Effects of Chlorinated Ethylenes on Expression of Rat CYP Forms: Comparative Study on Correlation between Biological Activities and Chemical Structures

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Chlorinated ethylenes (CEs) such as tetrachloroethylene (PCE), trichloroethylene (TCE), 1,1-dichloroethylene (1,1-DCE), *cis*-1,2-dichloroethylene (*cis*-DCE), *and trans*-1,2-dichloroethylene (*trans*-DCE) are members of the class of volatile halogenated hydrocarbons. Each was administered intraperitoneally at 0.5 g/kg alone or simultaneously with phenobarbital (PB, 80 mg/kg/d) to male Wistar rats weighing about 200 g (7-weeks-old). Microsomal fractions of livers and lungs removed from animals sacrificed 24 h after treatment were tested for monooxygenase activities and protein content of 4 cytochrome P450 (CYP) forms, *i.e.*, CYP1A1/2, 2B1/2, 2E1 and 3A1/2. In terms of constitutive expression, they were compensatory in liver and lung with CYP1A1/2, 2E1 and 3A1/2 being detected only in liver and CYP2B1 only in lung. All 5 CEs employed in this work suppressed hepatic CYP1A1/2, 2E1 and 3A1/2 in the descending order of 1,1-DCE > *cis*-DCE > *trans*-DCE > TCE > PCE. The magnitude of suppression of pulmonary CYP2B1 by CEs were compared as follows; PCE > *trans*-DCE > 1,1-DCE > *cis*-DCE > TCE. CYP1A1/2, CYP2E1 and CYP3A1/2 in hepatic microsomes from PB-treated animals responded to CEs similarly to those from PB-untreated animals. These 3 enzyme activities could not be detected in pulmonary microsomal fractions, irrespective of the PB-treatment. Hepatic CYP2B1/2 could be detected only when treated with PB in marked contrast to the constitutive expression of pulmonary CYP2B1. The suppression of PB-induced hepatic CYP2B1/2 was observed with all 5 CEs, with special emphasis on complete inhibition with 1,1-DCE. The adverse effects of TCE and PCE on pulmonary CYP2B1 from PB-treated rats were comparable with those from PB-untreated animals. In contrast, 3 dichloro-isomers were more suppressive to the enzyme in the absence than in the presence of PB. The protein levels of hepatic CYP forms were generally proportional to the enzyme activities in the case of 1,1-DCE-treatment. The amounts of pulmonary CYP2B1 apoprotein were in good accordance with the enzyme activities when treated with CEs individually.

Key words —— tetrachloroethylene, trichloroethylene, 1,1-dichloroethylene, *cis*-1,2-dichloroethylene, *trans*-1,2-dichloroethylene, cytochrome P450 form

INTRODUCTION

Chlorinated ethylenes (CEs) such as tetrachloroethylene (PCE), trichloroethylene (TCE), 1,1-dichloroethylene (1,1-DCE), *cis*-1,2-dichloroethylene (*cis*-DCE) and *trans*-1,2-dichloroethylene(*trans*-DCE) are volatile halogenated hydrocarbons. CEs have been widely used in industrial fields as metal-degreasing solvents and raw materials of consumer products. Due to carcinogenic potential, they are under strict legislative control in Japan.

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Severe liver and kidney-toxicities of CEs in rodents have been reported.[1] CEs are also well-known to be environmental pollutants.[2] Since lung is the primary organ exposed to CEs uptaken by respiratory systems, atmospheric pollution by CEs might be one of the risk factors for lung cancer.[3] It was reported that CEs were responsible for the increased carcinogenic risks in the liver and lymphohematopoietic tissue of exposed workers,[4] and cancer risks among people drinking water from wells polluted with TCE and more toxic PCE.[3] A portion of the hepatic tumors that developed in mice treated with CEs such as PCE, TCE and their metabolites, exhibited loss of heterozygosity in chromosome 6, suggesting the presence of tumor sup-

pressor genes.[5] Cytochrome P450 (CYP) is a principal monooxygenase superfamily that is involved in the phase I metabolism of a variety of endogenous and exogenous substrates. Wide substrate specificity of CYP is due to the existence of numerous forms that are regulated independently. The progression of toxicities and carcinogenicities of CEs are facilitated by CYP-mediated bioactivation as was shown by the fact that oxygenation of 1,1-DCE with cyp2e1 precedes the expression of pulmonary toxicities in mice.[6]

