Comparative Study on Correlation between Chemical Structure and Effect on Expression of Cytochrome P450 mRNAs in Rat among Chlorinated Ethylenes, Tetrachloroethylene, Trichloroethylene, 1,1-Dichloroethylene

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Three polychlorinated ethylenes (CEs), tetrachloroethylene (PCE), trichloroethylene (TCE) and 1,1-dichloroethylene (DCE), were comparatively studied for their effects on the expression of cytochrome P450 (CYP) mRNA forms in the liver and lung using 7-weeks-old male Wistar rats. The animals were i.p. inoculated with 0.5 g/kg of the 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. 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 observed around 12 to18 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 with 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 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 previously reported. Although PCE and TCE are under stringent legislative restriction in Japan as environmental pollutants owing to their relatively stable natures, they 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 (1,1-DCE > TCE > PCE), the more severe the acute toxicity.

Key words —— tetrachloroethylene, trichloroethylene, 1,1-dichloroethylene, CYP2B mRNA, CYP2E1 mRNA

INTRODUCTION

Phase I drug-metabolizing enzymes (DMEs), most of which represent cytochromes P450 (CYP), metabolically activate xenobiotics to genotoxic electrophilic intermediates, and phase II DMEs conjugate the intermediates to water-soluble derivatives, completing the detoxification cycle. The genetic differences in the regulation, expression and activity of the genes coding for phase I and phase II DMEs are crucial factors in defining the host susceptibility to the toxicity or carcinogenicity of environmental chemicals, also referred to as xenobiotics. Xenobiotics frequently induce DMEs involved in their detoxification. However, they are also suppressive to CYPs responsible for the formation of toxic intermediates.[1,2] Polycyclic aromatic hydrocarbons (PAHs) represented by 3-methylcholanthrene (3-MC) and chlorinated ethylenes (CEs) are among those metabolically detoxificated by DMEs, via the formation of toxic intermediates attributable to the function of CYPs. PAHs are metabolically activated by CYP1A, which can in turn be inducible by PAHs. However, the 3-MC -dependent induction of CYP1A was down-regulated in the presence of an excess amount of 3-MC by so far unknown mechanisms, by which the acute adverse effects of toxic intermediates are reversed.[3,4]

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The authors have been studying whether the putative host defense mechanisms against the acute toxicity of the metabolic intermediates of CEs are mobilized in CE-treated rats as in the case of PAHs.[5–9] When the animals were treated with 1,1-DCE for 24 hr, severe suppressive effects on the expression of phenobarbital (PB)-induced mRNAs of hepatic CYP2B and 2E1 were observed. In addition to mRNA, the PB-induced apoprotein of hepatic CYP2B was found to be highly susceptible to the suppressive effect of 1,1-dichloroethylene (DCE). 1,1-DCE was also suppressive on the expression of constitutive mRNAs of CYP2B and 2E1 of both hepatic and pulmonary origins to a lesser extent. In marked contrast with the cases of 1,1-DCE, highly chlorinated tetrachloroethylene (PCE) and trichloroethylene (TCE) did not show any marked effect on the expression of CYP2B and 2E1, irrespective of the coexistence of PB. However, the details remain unclear as to the divergent effects of CEs differentiated from each other by the numbers and positions of chlorinations. In order to answer these questions, in the present work the time dependent profiles of the effects of PCE, TCE and 1,1-DCE on the expression of CYP forms were studied comparatively at the level of transcription.

MATERIALS AND METHODS

Reagents —— PCE and TCE were purchased from Wako Pure Chemicals Ind. (Osaka, Japan). 1,1-DCE was from Aldrich Chemical Co. (U.S.A.) Hybond-N+ was from Amersham Pharmacia Biotech (U.K.). G3PDH cDNA control probe was purchased from Clontech Laboratories. BcaBEST labeling kit (TAKARA, Japan) and [α-32P]dCTP were used for the labeling of probes.

Animals and Treatment —— Male Wistar rats (Clea, Japan; 7-weeks-old) were i.p. injected with PCE, TCE or 1,1-DCE (0.5 g/kg body weight). The lungs and livers were removed from the animals 0, 6, 12, 18, 24 and 24 hr after the treatment, with 3–6 animals in each group.

Total RNA Preparation —— Total RNA was prepared with an RNeasy kit (Quiagen, Germany) from the liver and lung tissues. RNA yield and purity were assessed by A260/A280 ratio.

