J. Biochem. Mol. Biol. Vol. 30, No. 4, pp. 237~239 July 31, 1997

Effect of Trichloroethylene on the Induction of Rat Liver Microsomal Enzymes

Sung-Keun Chang*, Hyo Seok Jeong, Seok Chai, Ki-Woong Kim[1] and Sang Shin Park[1]

Department of Chemistry, College of Natural Science, Soonchunhyang University, Asan-Si 336-745, [1]Industrial Health Research Institute, Korea Industrial Safety Corporation, Incheon 403-120, Korea

(Received February 27, 1997)

Abstract : The effects of trichloroethylene (TRI) on the induction of cytochrome P-450 (CYP) and several other related enzymes in Sprague Dawley rats were investigated. Rats were treated with TRI 150, 300, 600 mg/kg body weight in corn oil intraperitoneally once a day for 2 days. The total contents of microsomal CYP and cytochrome b_5 (b_5) decreased with the increase of TRI concentration, but the activity of p-nitrophenol hydroxylase increased with the increase of TRI dosage ($p<0.05$). Western blot analysis which utilized monoclonal antibodies against CYP2E1 also showed a significant increase in the CYP2E band density. The increase of the activity of pentoxyresolufin-O-deethylase also was observed with the TRI treatment ($p<0.05$) although there was no significant increase in the cytochrome CYP2B1/2 in Western blotting. The TRI did not affect the induction of aryl hydrocarbon hydroxylase. These findings suggest that the CYP2E1 is the primary enzyme which could be induced by TRI treatment in rats.

Key words : cytochrome P-4502E, cytochrome P-450 monooxygenase, trichloroethylene

Trichloroethylene (TRI) is one of the most widely used organic solvents in industries. It is used to degrease metals and to extract many substances which are soluble in the solvent (Arlien-Soborg, 1992). Accordingly, TRI has been studied by many investigators. It was reported that TRI was metabolized by liver microsomal mixed-function oxidase (MFO) system containing cytochrome P-450 (CYP) to unstable reactive intermediates (Ikeda and Harada, 1980; Nakajima *et al.*, 1992) which were responsible for hepatotoxicity (Allemand *et al.*, 1978). The reactive intermediates were metabolized to trichloroethanol (TCE) and trichloroacetic acid (TCA) by alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) in liver and the metabolites were excreted in urine (Daniel, 1963; Fernandez *et al.*, 1977; Miller and Guengerich, 1982). TRI studies in humans and animals have shown that TRI caused some toxicity and morphological changes in liver and neurotoxicity (Kulig, 1987; Okino *et al.*, 1991). Okino *et al.* (1991) reported that cytochrome CYP content decreased even in the groups treated with less than 0.68 g TRI/kg body weight. Kim *et al.* (1994) also reported that TRI produced dose-dependent decreases in CYP content in liver microsomes, but the activity of ALDH of microsomes increased. Thus, this study was conducted to examine which CYP isozymes and other related enzymes would be affected by TRI treatment.

*To whom correspondence should be addressed.
Tel : 82-418-530-1240, Fax : 82-418-530-1247

Materials and Methods

Chemicals

Cytochrome C, NADPH, NADH, sucrose, m-xylene, ethoxyresorufin, 7-pentoxyresorufin and *p*-nitrophenol were purchased from Sigma Chemical Co. (St Louis, USA). Western immunoblotting kits was purchased from Bio-Rad Inc. (California, USA). All other chemicals were obtained commercially and they were of analytical grade.

Animals and preparation of liver microsomes

Male Sprague-Dawley rats (6 weeks old, body weight 170±10 g) were used for the source of liver microsomes. They were maintained in a temperature-controlled room with 12-h periods of light and darkness. Groups of rats were given intraperitoneal injections of 0, 150, 300, and 600 mg TRI/kg body weight in corn oil and ethanol group was orally treated with ethanol (4 g/kg body weight) once a day for two days. Rats were anaesthetized with sodium pentobarbital and the livers were quickly removed, placed in ice-cold 0.9% NaCl, homogenized in 0.25 M sucrose and subjected for centrifugation at 12,000 g for 40 min. The microsomal

fraction was obtained from the 12,000 g supernatant by centrifugation for 60 min at 105,000 g and resuspended in 0.25 M sucrose (Park and Kim, 1984).