In general, metabolites producted by phase I enzymes are detoxified by phase II enzymes and excreted into urine and/or bile; however, this may not be true for CEs. CEs once uptaken are first metabolized by CYP forms mainly in liver to such compounds as epoxides and other biologically active intermediates and these compounds in turn bind to cellular macromolecules (DNA, RNA, protein, *etc*.), being toxic and carcinogenic. By coincidence, the inducers of certain CYP forms, *e.g*., phenobarbital (PB) and ethanol, were shown to enhance CE toxicities.[7] Thus, CYPs play contradictory roles in animals administered with CEs. However, it is expected that CYP-expression will be controlled in the direction of the minimized CE-related toxicities. Although various effects of CEs on the expression of CYP forms have been reported,[8,9] there are few papers concerning the chemical structures of CEs with reference to their effects on the *in vivo* expression of CYP forms.

CEs are metabolized by CYP forms by way of putative active intermediate epoxides which are considered to be causes of hepatitis and liver cancer,[10] being followed by phase II enzymes to dichloroacetic acid in the case of 1,1-DCE or *trans*-DCE, and dichloroethanol for *cis*-DCE. Likewise, chloral hydrate and trichloroacetic acid correspond to TCE and PCE, respectively. Although activated intermediates are metabolized rapidly by abundant second phase enzymes such as epoxide hydrase and glutathione *S*-transferase to the excretory forms in liver, activated intermediates may have extended life spans in lung which, is devoid of many second phase enzymes. In fact, it was reported that more DCE epoxide was detected in lung than in liver.[11] Exposure to CEs occurs mainly by inhalation of contaminated air through respiratory organs represented by lung, and CEs in the blood circulation are, in turn, excreted mainly into expiratory air in the lung in unchanged forms. Taken these observations into consideration, the effects of CEs on pulmonary CYP forms were also examined in this study. Lung consists of alveolar type I cells, type II cells, fibroblasts, macrophages and Clara cells, *etc*., among which pulmonary CYP enzymes are reportedly localized in Clara cells.[12]

Although structure-dependency of the effects of environmental toxic compounds on CYP-expression has been studied in detail for polychlorodibenzo-*p*-dioxins and polychlorobiphenyls (PCBs),[13–15] there are few studies on the effects of CEs on CYP expression from the structural viewpoint.

In the previous paper,[16,17] the effects of PCE, TCE and 1,1,1-trichloroethane (TCA) on the expression of CYP forms in male and female rats were studied comparatively between lung and liver, revealing organospecific responses of CYP2B1/2 and 2E1 to these compounds with some sexual variations. In this paper, the *in vivo* responses of CYPs to di-, tri- and tetrachlorinated ethylenes will be described with special emphasis on the comparison between biological and structural features.

MATERIALS AND METHODS

Reagents —— Resorufin, 7-ethoxyresorufin, 7-pentoxyresorufin and erythromycin were purchased from Sigma Chemical Company (U.S.A.). PCE, TCE, *trans*-DCE, *p*-nitrophenol and 4-nitrocatechol were from Wako Pure Chemical Ind. (Osaka). 1,1-DCE and *cis*-DCE were purchased from Aldrich Chemical Co. (U.S.A.). Goat anti-rat CYP 1A1, 2B1, 2E1 and 3A2 sera and peroxidase-labeled anti-goat IgG serum were obtained from Daiichi Chemical Co. (Tokyo). All the other reagents were purchased from Wako Pure Chemical Ind. (Osaka).

Animals and Treatment —— Wistar rats (Nihon Clea, 7 weeks old) were divided into 12 groups, each consisting of 4–6 animals; PCE-treated, TCE-treated, 1,1-DCE-treated, *cis*-DCE-treated and *trans*-DCE-treated groups, 5 groups treated with individual CEs plus PB, control group and PB-treated group. Rats were i.p. injected with individual CEs (0.5 g/kg body weight) alone or simultaneously with corn oil or PB (80 mg/kg body weight/d).

