.051
3	nPAA	130.22	128.57	1.65	−77.404	−11.044	−0.055
4	nBAA	130.21	128.61	1.60	−82.791	−11.045	−0.055
5	iBAA	130.23	128.60	1.63	−82.435	−11.042	−0.053
6	LAA	130.24*	—	1.72♭	−126.139	−11.049	−0.061
7	HEAA	127.02*	—	7.64♭	−118.870	−10.717	−0.369
8	HPAA	126.95*	—	6.15♭	−126.224	−10.710	−0.341
9	MMA	125.23	136.15	10.92	−74.768	−10.548	−0.058
10	EMA	124.97	136.51	11.54	−79.542	−10.524	−0.027
11	nPMA	124.95	136.52	11.57	−84.767	−10.529	−0.033
12	nBMA	124.70	136.41	11.71	−90.156	−10.530	−0.034
13	iBMA	124.98	136.52	11.54	−89.832	−10.523	−0.031
14	LMA	125.23*	—	10.92♭	−133.504	−10.533	−0.038
15	HEMA	123.89	135.96	10.07	−127.014	−10.573	−0.107
16	HPMA	126.95*	—	7.76♭	−126.224	−10.710	−0.341
17	BZMA	125.66	136.21	10.55	−49.295	−9.795	−0.034
18	4META	126.13	135.58	9.45#	−230.290	−10.711	−1.625
19	NPGDMA	125.49*	—	10.47♭	−155.120	−10.558	0.223
20	EDMA	125.9	136.1	10.2#	−141.164	−10.570	−0.102
21	DEGDMA	125.57*	—	10.31♭	−182.947	−10.567	−0.300
22	TEGDMA	125.4	136.3	10.9#	−153.155	−10.455	−0.271
23	bis-GMA	126.2	135.9	9.7#	−267.921	−9.015	−0.311
24	UDMA	—	—	—	−348.278	−9.782	0.004

The abbreviation of monomers is shown in the text. * $\delta_{C\beta}$: $H_2C_\beta=C_\alpha$ (H or Me)−R, Ref[20]; *The chemical-shifts was determined in CDCI, by NMR spectroscopy (JEOLJNM-A500) and was converted into the TMS scale. ♭determined using Eq.(1) (see Table 3); #determined using Eq.(2) (see Table 3).

shown between E_{HOMO} and $\delta_{C\beta}$ for the acrylates, methacrylates and, dimethacrylates — except for the aromatic methacrylates, 4-META, BZMA, and bis-GMA. Similarly, a good correlation is seen between and $|\delta_{C\alpha}-\delta_{C\beta}|$ and E_{HOMO} in Eq. (2).

As shown in Table 1, the $\delta_{C\beta}$ values of monomers decreased linearly with increasing E_{HOMO}. The chemical shifts of the carbons in methacrylate monomers depend on the π-electron density. Therefore, it was expected that $\delta_{C\beta}$ and E_{HOMO} (which represents the electron-donating power of the molecule) would exhibit a linear relationship. Indeed, as indicated in Table 1, E_{HOMO} became larger as $\delta_{C\beta}$ moved to a lower magnetic field and $\delta_{C\alpha}$ to a higher magnetic field.

The chemical shifts of β-carbon for acrylates were greater than those for methacrylates. However, the absolute values of shift difference for methacrylates were greater than those for acrylates. Further, as the E_{HOMO} value increased, the absolute value also increased. As seen in Table 1, the E_{HOMO} values of acrylates were lower than the corresponding methacrylates. 4-META, BZMA, and bis-GMA were removed from Eq. (1) because these were outlier compounds — BZMA and bis-GMA possessed greater E_{HOMO} values, whereas 4-META possessed a markedly lower E_{LUMO} value (Table 1).