Northern Blotting —— Total RNA (30 μg) was separated electrophoretically on a denatured 1.2% agarose/2.2 M formaldehyde gel and stained with ethidium bromide for the analysis of mRNA. RNA was transferred onto Hybond-N+ using a capillary blotting unit (Scotlab, U.K.). The membrane was hybridized separately with either a CYP2B, CYP2E1 or G3PDH cDNA probe as described previously.[5] The membrane was exposed to an Imaging Plate and analyzed by Storm TM830.

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

RESULTS

Time Profiles of the Expression Levels of CYP2B mRNA in the Livers of CE-Treated Rats

A temporary 3.5-fold increase in the expression of CYP2B mRNA in the livers of rats treated with PCE was observed 6 hr after the treatment, returning to the control level by 18 hr (Fig. 1A). Although TCE did not show any marked effect on the expression of hepatic CYP2B mRNA (Fig. 1B), a potent suppressive effect of 1,1-DCE was noted as early as 6 hr after the treatment, reaching a maximum value of 85% inhibition at 18 hr and returning to the control level by 30 hr (Fig. 1C).

Time Profiles of the Expression Levels of CYP2B mRNA in the Lungs of CE-Treated Rats

In the presence of PCE and TCE, a temporary increase in the expression of CYP2B was observed at 6 hr, although it was not significant in the case of PCE, followed by a gentle suppression with the maximum inhibition of 20–35% at 18 hr (Figs. 2B and 2C). In contrast, 1,1-DCE showed a much more severe effect on the mRNA expression, with a maximum inhibition of 85% observed at 12 hr. The suppressive effect of 1,1-DCE recovered gradually, however, ca. 45% inhibition still remained at 30 hr (Fig. 2C).

Time Profiles of the Expression Levels of CYP2E1 mRNA in the Livers of CE-Treated Rats

The expression of hepatic CYP2E1 mRNA suffered from the suppressive effect of all three CEs continuously, from soon after the treatment up to 30 hr in an ascending order of magnitude of PCE (the highest inhibition of 35% shown at 18 hr, Fig. 3C), TCE (50% at 18 hr, Fig. 3B) and 1,1-DCE (85% at 18 hr, Fig. 3C).

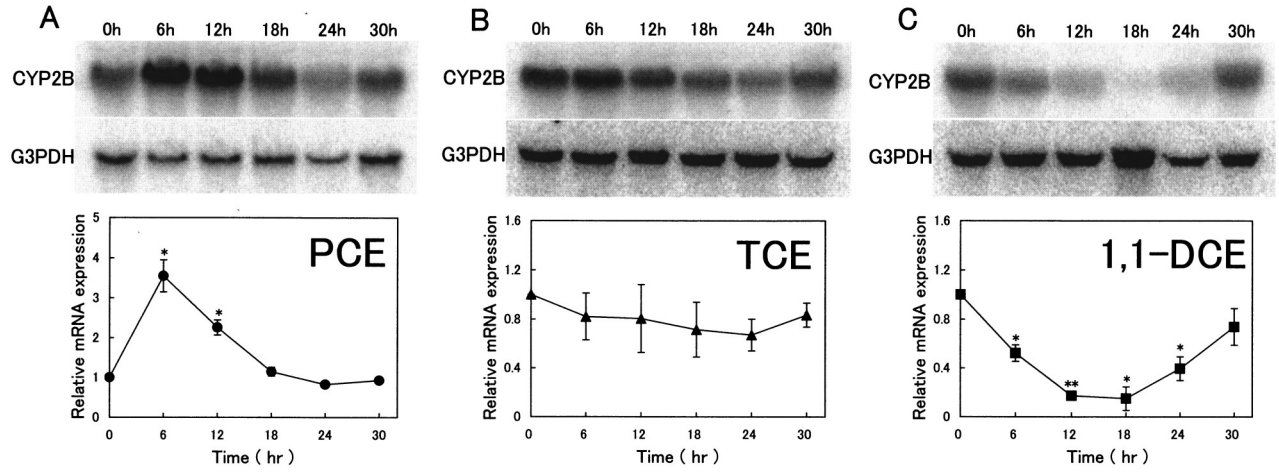

Fig. 1. Time-Course of the Expression Levels of CYP2B mRNA in the Livers of CE-Treated Rats

Total RNA was isolated from the livers of animals i.p. injected with individual CEs (0.5 g/kg body weight) and a 30 μg-aliquot was used for Northern blots. The readings are the ratios of the band intensity of CYP2B mRNA to the band intensity of G3PDH mRNA normalized by the ratios of the untreated rats, representing the mean +/– S.E. for 3–6 animals. Panel A, PCE-treated rats; Panel B, TCE-treated rats; Panel C, 1,1-DCE-treated rats. * and **: significantly different from the 0-hr control ($p < 0.05$ and $p < 0.01$, respectively).