Preparation of Microsomes —— Lungs and livers were removed from animals 24 h after the treatment, and the wet weights of these organs were measured. Lungs and livers were homogenized in four volumes of cold 1.15% KCl–50 mM Tris·HCl (pH 7.4)–1 mM EDTA. The homogenates were centrifuged at $900 \times g$ for 5 min and then $9000 \times g$ for 15 min at 4°C. Mi-

crosomal pellets were obtained from the 9000 × *g*-supernatants by centrifugation at 105000 × *g* for 60 min at 4°C. The microsomal pellets were homogenized in cold 1.15% KCl–50 mM Tris·HCl (pH 7.4)–1 mM EDTA containing 20% glycerol. The microsomes were kept at – 80°C until use. Protein content was evaluated using Miller's modification of Lowry's method.[18]

Enzyme Assays —— The details of the assay procedures for ethoxyresorufin *O*-deethylase (EROD),[19] pentoxyresorufin *O*-dealkylase (PROD),[20] *p*-nitrophenol hydroxylase (PNPH)[21] and erythromycin *N*-demethylase (EMND)[22] were described previously.[16,17]

Western Immunoblot —— Immunoblot analysis of microsomes was performed to detect CYP form apoproteins. SDS-PAGE was carried out with 10% acrylamide according to the method of Laemmli.[23] CYP apoproteins were transferred electrophoretically with the help of a blotting apparatus (BioRad, Transblot-SD) from the gel to a nitrocellulose sheet. The sheet was treated with goat anti-rat CYP form sera and peroxidase-labeled anti-goat IgG. Color development was achieved using a HRP Conjugate Substrate Kit (BioRad). The protein band-images read by the image scanner were analyzed by NIH-image software.

Statistics —— Statistical significance was determined using Student's *t*-test. The 0.05 level of probability was the criterion of significance.

RESULTS

Effects of CEs on Whole Body and Wet Organ Weights

The changes in the weights of whole bodies and wet organs of rats treated with CEs in the absence or the presence of PB are shown in Table 1, as well as those of animals in the control and PB-monotreatment groups. In contrast to steady gain in body weight in the control group, reduced gain or loss in body weight was observed in all the CE-treated groups. The magnitude of relative loss of body weight of animals treated with CEs to that of the control group was in the descending order 1,1-DCE > *cis*-DCE > TCE > PCE > *trans*-DCE. When combined with PB, only treatment with 1,1-DCE resulted in loss in body weight compared to the PB-monotreatment group. Thus, the most potent toxicity in terms of body weight loss was shown by 1,1-DCE among the 5 CEs in the absence or presence of PB. The highest degree of hepatic hypertrophy was observed in rats treated with a combination of TCE and PB (+ 33%). In contrast, pulmonary hypertrophy was significant when treated with 1,1-DCE (+ 47%) and PCE (+ 25%).

Effects of CEs on CYP Form-Specific Monooxygenase Activities

Rats were treated with individual CEs and the animals in the control group were given corn oil vehicle. Using microsomal fractions prepared from lung and liver removed from each animal, CYP form-specific monooxygenase activities were measured; EROD, PROD, PNPH and EMND correspond to the functions of CYP1A1/2 (CYP1A), 2B1/2 (CYP2B), 2E1 and 3A1/2 (CYP3A), respectively.[13–16] The results are shown in the upper part of Table 2.

As shown in the previous paper,[10,11] the expression profiles of constitutive CYP forms (and enzyme activities in the control group) were of marked divergence between liver and lung. PROD activity was exclusively detected in pulmonary microsomes and the activities of the other three enzymes, EROD, PNPH and EMND, were detected, without being accompanied by PROD activity, in hepatic microsomes.

The decrease in hepatic EROD activity was greater than 50% in CE-treated groups when compared with the control group, being over 90% especially in the case of 1,1-DCE. Hepatic PROD activity was not detected in rats treated with CEs, as well as in the control animals. It is noteworthy that hepatic PNPH activity was inhibited completely by 1,1-DCE while it was not affected by the other CEs. A marked decrease in hepatic EMND activity was observed in animals treated with dichloro-isomers (% inhibition); 1,1-DCE (62), *cis*-DCE (40) and *trans*-DCE (18). In lung, PROD activity was exceptionally over the detection limit, being severely suppressed by all 5 CEs with % inhibitions in the descending order of 99, 96, 90, 73 and 44 for PCE, *trans*-DCE, 1,1-DCE, *cis*-DCE and TCE, respectively.