RP-HPLC log P

The hydrophobicity (log P) of methacrylates is one of the most important factors in the evaluation of their biological activity[10]. In a previous study, we investigated the log P values of methacrylates using RP-HPLC[21,22]. In the present study, results of the previous studies were re-examined using the PM3 method, and the findings thereof are shown in Table 2. Except for bis-GMA, a linear correlation between log P derived from retention time (t_r) and log P based on MOPAC (PM3) program, log P (PM3), was found (n=12, r^2=0.845). With bis-GMA, its log P (PM3) value was greater than that obtained with RP-HPLC in a mobile phase of 85:15 methanol:water. Many investigators have used capacity factors extrapolated to 100% water (log k_w) to eliminate the effects of organic solvents[23]. In general, extrapolation to 100% water is based on a parabolic relationship between

Table 2 Log P, RP-HPLC retention time (t_r), electronegativity (χ), median effective dose (ED_{50}) and median lethal dose (LD_{50}) for acrylates, methacrylates and dimethacrylates.

No.	Monomers	[a]log P	[b]Exptl log P	[c]logt_x min	change log P[b]−log P[a]	χ	[d]ED_{50} mmol/L	[e]Oral LD_{50} mmol/kg
1	MAA	0.62	0.80	0.54	0.18	5.574	2.51	9.60
2	EAA	0.96	1.33	0.56	0.37	5.545	3.70	17.97
3	nPAA	1.43	—	—	—	5.549	1.74	—
4	nBAA	1.82	2.36	—	0.54	5.550	1.11	59.98
5	iBAA	1.83	2.22	—	0.92	5.547	1.35	47.63
6	LAA	4.99	—	—	—	5.555	0.10	—
7	HEAA	0.55	−0.21	—	−0.76	5.632	0.19	5.177
8	HPAA	0.59	0.35	—	−0.24	5.543	0.58	8.11
9	MMA	0.89	1.38	0.56	0.49	5.303	89.89(70.1[#])	51.97
10	EMA	1.24	1.94	0.59	0.7	5.276	29.26	67.64
11	nPMA	1.71	—	—	—	5.281	10.22	—
12	nBMA	2.10	2.88	0.68	0.78	5.282	2.71(2.5[#])	147.70
13	iBMA	2.11	2.66	0.65	0.55	5.277	2.94	83.14
14	LMA	5.28	6.45**	—	1.17	5.286	0.67	—
15	HEMA	0.83	0.47	0.53	−0.36	5.340	10.07	45.24
16	HPMA	0.86	0.97	—	—	5.526	8.67	55.24
17	BZMA	2.67	2.77*	0.66	0.1	4.915	0.64	—
18	4META	1.80	2.13***	—	0.33	6.168	5.11	—
19	NPGDMA	2.77	3.39*	0.70	0.62	5.168	0.65	—
20	EDMA	1.88	1.84*	0.60	−0.04	5.336	1.06	—
21	DEGDMA	1.72	1.38*	0.57	−0.34	5.133	1.34	—
22	TEGDMA	1.55	1.53*	0.58	−0.02	4.997	1.5(1.3[#])	—
23	bis-GMA	5.07	3.84*	0.73	−1.23	4.663	0.03(0.17[#])	—
24	UDMA	3.80	1.53[Ali]	0.58	−2.27	4.889	0.09	—

Monomers see the text. [a]log P based on PM3 method; [b]fom Ref[4]; [c]fom Ref[21]; *log P calculated from the formulation: log P=−7.43+15.45log t_x (n=7, r^2=0.952, p<0.001); [d]50% cytotoxic concentration(ED_{50}) for HeLA S3 cells, Ref[6]; [#]ED_{50} for HGF cells; [e]acute oral LD_{50} using male dd Ymice, Ref[4]; **from Ref[1]; ***from Ref[22]; χ, electronegativity.

the isocratic capacity factor log k′ and the volume fraction (ϕ) of organic solvents. However, for a limited volume fraction, a linear relationship is given as follows:

$$\log k' = -S\phi + \log k_w$$

where −S is the slope, log k_w is the intercept of the regression curve, and ϕ is the volume fraction of methanol in water in the mobile phase.