Enzyme assays

The CYP content in liver microsomal fraction was determined by the method of Omura and Sato (1964).

Ethoxyresorufin-*o*-deethylase (EROD) activity was spectrophotometrically measured as described by Klotz *et al.* (1984). *p*-nitrophenol-hydroxylase (*p*NPH) and pentoxyresolufin-o-deethylase (PROD) activities were determined by the method of Koop (1986) and the method of Lubet *et al.* (1985), respectively. Aryl hydrocarbon hydroxylase (AHH) activity was measured by the method of Nebert and Gelboin (1968). NADPH-Cytochrome P-450 (P-450) reductase and NADH b_5 reductase activities were determined by the methods of Masters *et al.* (1967) and Hultqust (1978), respectively.

Immunoblot analysis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE, 8.5%) was done by the method of Laemmli (1970) using a Bio-Rad Protein II xi Cell aparatus. Western Immunoblotting of P450 isozymes were carried out as described previously (Ko *et al.* 1987) using monoclonal anti-mouse CYP2B1/2 (MAb 2-66-3)(Park *et al.* 1983) and CYP 2E1/2 (MAb 1-98-1) antibodies (Ko *et al.* 1987). Liver microsomal proteins were separated by 8.5% SDS PAGE and electrophoretically transferred to nitrocellulose membranes. The membranes were incubated with either CYP2B1/2 and CYP2E1/2 antibodies (ascite, 500 μg/ml). Alkaline phosphatase-conjugated goat anti-mouse IgG (Bio-Red) was used as the secondary antibody for CYP2B1/2 and CYP2E1/2. Colour was developed using BCIP/NBT solution (Sigma Chemical Co.). Colour densities were read at 575 nm utilizing a UV/VIS Spectrophotometer (Beckman, USA).

Results

The effects of increasing dose of TRI on the total CYP and b_5 contents are presented in Table 1. Administration of TRI to rats resulted in a dose-dependent decrease of the total content of CYP and b5, but it was not statistically significant ($p<0.05$). The enzymatic activities of CYP, pNPH, PROD, AHH and EROD are shown in Table 2.

*p*NPH activity increased drastically with the increase of TRI administration dose ($p<0.05$), and PROD and EROD activities also increased in the treated groups, but the statistical significance was found only in the PROD activity ($p<0.05$). NADPH-P-450 reductase activity was decreased, and NADH-b_5 reductase increased with the increase of TRI administration dosage, but the statistical significance was found only in the groups of 300 and 600 mg/kg of TRI administration ($p<0.05$) (Table 3). Immunoblot analysis of CYP2E1 is shown in Fig. 1. The immunoblot densities of CYP2E1 was enhanced by the increase of TRI treatments, 300 and 600 mg/kg body weights which indicated that TRI cuould induce CYP2E1. We also observed the increase of PROD activity by the treatment of TRI., but we could not observe clearly the effect of TRI on the CYP2B1/2 band densities (data not shown).

Table 1. Effect of trichloroethylene on the content of hepatic proteins

TRI (mg/kg)	Microsomal Protein (mg/ml)	Cytochrome P-450 (nmol/mg)	Cytochrome b_5 (nmol/mg)
Control	5.81±0.541	1.03±0.356	0.32±0.067
150	5.65±0.533	0.98±0.253	0.30±0.066
300	5.69±0.718	0.85±0.131	0.27±0.068
600	5.92±0.984	0.75±0.106	0.24±0.042

Values in the table are mean±S.D.
All F-values are not statistically significant.