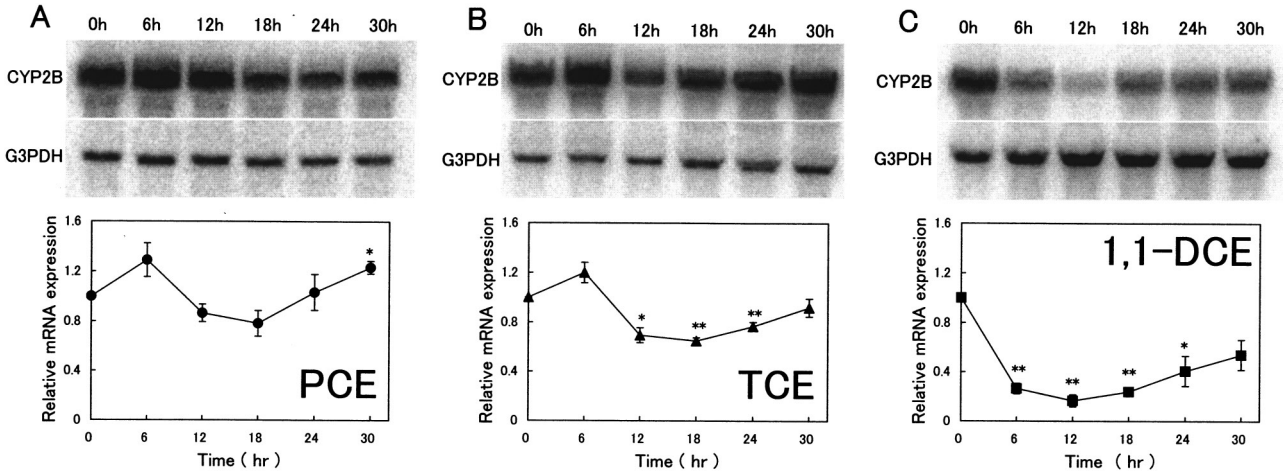

Fig. 2. Time-Course of the Expression Levels of CYP2B mRNA in the Lungs of CE-Treated Rats

The experimental procedures are the same as those shown in the legends to Fig. 1 except that lungs were used instead of livers.

DISCUSSION

The authors report that the expression of hepatic CYP2B was suppressed by 1,1-DCE transcriptionally, and that of hepatic CYP2E1 both transcriptionally and post translationally. In general, the detection of CYP2B in rat liver remains to be induced in terms of ethoxyresorufin *O*-deethylase (EROD) activity, in marked contrast to its constitutive pulmonary counterpart.[2] Since the expression of PB-induced CYP2B mRNA was susceptible to the inhibitory effect of 1,1-DCE to a much greater extent than the basal constitutive one, the signal transduction triggered by PB was proposed as a candidate for the targeting of 1,1-DCE.[5]

In rat hepatocyte primary culture, CEs including 1,1-DCE were apt to work as inducers of CYP forms (Nakahama *et al.*, unpublished). Therefore, the suppressive effects of CEs, particularly 1,1-DCE, observed in the whole animals were not really considered to be direct effects. Abdel-Razak *et al.* (1995)[10] reported that IL-1β strongly inhibited basal CYP1A and 2B activities as well as their induction by PB dose–dependently. Furthermore, IL-1β was found to be suppressive on the PB-induced mRNA expression of all kinds of CYP forms tested, *i.e.*,