Plots of k_w *versus* the volume fraction (ϕ) within the range of $0.5 \leqq \phi \leqq 0.85$ for each monomer (HEMA, MMA, EMA, nBMA, and bis-GMA) showed a linear relationship (data not shown). The r^2 value for HEMA was 0.80, whereas for other monomers it ranged between 0.95 and 0.99. The S values (log k′) of HEMA, MMA, EMA, nBMA, and bis-GMA were 0.83 (0.05), 2.33 (0.363), 2.76 (0.44), 4.27 (0.63), and 17.64 (1.20) respectively. Hydrophobic bis-GMA showed the largest S value, whereas hydrophilic HEMA gave the smallest value. From the data generated by analyzing four methacrylates with experimental log P values (Table 2), a correlation is shown in Eq. (3) (Table 3).

The log P value of bis-GMA calculated using Eq. (3) was 4.82, which agreed comfortably with that of log P (PM3) at 5.07. It is noteworthy that if the isocratic log t_r value were measured at an inappropriate point on the slope of ϕ, it might result in an unreliable log P value. For this reason, log P (PM3) values were used in this study as the parameter for hydrophobicity.

The partition coefficient between two immiscible phases is dependent on the solvation energy difference of the solute between the organic and water phases. A highly informative interpretation for the retention mechanisms of RP-HPLC stationary phase can be obtained by linear solvatochromic parameters[24]. In this respect, VDW_{area}, μ. and E_{HOMO} significantly accounted for the variation of log P values among the monomers. In general, monomers used as dental materials — except for dental bonding agents — are neutral organic compounds. In the present study, VDW_{area}, μ, and E_{HOMO} for seven monomers commonly used in dentistry were calculated using the PM3 method. The VDW_{area} values calculated for bis-GMA, NPGDMA, EDMA, TEGDMA, DEGDMA, EMA, and MMA were 196.40, 124.87, 109.60, 133.26, 123.01, 74.64, and 66.62 respectively. The HOMO values of these monomers are also shown in Table 1. It is noteworthy that linear relationships between log P and VWV_{area} (r^2<0.816) or E_{HOMO} (r^2<0.810) were observed for these seven monomers (correlations not shown). In particular, two molecular parameters, VDW_{area} and E_{HOMO}, contributed significantly to the variation of log P values (r^2<0.872) (correlations not shown).

Correlation between toxicity and descriptors

In the present study, relationship between log $1/LD_{50}$ and χ was investigated for 12 monomers. Table 3(B) shows the SAR results thereof. As shown in Eq. (4), a linear relationship was found between log $1/LD_{50}$ and χ for these 12 methacrylates. Further, as shown in Eq. (5), a good linear relationship was observed between log $1/LD_{50}$ and log P for the aliphatic acrylates (r^2<0.970).

It should also be mentioned that a significant linear correlation was found between log $1/LD_{50}$ and $\delta_{C\beta}$ for the acrylates (MAA, EAA, nPAA, nBAA, and iBAA; n=5, r^2=0.685) and methacrylates (MMA, EMA, nPMA, nBMA, and iBMA; n=5, r^2=0.944) (correlations not shown). It is noteworthy that $\delta_{C\beta}$ significantly influenced the toxicity of these esters in mice.

Next, the relationships between log EC_{50} and log P or E_{HOMO} were investigated for the test monomers (Table 3). Each acrylate, methacrylate and dimeth-

Table 3 SARs for physicochemical properties (A) and toxicity (B) of methacrylates.