Effects of CEs on CYP Form-Specific Monooxygenase Activities in Combination with PB

The results for rats treated with individual CEs in combination with PB are summarized in the lower part of Table 2. As previously described,[10,11] hepatic PROD activity became detectable in the presence of PB, which did not affect PROD activity expressed

Table 1. Effect of Treatment with Chlorinated Ethylenes on Body Weight and Organ Wet Weight

Treatment	Body weight change[a)] (%)	Liver wet weight (g/100 g body wt.)	Lung wet weight (g/100 g body wt.)
Single-administration			
Control[b)]	3.16 ± 0.77	4.13 ± 0.10 (100)	0.36 ± 0.03 (100)
PCE	0.77 ± 0.80	4.68 ± 0.08 (113)**	0.45 ± 0.01 (125)*
TCE	−0.08 ± 0.68*	4.91 ± 0.13 (119)**	0.42 ± 0.01 (117)
1,1-DCE	−9.42 ± 0.63***	4.59 ± 0.08 (111)**	0.53 ± 0.02 (147)**
cis-DCE	−0.41 ± 0.61**	4.52 ± 0.14 (109)	0.41 ± 0.00 (114)
trans-DCE	1.89 ± 0.45	4.60 ± 0.16 (111)	0.41 ± 0.01 (114)
Co-administration			
PB	1.12 ± 0.53	4.59 ± 0.12 (111)	0.41 ± 0.01 (114)
PB+PCE	2.01 ± 0.36	5.11 ± 0.10 (123)§	0.43 ± 0.01 (119)
PB+TCE	2.93 ± 0.60	5.50 ± 0.06 (133)§§§	0.43 ± 0.01 (119)
PB+1,1-DCE	−5.66 ± 0.69§§§	4.32 ± 0.07 (105)	0.45 ± 0.03 (125)
PB+*cis*-DCE	1.92 ± 1.09	4.83 ± 0.09 (117)	0.39 ± 0.01 (108)
PB+*trans*-DCE	2.82 ± 0.87	4.85 ± 0.12 (117)	0.40 ± 0.00 (111)

Rats were treated with a i.p. injection of each chlorinated ethylene (0.5 g/kg body wt.) and/or phenobarbital (80 mg/kg) . The body weights were measured at the time of PB and chlorinated ethylene-injection and the body weights and organ wet weights were measured after 24 h. Data are shown as the mean ± S.E. for 4–6 individual rats with statistically significant differences compared to the control group or PB-treated group at $p < 0.05$(*), (§), $p < 0.01$(**), (§§) and $p < 0.001$(***), (§§§). Figures in parentheses are the percentage of control. *a*) (B−A)/A × 100, where A is the body weight at the start of treatment and B atthe time of sacrifice. *b*) Corn oil dosing.

constitutively in lung. All the tested CYP form-related enzyme activities were markedly suppressed by 1,1-DCE and less significantly by *cis*-DCE. The residual activities of EROD, PROD and PNPH were lower than or close to their detection limits when treated with 1,1-DCE in combination with PB. *trans*-DCE was the least potent inhibitor of hepatic enzymes among 3 dichloroethylenes. It is noteworthy that pulmonary PROD activity was completely suppressed by PCE in the presence of PB, to which the responses to the other CEs were made moderate. In general, CYPs were less insensitive to the suppressive effects of CEs in the presence of PB than in the absence of PB.

Effects of CEs on the Expression of CYP Form Apoproteins

The CYP form apoproteins were detected by immunoblotting and the results are shown in Fig. 1 and Table 3, leaving CYP1A apoprotein undetectable under the conditions used in the present study.