Table 2. Effect of trichloroethylene on the catalytic activities of hepatic microsomal P-450 of rats

TRI (mg/kg)	Catalytic activity (pmole/mg protein/min)			
	AHH[a]	EROD	PROD	pNPH
Control	18.4±0.14	3.80±0.69	7.5±1.40	134.6±12.80
150	18.9±0.57	4.50±1.17	17.9±2.82[b]	291.3±14.85[b]
300	19.5±0.28	4.45±0.58	28.3±3.57[b]	352.9±15.28[b]
600	17.9±0.28	4.90±0.81	19.3±5.71[b]	643.8±23.41[b]

Values represent the mean±S.D.
[a]1 unit corresponds to 18 pmol/mg protein/min AHH, aryl hydrocarbon hydroxylase; EROD, ethoxyresolufin-O-deethylase; PROD, pentoxyresolufin-O-deethylase; *p*NPH, *p*-nitrophenol hydroxylase.
[b]Significant difference from control values ($p<0.05$).

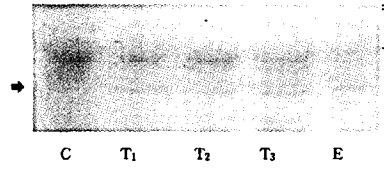
Table 3. The contents of NADPH-Cytochrome P-450 reductase and NADH-Cytochrome b_5 reductase in liver microsomes from rats treated with trichloroethylene

TRI (mg/kg)	NADPH P-450 reductase (μmol/min/mg)	NADH-b_5 reductase (μmol/min/mg)
Control	0.75±0.096	103.40 ±17.393
150	0.72±0.137	116.34 ±15.497
300	0.69±0.102	139.62[a]±30.249
600	0.68±0.108	144.02[a]±17.411

Values represent the mean±S.D of four rats.
[a]Significant difference from control values ($p<0.05$).

Discussion

The toxicity and pharmacokinetics for absortion, dis-



Relative absorbance of CYP2E1 bands at 575 nm

Groups	Abs
Control	0.42±0.099
T_1	0.42±0.016
T_2	0.64±0.018
T_3	0.58±0.053

Fig. 1. Western immunoblot analysis of liver microsomes of rats treated with TRI utilizing monoclonal anti-mouse rat CYP 4502E1 antibodies. Liver microsomes (200 μg) were loaded for control and TRI treated groups (150, 300, 600 mg TRI/kg body weight, 2 consecutive days, killed 24 h after the last injection). C, control; T_1, 150 mg/kg; T_2, 300 mg/kg; T_3, 600 mg/kg; E, Ethanol 4 g/kg; ➡, CYP2E1 band identified with monoclonal antibody against CYP2E1. Relative absorbance of CYP 2E1 bands represents the mean±S.D for three measurments.

tribution, metabolism and excretion of TRI in animals and humans have been studied as the subjects of many investigators (Miller and Guengerich, 1982; Kulig, 1987; Okino *et al.*, 1991; Kim *et al.*, 1994). Costa *et al.* (1980) reported that CYP metabolized TRI and generated reactive intermediates which were transformed to TCE-OH and TCA by ADH and ALDH, respectively and they were excreted in urine. The TCE-OH was the major metabolite of TRI. Although the involvement of CYP for the metabolism of TRI was known, it is not known which CYP isozyme is responsible for TRI metabolism.

Kim *et al.* (1994) reported that TRI treatment caused a decrease in the hepatic microsomal NADPH-P-450 reductase activity and the contents of CYP and b_5. Rouisse and Chakrabarti (1986) reported that the activities of aniline hydroxylase and aminopyrine N-demethylase were decreased in the rats treated with TRI. Guengerich *et al.* (1991) showed that the human CYP2E1 is involved in TRI metabolism. Nakajima *et al.* (1992) reported that the CYP2E1, CYP1A1/2, CYP2C 6/11 and CYP2B1/2 were involved in the metabolism of benzene, toluene and TRI. We also found that the content of CYP, b_5 and the activity of NADPH-P-450 reductase were decreased by the treatment of TRI. In this study, we observed that PROD and pNPH activities were increased by TRI treatment. But AHH and EROD activities were not affected.

