— **Minireview** —

Mode of Action of Chlorinated Ethylenes on the Expression of Rat Cytochrome P450 forms and Specificity in the Metabolic Activation of CEs by CYPs

Yoshio Inouye*

School of Pharmaceutical Sciences, Toho University, 2–2–1 Miyama, Funabashi,Chiba 274–8510, Japan

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Chlorinated ethylenes (CEs) including tetrachloroethylene (PCE), trichloroethylene (TCE) and 1,1-dichloroethylene (DCE) were comparatively evaluated for their effects on the expression of cytochrome P450 (CYP) forms of subfamilies 1A, 2B, 2E and 3A as well as their relative suitability as substrates of these CYPs. The magnitudes of inhibition of the enzyme activities were as follows in descending order: 1,1-DCE > TCE > PCE against hepatic CYPs and PCE > 1,1-DCE > TCE against pulmonary CYP2B1. These organ-specific profiles in the sensitivities to the adverse effects of CEs were partly attributable to the differential expression patterns of CYP forms by which they were metabolically activated. The expression of hepatic and pulmonary CYP2B mRNA was severely suppressed in the presence of 1,1-DCE during the entire observation period until 30 hr after the CE-treatment, in marked contrast to the temporarily enhanced expression at 6 hr followed by a moderate suppression in the cases of PCE and TCE with the trough values being observed at 18 hr. In addition to CYP2B, 1,1-DCE in advance of the transcriptional stage, when simultaneously treated with phenobarbital, also exclusively suppressed CYP2E1. These general suppressive effects of 1,1-DCE on the expression of divergent CYP mRNAs *in vivo* resembled the published findings in primary cultured hepatocytes treated with inflammatory cytokines such as IL-1β, TNF-α and IL-6, implying the highly inflammatory nature of 1,1-DCE.

Key words —— cytochrome P450, tetrachloroethylene, trichloroethylene, 1,1-dichloroethylene, inflammatory cytokine, nuclear receptor

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), and 1,1,1-trichloroethane (TCA) are members of the volatile halogenated hydrocarbons (VHHs). VHHs have been widely used in industrial fields as metal-degreasing solvents and raw materials of industrial products. However, the production of TCA has been internationally restricted as it is an ozone-depleting substance (the 1985 Vienna Convention, the Montreal Protocol), while CEs are under strict legislative control in Japan due to their carcinogenic potentials proved by the increased carcinogenic risks in the liver and lymphohematopoietic organs among exposed workers.[1)] They are also known as long-lived environmental pollutants resulting from their refractoriness to microbial degradation. CEs are metabolized by intracellular enzymes represented by cytochromes P450 (CYPs) and excreted into the urine as highly hydrophilic compounds such as trichloroethanol and trichloroacetic acid *via* the CYP-dependent formation of epoxide intermediates, which are responsible for their toxicities by forming complexes with cellular components.[2)] Thus, the epoxidation of CEs by CYPs, especially the members in the subfamilies of 1A, 2B and 2E has bi-directional effects on the host animals, *i.e.*, disadvantageous interaction with cellular molecules and advantageous urinary excretion.

*To whom correspondence should be addressed: School of Pharmaceutical Sciences, Toho University, 2–2–1 Miyama, Funabashi, Chiba 274–8510, Japan. Tel.: +81-47-472-2532; Fax: +81-47-476-6195; E-mail: yinouye@phar.toho-u.ac.jp

In contrast, the expression of CYPs might be affected by CEs either suppressively or inductively as in the cases of other xenobiotics including foods, drugs and environmental pollutants. It is well known that the pharmacokinetic interactions between two lipophilic compounds including drugs are often provided by the functions of CYPs; the adaptive increase of CYP gene expression (induction) by one chemical leads to the enhanced metabolism and clearance of the other. Taking these into consideration, the correlations between CEs and CYPs were analyzed in the absence or presence of the antiepileptic drug phenobarbital (PB), a well-known inducer of CYP2B.[3)]