As in the case of monotreatment with CEs, the amount of hepatic CYP2B-apoprotein increased twofold by PCE and TCE. 1,1-DCE was found to be suppressive to the expression of hepatic CYP2B-, CYP2E1-, and CYP3A-apoproteins. When combined with PB, 1,1-DCE decreased hepatic CYP-apoproteins to a much greater extent than alone. Selective reduction in the amount of CYP3A-apoprotein was observed with the other dichloroisomers. In marked contrast to hepatic proteins, all 5 CEs suppressed the expression of pulmonary CYP2B-apoprotein when compared with control. However, the response of CYP2B-apoprotein to CEs was indulgent in combination with PB, in accordance with the observations associated with the enzyme activity.

DISCUSSION

In this study, experimental animals were treated with PCE, TCE, *cis*-DCE, *trans*-DCE and 1,1-DCE, i.p. at 0.5 g/ kg body weight, to investigate the correlation between effects on the expression of CYP forms and differences in chlorination patterns (number and site of substitutions) on the ethylene core structure.

The toxicities of CEs were examined in terms of the rate of body weight gain and the change in organ wet weight (Table 1). No visible change in animal health condition was observed when the animals were sacrificed 24 h after treatment. Toxicity in terms of loss or decreased gain of body weight was observed when treated with TCE, 1,1-DCE and *cis*-DCE.

The treatment with PCE, TCE and 1,1-DCE resulted in hepatic hypertrophy, whereas pulmonary

Table 2. Effects of Chlorinated Ethylenes and/or PB on P450-Dependent Monooxygenase Activities in Microsomes of Rat Livers and Lungs

Enzyme activity		Liver		Lung	
Single-administration					
Ethoxyresorufin *O*-deethylase	Control	53.9 ± 7.4[a)]		n.d.	
(pmol/min per-mg protein)	PCE	20.5 ± 3.9	(38.1)**	n.d.	
	TCE	20.6 ± 1.7	(38.3)*	n.d.	
	1,1-DCE	4.9 ± 4.2	(9.0)**	n.d.	
	cis-DCE	17.0 ± 3.3	(31.6)**	n.d.	