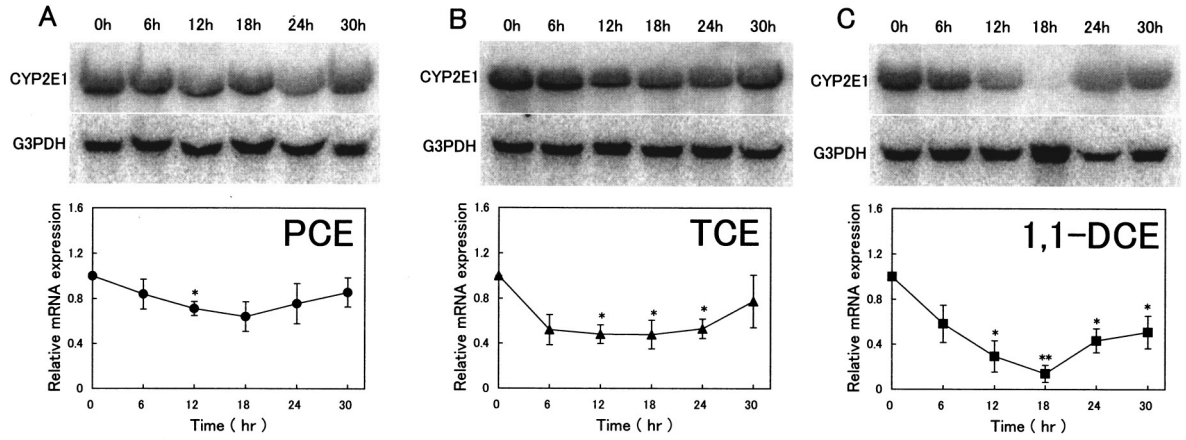

Fig. 3. Time-Course of the Expression Levels of CYP2E1 mRNA in the Livers of CE-Treated Rats

The experimental procedures are the same as those shown in the legends to Fig. 1 except that CYP2E1 mRNA was analyzed instead of CYP2B mRNA.

CYP1A, 2B, 2C, 2E and 3A, in cultured hepatocytes. According to the report of Carlson *et al.* (1996), the suppressive effects of IL-1β and TNF-α on the expression of CYP2B and 3A2 proteins were blocked by the inclusion of nitric oxide synthase (NOS) inhibitors, suggesting the down-regulation of CYP forms *in vitro* is directly associated with NO production, whereas the CYP down-regulation by IL-6 seemed dissociated with NO production.[11]

Taking into consideration the similarity between the phenomena observed with 1,1-DCE in the whole animals and IL-1β in cultured primary hepatic cells, the authors proposed the following explanations. 1,1-DCE was easily metabolically activated by CYP forms *in vivo*, causing acute toxicities accompanied by severe inflammation, which in turn induced the release of various cytokines represented by IL-1β, which were responsible for the nonspecific down-regulation of the expression of various CYP forms as well as the PB-induced ones.

In order to study the *in vivo* events caused by CEs in more detail, the time-dependent effects of CEs on the expression of CYP forms *in vivo* were partly clarified. The reduced expression levels of mRNAs of hepatic CYP2B and 2E1 and pulmonary CYP2B were observed as early as 6 hr post-treatment in the case of 1,1-DCE. However, about a 3.5-fold increase in the expression of hepatic CYP2B mRNA was noted when the animals were treated with PCE, and the effects on the other CYP mRNA levels were negligible. The results for TCE were intermediate between those of 1,1-DCE and PCE. Highly chlorinated CEs, PCE and TCE, are well known environmental contaminants due to their long-lived natures, with PCE being more stable than TCE. Their relative environmental stabilities were well conserved in their *in vivo* behaviors; PCE was most stable and 1,1-DCE was most labile. In cultured primary hepatocytes and hepatoma cell lines, CEs generally work as the inducers of CYP forms by which they are metabolically intoxicated (Nakahama *et al.*, unpublished data). Therefore, CEs could be the inducers of certain CYP forms in nature, even *in vivo* as was shown by the results for PCE. However, the active intermediates of CEs metabolized by CYP forms could be inflammatory *in vivo* if a given CE were toxic in an acute phase. Because the inflammatory events were highly suppressive on the expression of CYP forms, it could be considered as a kind of homeostatic response to the toxic xenobiotics.

The irresponsiveness of pulmonary CYP2B mRNA to PCE was in marked contrast to the inductive response of its hepatic counterpart, as was the case with PB-treatment.[5] As well as hepatic CYP2B, hepatic CYP2E1 and pulmonary CYP2B were susceptible to the suppressive effect of 1,1-DCE. Together with the lack of CYP form-specificity, the long-lasting suppressive effect of 1,1-DCE might be mediated by the inflammatory cytokines such as IL-1β and TNFα. Although the present study was concerned with the acute phase, the chronic toxicity should be more important from the viewpoint of environmental pollution, by which human life has been gradually threatened. Therefore, the negligible effect of PCE and the intermediate effect of TCE in an acute phase do not preclude their potent risk as biohazards.

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