Effects of CEs on the Monooxygenase Activities and Protein Contents of CYP Forms in Rats

Individual CEs were administered intraperitoneally at 0.5 g/kg alone or simultaneously with PB (80 mg/kg) to 7-week-old, male Wistar rats weighing about 200 g. Microsomal fractions of the livers and lungs removed from animals sacrificed 24 hr after treatment were tested for monooxygenase activities and protein contents of four CYP forms, *i.e.*, CYP1A1/2, 2B1/2, 2E1 and 3A1/2.[4–6)] In terms of constitutive expression, they were compensatory in the liver and lung with CYP1A1/2, 2E1 and 3A1/2 being detected only in the liver and CYP2B1/2 only in the lung. All five CEs suppressed hepatic CYP1A1/2, 2E1 and 3A1/2 in the descending order 1,1-DCE > *cis*-DCE > *trans*-DCE > TCE > PCE. The magnitude of suppression of pulmonary CYP2B1/2 by CEs was 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. The activities of these three enzymes 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 CYP2B1/2. The suppression of PB-induced hepatic CYP2B1/2 was observed with all five CEs, with special emphasis on complete inhibition with 1,1-DCE. The adverse effects of TCE and PCE on pulmonary CYP2B1/2 from PB-treated rats were comparable with those from PB-untreated animals. In contrast, three 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 CYP2B1/2 apoprotein were in good accordance with the enzyme activity when treated with CEs individually.

Effects of CEs on the mRNA Expression of CYP Forms in Rats

PCE, TCE and 1,1-DCE were comparatively studied for their effects on the *in vivo* mRNA expressions of CYP2B and 2E1 after 24 hr of treatment.[7)] As in the case of apoproteins, the expression of hepatic CYP2B mRNA remained poor until it was induced with PB, in comparison with that of the pulmonary one of constitutive and PB-insensitive nature. 1,1-DCE suppressed the amount of PB-induced CYP2B mRNA to the same level as that of the constitutive one, suggesting that 1,1-DCE affected the expression of CYP2B mRNA transcriptionally in the absence of PB and both transcriptionally and pretranscriptionally in the presence of PB. Concerning hepatic CYP2E1, no alternation was observed in the level of mRNA by any of the CEs in the absence or presence of PB, except for a marked decrease in the amount of mRNA when the rats were treated with 1,1-DCE in combination with PB. In the lung, the CYP2B mRNA level was lowered by *ca.* 50% exclusively by 1,1-DCE irrespective of the PB coexistence, while CYP2E1 mRNA was not detected under the given assay conditions. Based on these findings, in combination with those for the enzyme activity and protein levels, it is suggested that 1,1-DCE suppresses the induction of hepatic CYP2B and 2E1 in advance of the transcriptional stage. The expression of pulmonary CYP2B was obstructed by PCE posttranslationally in the absence of PB.

In addition, both hepatic and pulmonary CYP2Bs were identified as CYP2B1, although they were clearly distinguishable from each other by their sensitivities to PB-induction.[7)]

Time-Dependent Effects of CEs on the Expression of CYP mRNAs in Rats

The animals were i.p. inoculated with individual CEs and sacrificed at 6-hr intervals for 30 hr for the determination of the mRNA levels of hepatic and pulmonary CYP2B and hepatic CYP2E1.[8)] A 3.5-fold increase in the expression of hepatic CYP2B mRNA was noted transiently at 6 hr in the presence of PCE. In contrast, 1,1-DCE was suppressive and started within 6 hr and lasted for more than 30 hr, with a trough value of 15% of the control being ob-

served around 12 to 18 hr, while there was no marked effect observed in the case of TCE-treatment. The expression of pulmonary CYP2B mRNA was severely suppressed in the presence of 1,1-DCE during the entire observation period as was the case of its hepatic counterpart, while the temporarily enhanced expression of mRNA at 6 hr by PCE and TCE was followed by a moderate suppression, with the trough values of *ca*. 80 and 65% of the control, respectively, at 18 hr. Concerning the hepatic CYP2E1, the expression of mRNA was adversely affected by all the CEs with a descending order of magnitude of 1,1-DCE, TCE and PCE with the peak inhibition values of 85, 50 and 35%, respectively. The nonselective suppressive effect of 1,1-DCE on the expression of divergent CYP mRNAs was well correlated with the IL-1β-dependent suppression of various types of CYP mRNA in primary cultured hepatocytes. TCE and PCE are less toxic in terms of the inhibition of the mRNA expression of CYP forms in acute phases than short-lived 1,1-DCE, which is inflammatory to the host animals; the shorter the environmental life span, the more severe the acute toxicity.