	trans-DCE	24.0 ± 1.9	(44.6)*	n.d.	
Pentoxyresorufin *O*-dealkylase	Control	n.d.		75.0 ± 6.1	
(pmol/min per mg-protein)	PCE	n.d.		1.1 ± 1.1	(1.4)**
	TCE	n.d.		42.1 ± 3.2	(56.1)**
	1,1-DCE	n.d.		7.6 ± 1.4	(10.1)**
	cis-DCE	n.d.		20.1 ± 4.8	(26.8)***
	trans-DCE	n.d.		2.9 ± 1.9	(3.9)***
p-Nitrophenol hydroxylase	Control	350.6 ± 39.9		n.d.	
(nmol/min per mg-protein)	PCE	303.6 ± 23.3	(86.6)	n.d.	
	TCE	345.3 ± 6.3	(98.5)	n.d.	
	1,1-DCE	n.d.	(0.0)**	n.d.	
	cis-DCE	417.0 ± 53.4	(119.0)	n.d.	
	trans-DCE	393.3 ± 38.3	(112.2)	n.d.	
Erythromycin *N*-demethylase	Control	0.85 ± 0.05		n.d.	
(nmol/min per mg-protein)	PCE	0.87 ± 0.05	(102.4)	n.d.	
	TCE	0.67 ± 0.06	(78.8)	n.d.	
	1,1-DCE	0.32 ± 0.03	(37.6)***	n.d.	
	cis-DCE	0.51 ± 0.04	(60.0)***	n.d.	
	trans-DCE	0.70 ± 0.03	(82.4)*	n.d.	
Co-administration					
Ethoxyresorufin *O*-deethylase	PB	101.3 ± 17.8		n.d.	
(pmol/min per mg-protein)	PB+PCE	67.6 ± 4.9	(66.7)	n.d.	
	PB+TCE	52.2 ± 6.1	(51.5)	n.d.	
	PB+1,1-DCE	0.0 ± 0.0	(0.0)§§	n.d.	
	PB+*cis*-DCE	32.4 ± 4.4	(32.0)§§	n.d.	
	PB+*trans*-DCE	33.8 ± 4.0	(33.4)§	n.d.	
Pentoxyresorufin *O*-dealkylase	PB	354.3 ± 69.1		38.5 ± 4.6	
(pmol/min per mg-protein)	PB+PCE	178.4 ± 14.8	(50.4)	n.d.	(0.0)§§§
	PB+TCE	331.1 ± 45.3	(93.5)	22.6 ± 3.6	(58.8)§
	PB+1,1-DCE	n.d.	(0.0)§§	24.9 ± 2.7	(64.5)
	PB+*cis*-DCE	135.0 ± 10.9	(38.1)§	37.8 ± 1.0	(98.2)
	PB+*trans*-DCE	123.9 ± 13.6	(35.0)§	18.0 ± 0.9	(46.7)§§
p-Nitrophenol hydroxylase	PB	656.1 ± 26.5		n.d.	
(nmol/min per mg-protein)	PB+PCE	568.3 ± 28.1	(86.6)	n.d.	
	PB+TCE	667.2 ± 33.0	(101.7)	n.d.	
	PB+1,1-DCE	n.d.	(0.0)§§§	n.d.	
	PB+*cis*-DCE	481.9 ± 33.0	(73.5)§§	n.d.	
	PB+*trans*-DCE	453.3 ± 18.9	(69.1)§§§	n.d.	
Erythromycin *N*-demethylase	PB	1.03 ± 0.04		n.d.	
(nmol/min per mg-protein)	PB+PCE	1.10 ± 0.02	(106.8)	n.d.	
	PB+TCE	1.05 ± 0.05	(101.9)	n.d.	
	PB+1,1-DCE	0.42 ± 0.02	(40.8)§§§	n.d.	
	PB+*cis*-DCE	0.72 ± 0.06	(69.9)§§	n.d.	
	PB+*trans*-DCE	0.94 ± 0.03	(91.3)	n.d.	