A)	
$\delta_{c\beta} = 22.96(\pm 0.42) - 9.71(\pm 0.39)E_{HOMO}$ (n=13 r^2=0.982, p<0.01)	Eq. (1)
$\mid \delta C\alpha - \delta C\beta \mid = 198.72(\pm 0.63) + 17.83(\pm 0.70)E_{HOMO}$ (n=13, r^2=0.983, p<0.001)	Eq. (2)
log P = 0.13 + 4.14 log' (n=5, r^2=0.967, p<0.01)	Eq. (3)
B)	
log $1/LD_{50} = -14.63(\pm 0.32) + 2.40(\pm 0.69)\chi$ (n=12, r^2=0.546, p<0.01)	Eq. (4)
log $1/LD_{50} = -0.738(\pm 0.11) + 0.35(\pm 0.03)$ log P (n=6, r^2=0.970, p<0.001)	Eq. (5)
log $1/ED_{50} = 1.78(\pm 0.52) + 0.45(\pm 0.09)$ log P (n=20, r^2=0.600, p<0.001, outlier No. 7, 8, 15 and 16)	Eq. (6)
log $1/ED_{50} = 2.00(\pm 0.13) + 0.51(\pm 0.04)$ log P (dimethacrylates: n=6, r^2=0.974, p<0.01)	Eq. (7)
log $1/ED_{50} = 14.28(\pm 0.22) + 1.07(\pm 0.15)$ E_{HOMO} (dimethacrylates: n=6, r^2=0.974, p<0.01)	Eq. (8)

Eqs(1) and(2): aliphatic (meth)acrylates (No.1-5, 9-13, 20 and 22); Eq.(3):compounds(No.9,10,12,15 and 23); Eq.(4):aliphatic (meth)acrylates(No.1.2,4,5,7-10,12,13,15 and 16);Eq.(5):aliphatic acrylates(No.1,2,4,5,6 and 7); Eq.(6):compounds(No.1-6,9-14 and 17-24); Eqs(7) and(8): dimethacrylates(No.19-24). No. of compounds and parameterssee Tables 1 and 2. ED_{50} represents the molar concentration of(meth)acrylates.

acrylate series comprised six monomers. For acrylates and methacrylates with a hydroxy group — HEAA and HPAA for acrylates in conjunction with HEMA and HPMA for methacrylates, they were omitted as outlier compounds. As shown in Eq. (6), a linear relationship between log $1/ED_{50}$ and log P was found for the methacrylate series of 20 compounds. For acrylates and methacrylates, no correlations were found between $1/ED_{50}$ and E_{HOMO} as well as other molecular descriptors.

For the dimethacrylates, linear regression equations between log $1/ED_{50}$ and log P or E_{HOMO} were shown in Eqs. (7) and (8) respectively. Further, a multiple linear regression equation was found for log P and E_{HOMO} (correlation not shown), but the correlation coefficient was similar to that in Eqs. (7) and (8). It is also noteworthy that a poor correlation coefficient between log $1/ED_{50}$ and χ ($r^2 = 0.694$) was observed for the dimethacrylates.

In the present study, acrylates and dimethacrylates (especially the latter) were found to play a partial role in the cytotoxic activity of the esters. For methacrylates, it is interesting to note that their toxicity-induction effects in relation to log P showed a great difference between the animal and tissue culture tests. As log P increased, the cytotoxic effect on HeLa cells became enhanced. Conversely, induced toxicity in mice declined as log P increased. This might indicate the importance of the hydrophobicity of methacrylates in eliciting the acute toxicity effect.

It is well known that log P influences VDW_{area}. For this reason, we investigated the relationship between log $1/ED_{50}$ and VDW_{area} for seven monomers. As expected, a good linear equation (r^2=0.974) was obtained (correlation not shown). Therefore, VDW_{area} — a bulk descriptor — was correlated with log $1/ED_{50}$, but electronic descriptor (μ) and energy descriptors (E_{HOMO}, E_{LUMO}) showed no such correlations.

Except for bis-GMA, the cytotoxicity of MMA, BMA, and TEGDMA against HGF cells was similar to that exhibited against HeLa cells (Table 2). We then examined the cytotoxicity of a purified bis-GMA against HGF cells. This is because the great difference in cytotoxicity of bis-GMA against HeLa and HGF cells could lie in the type of bis-GMA used. In particular, commercial bis-GMA products — which contain Bis-GMA isomers and unpurified compounds — have posed difficulties for purification.