Specificity in the Metabolic Activation of CEs by CYP Forms in Primary Rat Hepatocytes

In order to study the roles of CYPs, which have a wide range of substrate specificities, in the metabolic activation of PCE, TCE and 1,1-DCE, the cytotoxicity of individual CEs was tested in primary cultured hepatocytes established from animals treated with various CYP-inducers such as 3-methylchoranthrene (inducer of CYP1A1/2), phenobarbital (CYP2B1/2) and pyridine (CYP2E1). The cytotoxicity of CEs measured by lactate dehydrogenase leakage after 24 hr was enhanced in different fashions, depending on the CYP inducers used. The results are summarized as follows: CYP1A1/2 and 2B1/2 raised the cytotoxicity of PCE; CYP2B1/2 was exclusively associated with the enhanced cytotoxicity of TCE; and CYP2E1 and 2B1/2 were proved to potentiate 1,1-DCE.[9] Based on these observations, CEs differing in the number of chlorine substitutions were disclosed to have divergent preferences for CYPs, by which they were metabolically activated.

Putative Molecular Basis for the Inhibition by 1,1-DCE of PB-Induced Hepatic CYP2B1 mRNA

Recently, the PB-induction of CYP2B mRNA was found to be mediated by constitutively activated

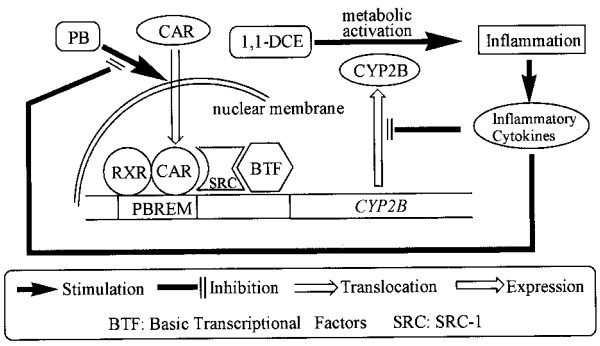

Fig. 1. Model for the Inhibition of PB-Dependent Induction of Hepatic CYP2B mRNA by 1,1-DCE

receptor (CAR), a member of the nuclear receptor (NR) gene superfamily.[10] After being nuclear translocated in the presence of PB, CAR dimerizes with another NR member refered to as retinoid X receptor (RXR) to transactivate the expression of CYP2B by binding to the PB-responsive enhancer module (PBREM) located upstream of the CYP2B genes.[11–13] The irresponsiveness of pulmonary CYP2B1 to the PB-induction was accounted for by the poor expression of CAR in this organ in comparison with the liver (Mizuno *et al*., unpublished data).

As described above, 1,1-DCE suppressed the expression of CYP2B mRNA *in vivo* transcriptionally in the absence of PB and both transcriptionally and pretranscriptionally in the presence of PB.[7] In contrast, 1,1-DCE was found to potentiate the expression of CYP2B mRNA in primary cultured hepatocytes (Nakahama *et al*., unpublished data). Reportedly, the inflammatory cytokines adversely affect the expression of CYPs non-specifically in primary cultured hepatocytes.[14,15] Therefore, 1,1-DCE would be much more inflammatory to the host animals than TCE and PCE, and the observed effects of 1,1-DCE on the expression of CYP2B1 *in vivo* would be attributable to the blocked function of CAR indirectly by newly mobilized inflammatory cytokines (Fig. 1).

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