Rats were treated with i.p. injection of trichloroethylene (0.5 g/kg) and/or phenobarbital (80 mg/kg) . *a*) Data are shown as the mean ± S.E. for 4–6 individual rats with statistically significant differences compared to the control group or PB-treated group at $p < 0.05$(*), (§), $p < 0.01$(**), (§§) and $p < 0.001$(***), (§§§). Figures in parentheses are the percentage of control group or PB-treated group. n.d.: not detected. (EROD, PROD < 1 pmol/min/mg-protein, PNPH, EMND < 0.1 nmol/min /mg-protein).

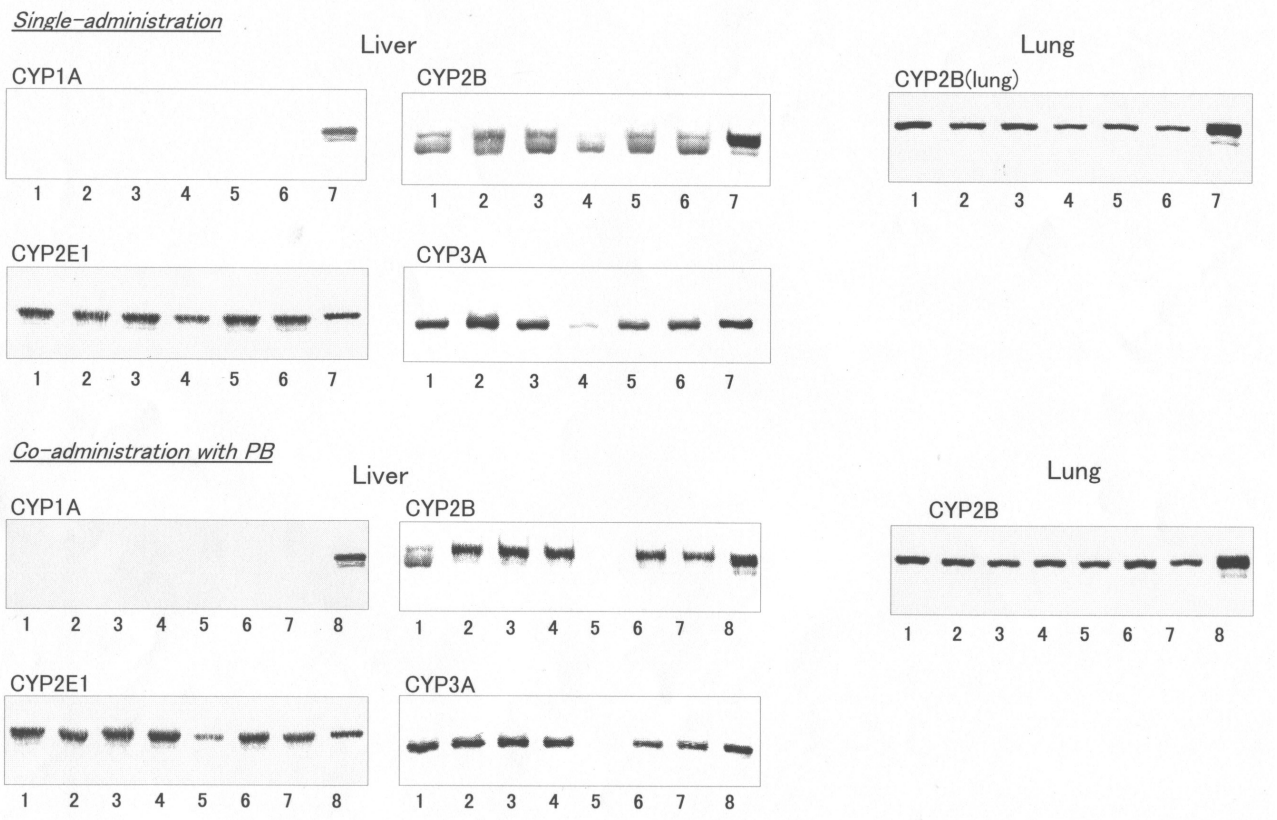

Fig. 1. Immunoblotting of Liver and Lung Microsomes from Chlorinated Ethylene-Treated Rats with Anti-rat CYP1A1, CYP2B1, CYP2E1 and CYP3A2 Antibodies

Each lane was loaded with a mixture of microsomal samples prepared from 5 individual animals. Liver microsomes from rats given 3-methylcholanthrene, phenobarbital, pyridine and dexamethasone were used as the standards for CYP1A, 2B, 2E1, 3A, respectively. Single-administration (upper part): Lane 1, control; lane 2, PCE; lane 3, TCE; lane 4, 1,1-DCE; lane 5, *cis*-DCE; lane 6, *trans*-DCE; lane 7, standard microsome. Microsomal protein levels are 100, 20, 20, 50 and 20 μg (lanes 1–6) for liver CYP1A, 2B, 2E1, 3A and lung CYP2B, respectively, and 4 μg (lane 7). Co-administration with PB (lower part): Lane 1, control; lane 2, PB, lane 3 PCE; lane 4, TCE; lane 5, 1,1-DCE; lane 6, *cis*-DCE; lane 7, *trans*-DCE; lane 8, standard microsome. Microsomal protein levels are 100, 20, 20, 50 and 20 μg (lane 1), 100, 20, 20, 50 and 20 μg (lanes 2–7) for liver CYP1A, 2B, 2E1, 3A and lung CYP2B, respectively, and 4 μg (lane 7).

hypertrophy was observed in the animals treated with PCE and 1,1-DCE.

In hepatic microsomes, 1,1-DCE and *cis*-DCE suppressed all the tested CYP forms. The suppressive effects of PCE, TCE and *trans*-DCE were confined to CYP1A and 2B, not affecting CYP2E1 and 3A (Table 2). In general, CEs were suppressive but not inductive of the enzyme activities of CYP forms, with marked variations in their suppressive effects.