DISCUSSION

This study demonstrated a good correlation between the chemical shift $\delta_{C\beta}$ and E_{HOMO} for methacrylates. Moreover, the difference between $\delta_{C\alpha}$ and $\delta_{C\beta}$ correlated well with E_{HOMO}. E_{HOMO} represents the electron-donating power of a molecule, while the magnitude of NMR chemical shift depends on the electron donating power of a monomer. In the present study, the E_{HOMO} values of acrylates were lower than the corresponding methacrylates, whereas the χ values of the former were greater than the latter's. Acrylates are more toxic than their corresponding methacrylates, thereby indicating a causal link between the manifestation of acute toxicity and these parameters. For this matter, a significant multiple linear correlation between the acute toxicity of methacrylates in mice and the parameters of χ and log P suggested that both electronegativity and hydrophobicity played an important role in SARs.

A previous study has shown that the toxicity of acrylates is significantly related to their reactivity with glutathione (GSH)[4], stemming from its correlation with χ as shown in this study. In another study which investigated the relationship between the structures and cytotoxicity of acrylates and methacrylates, a linear relationship between the log ED_{50} for HeLa S3 cells and log P was found for these monomers[6]. In the present study, these results were re-examined using quantum molecular descriptors. A significant multiple linear equation for the function of parameters log P and E_{HOMO} was observed for both the acrylates and dimethacrylates, except for acrylates with a hydroxy group. In contrast, the cytotoxicity of methacrylates was related to log P alone.

The dependence of dimethacrylates on E_{HOMO} or χ was probably because they have twice as many double bonds in the molecules. As for monomers HEAA, HPAA, HEMA, and HPMA, they were omitted from the present ester series for log EC_{50} because they exhibited prominent outlier behavior in the regression equations. These monomers contained a hydroxy group as a primary alcohol, and the hydrolysis of the ester of these compounds in tissues could be caused by esterase enzymes. Therefore, the biological activity of these compounds may be related to a mechanism that involves ester hydrolysis. In particular, the high toxicity of the acrylates, HEAA and HPAA (Table 2), could be caused by these decomposition products[25]. Hydrolyzed components derived from methacrylates in addition to the original compounds could be implicated in the induction of toxicity under high concentrations of monomers.

The RP-HPLC method involves the determination of retention times (t_r) for methacrylates, followed by the calculation of their capacity factors. In this study, the log P value of bis-GMA, which was determined using a mobile phase of 85:15 methanol: water, showed a lower value than did RP-HPLC log P using isocratic k_w, which entailed extrapolation to 100% water. The capacity factor obtained by changing the solvent ratio of methanol-water, which is based on intramolecular hydrophobic interaction,

affected the RP-HPLC log P values for hydrophobic compounds. In RP-HPLC, the driving force for retention is the unfavorable interaction of a solute with the surrounding water molecules present in the mobile phase. Therefore, the calculated PM3 log P values for highly hydrophobic compounds such as bis-GMA might be expectedly different from the experimental values obtained using a flask-shaking method and RP-HPLC technique.

The E_{HOMO} of a molecule represents its electron-donating power and is thus related to hydrogen bond basicity as proposed by Taft *et al.*[26]. This meant that E_{HOMO} is an important factor to the partitioning of a molecule in the water phase because it is assumed that log P comprises polarization, electrostatic, and electronic terms[26]. The three properties, VDW volume or area, μ, and E_{HOMO} are known to influence the interaction of molecules with the RP-HPLC stationary-mobile phases[27]. In the present study, it was found that VDW_{area} (molecular bulk) and HOMO energy (reactivity), but not μ (molecular polarity), of the monomers widely used in dental materials were linearly correlated with log P. A good correlation between log $1/ED_{50}$ and VDW_{area} of the test monomers was observed, suggesting that VDW_{area} as well as log P were reliable for use in SAR studies of methacrylates. In conclusion, the NMR chemical shifts of β-carbon and semiempirical molecular descriptors, in addition to log P, may help to reveal the toxic potentials of new medical and dental materials, thereby facilitating the synthesis of less toxic monomers.

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