The immunoblot analysis which utilized monoclonal antibodies against CYP2E1 also showed a significant increase in the CYP2E1 band density at 300 mg TRI/kg body weight, but it was not doubled at 600 mg TRI/kg body weight indicating that the high dose of TRI gave some toxic effect on the induction of CYT2E1. The increase in the activity of PROD by TRI was significant ($p<0.05$) but there was no significant increase in the CYP2B1/2 band densities in immunoblotting. Therefore the activity of PROD might be due to some other CYP isozymes. This study suggested that the CYP2E1 is the primary enzyme which could be induced by TRI and metabolize TRI in rats.

Acknowledgements

This research was supported in part by Korean Ministry of Education through Research Fund (BSRI-96-3447).

References

Allemand, H., Pessayre, D., Descatoire, V., Deggott, C., Fildmann, G. and Benhamou, J. P. (1978) *J. Pharmacol. Ther.* **204**, 714.

Arlien-Soborg, P (1992) in Solvent neurotoxicity (Arlien-Soborg, P. ed.) pp 259-284, CRC Press, Florida.

Costa, A. K., Katz, I. D. and Ivantich, K. M. (1980) *Biochem. Pharmacol.* **29**, 433.

Daniel, J. W. (1963) *Biochem. Pharmacol.* **12**, 795.

Fernandez, J. G., Droz, P. O., Humbert, B. E., and Caperos, J. R. (1977) *Br. J. Ind. Med.*, **34**, 43.

Guengerich, F. P., Kim, D. H. and Iwasaki, M. (1991) *Chem. Res. Toxicol.* **4**, 168.

Hultqust, D. E. (1978) *Methods Enzymol.* **52**, 463.

Ikeda, M. and Harada, I. (1980) *Jap. J. Ind. Health.* **22**, 3.

Kim, K. W., Kang, S. K., Yang, J. S., Park, I. J. and Moon, Y. H. (1994) *Korean Ind. Hyg. Assoc. J.* **4**, 148.

Klotz, A. V., Stegeman, J. J., Walsh, C. (1984) *Anal. Biochem.* **140**, 138.

Ko, I. Y., Park, S. S., Song, B. J., Patten, C., Tan, Y., Hah, Y. C., Yang, C. S. and Gelboin, H. V. (1987) *Cancer Res.* **47**, 3101.

Kulig, B. M. (1987) *Neurotoxicol. Teratol.* **9**, 171.

Laemmli, U. K. (1971) *Nature* **227**, 680.

Lubet, R. A., Meyer, R. T., Cameron, J. W., Nims, R. W., Burke, M. D., Wolff, T. and Guengerich, R. P. (1985) *Arch. Biochem. Biophys.* **238**, 43.

Masters, B. S. S., Williams, C. H. Jr. and Kamin, H. (1967) *Methods Enzymol.* **10**, 565.

Miller, R.E. and Guengerich, F. P. (1982) *Biochemistry* **21**, 1090.

Nakajima, T., Wang, R. S., Elovaara, E., Park, S. S., Gelboin, H. V. and Vainio, H.(1992) *Biochem. Pharmacol.* **43**, 251.

Nebert, D. W. and Gelboin, H. V. (1988) *J. Biol. Chem.* **234**, 6242.

Okino, T., Nakajima, T. and Nakano, M. (1991) *Toxicol. Appl. Pharmacol.* **108**, 379.

Omura, T. and Sato, R. (1964) *J. Biol. Chem.* **239**, 2370.

Park, K. H. and Kim, C. R. (1984) *Korean Biochem. J. (presently J. Biochem. Mol. Biol.)* **17**, 10.

Park, S. S., Fujino, T., Miller, H., Guengerich, F. P., and Gelboin, H. V. (1983) *Biochem. Pharmacol.* **33**, 2071.

Rouisse, L. and Chakrabarti, S. K. (1986) *Environ. Res.* **40**, 450.