Taking into consideration the suppression of enzyme activities of hepatic CYP forms by 1,1-DCE, *cis*-DCE, *trans*-DCE, TCE and PCE in the descending order of potency, less chlorine-substituted compounds might be more suppressive to CYP activities. On the other hand, pulmonary CYP2B was suppressed by PCE, *trans*-DCE, 1,1-DCE, *cis*-DCE and TCE in descending order. The congeners lacking electric dipole moment, PCE and *trans*-DCE, demonstrated higher potencies in lung compared with liver. Thus, there were organospecificities not only in the expression profiles of CYPs in accordance with the previous results but also in the susceptibilities of CYPs to CEs between liver and lung. Furthermore, pulmonary CYP2B seemed to be more susceptible to CEs than hepatic enzymes, giving PCE and *trans*-DCE highly selective effects to pulmonary CYP2B. CEs are metabolized in rat by such CYP forms as CYP2E1, 2B, 2C11 and 1A, among which CYP2E1 seems to play the most important role. In lung, where CYP2E1 is deficient, CYP2B might take the place of CYP2E1 in liver. The difference in the behavior of CEs between liver and lung are in part attributable to differences in the substrate specificities of CYP2E1 and CYP2B.[10]

The expression of CYP forms is affected by xenobiotics at transcriptional, translational and/or post-translational stages. The hepatic CYP2E1 and CYP3A-apoprotein contents decreased in parallel

Table 3. Effect of Treatment with Chlorinated Ethylenes on P450 Protein Levels in Rat Liver and Lung Microsomes

CYP-apoproteines		Treatment					
		Control	PCE	TCE	1,1-DCE	*cis*-DCE	*trans*-DCE
Single-administration							
Liver	CYP1A	TR	TR	TR	TR	TR	TR
	CYP2B	1.00	2.12	1.80	0.21	1.32	1.19
	CYP2E1	1.00	0.84	1.07	0.67	1.14	1.04
	CYP3A	1.00	1.42	1.04	0.12	0.74	0.94
Lung	CYP2B	1.00	0.74	0.84	0.51	0.75	0.59
Co-administration with PB							
Liver	CYP1A	TR	TR	TR	TR	TR	TR
	CYP2B	1.00	1.23	1.18	TR	0.99	0.90
	CYP2E1	1.00	1.24	1.30	0.35	1.12	0.91
	CYP3A	1.00	1.06	0.93	TR	0.54	0.64
Lung	CYP2B	1.00	0.90	1.02	0.80	1.15	0.79

The western immunoblotting results were normalized to those of the control group or the PB-treated group. TR: trace.

with the reduction in their enzyme activities in the case of 1,1-DCE-treatment, indicating pre-translational effects. In the presence of PCE and 1,1-DCE, the respective decrease in pulmonary CYP2B-apoprotein content were equivalent each other, although the corresponding enzyme activity was markedly reduced only with PCE, suggesting the existence of differential effects by these compounds. PCE seemed to adversely affect pulmonary CYP2B expression post-translationally. In fact, the covalent binding of active metabolites of CEs to CYP apoproteins is known. Since a parallel decrease in enzyme activity and apoprotein level of pulmonary CYP2B was observed in animals treated with CEs except for PCE, they are considered to work at the steps prior to translation. PCE epoxide seems to be relatively stable as the molecular electron is not localized, and this fact in turn supports the higher probability of PCE epoxide-binding to cellular proteins and lipids compared with those for the other CEs, due to the putative extended life span of PCE epoxide. In contrast, the epoxides of 1,1-DCE and *trans*-DCE are highly reactive and unstable and are considered to suppress CYP2B expression strongly at the steps preceding translation. In lung, which is deficient in phase II enzymes, generation of such a reactive intermediate might result in the rapid suppression of CYP2B-expression.

With regards to cotreatment with PB, no marked difference was observed in comparison with the cases of monotreatment, though the detection of hepatic CYP2B was fully dependent on PB-induction. In combination with PB, pulmonary CYP2B was suppressed by CEs to the same order of magnitude as in the case of monotreatment. However, the sensitivity to CEs was partially reversed in the presence of PB, suggesting the existence of either direct or indirect interactions between PB and CEs. A difference in the response profiles of CYP2B to CEs was observed between lung and liver. 1,1-DCE marked the highest suppression in liver while PCE was the most potent suppressor in lung, suggesting the organospecific effect on the expression of CYP 2B1 (pulmonary CYP 2B1 data is not shown).

The results obtained in the present study together showed that the expression of CYPs in both lung and liver were negatively controlled by CEs at the tested doses, irrespective of the presence of PB, at various stages depending on the chemical natures of the CE isomers with certain organospecificities. Thus, the detailed mechanism of CE-dependent suppression of CYPs remain to be elucidated in the future.

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