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Lipid Peroxidation Induced by Trichloroethylene in Rat Liver

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It is well-known that trichloroethylene (TRI) is metabolized by cytochrome P-450 to TRI oxide, which binds irreversibly to cell macromolecules to generates hepatic damage (Bolt and Filser 1977; Leibman 1965; Ikeda et al. 1980). TRI oxide was metabolized to Chloral (Powel 1945) and Chloral hydrate (Byington and Leibman 1965; Leibman 1965; Costa et al. 1980; Ikeda et al. 1980; Nakajima et al. 1990) as an intramolecular rearrangement product of TRI oxide. However, recent studies have demonstrated that TRI oxide is not an obligate intermediate in the conversion of TRI to chloral (Miller and Guengerich 1982, 1983). Therefore, there is no satisfactory explanation about the hepatic toxicity of TRI.

On the other hand, the hepatic toxicity of halogenated compounds, e.g. carbon tetrachloride and bromotrichloroethane, are said to be closely related to lipid peroxidation (Slater 1966; Comporti 1986). TRI enhances carbon tetrachloride hepatotoxicity in association with lipid peroxidation (Pessayre et al. 1982). There are few reports on TRI and lipid peroxidation because TRI-induced hepatotoxicity is mild. In this report, we studied the effect of TRI on lipid peroxidation in vivo and in vitro.

MATERIALS AND METHODS

Male Wistar rats each weighing 200 to 250 g were used. TRI (2000 mg/kg, intraperitoneally, in olive oil) was administered. Blood was collected from the aorta at various time intervals under light ether anesthesia. Serum glutamic pyruvic transaminase (GPT) was estimated using commercial reagent kits of Boehringer, Mannheim. The liver was perfused with ice-cold NaCl solution and a part was homogenized in 1.15% KCl solution and lipid peroxide level in the liver was measured by the thiobarbituric acid (TBA) method (Ohkawa et al. 1979). The liver of untreated rat was perfused with ice-cold NaCl solution and a part was homogenized in 3 volumes of ice-cold 1.15% KCl with 50 mM Tris HCl, pH 7.4. The homogenate was centrifuged for 20 min at 9000 x g and the supernatant was recentrifuged at 105,000 x g for 90 min.

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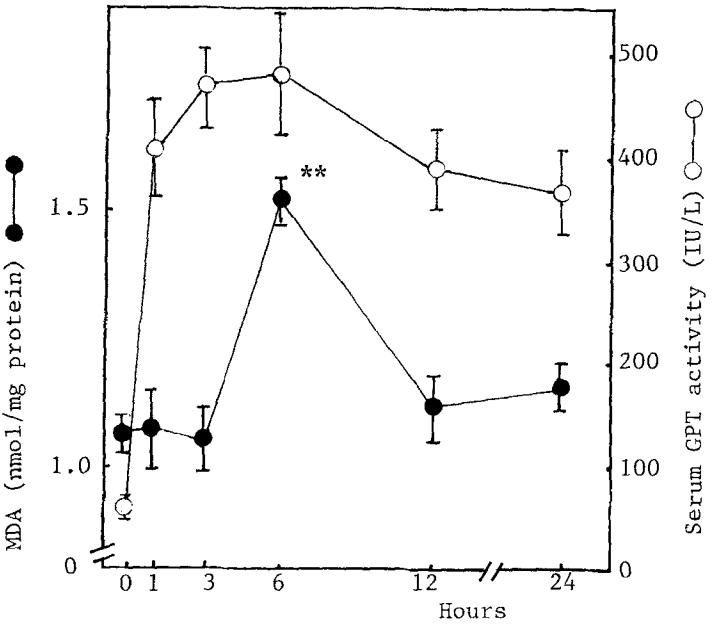

Figure 1. Changes in hepatic malondialdehyde and serum GPT activity after administration of trichloroethylene. Each value represents the mean ± SE of 6 rats. **p < 0.01

The microsomal pellet was washed twice and resuspended in homogenate buffer. Liver microsomes (3 mg protein/ml) were incubated with 0-10 mM TRI (5 µl dimethylsulfoxide) and a freshly prepared NADPH-generating system in 50 mM Tris HCl, pH 7.4. The NADPH-generating system contained NADP, 0.17 mM; glucose-6-phosphate, 3.3 mM; $MgCl_2$, 8.3 mM; nicotinamide, 3.3 mM; and 2 units of D-glucose -6-phosphate dehydrogenase. After a 30-min incubation at 37°C, the lipid peroxide level was measured as malondialdehyde (MDA) formation using the thiobarbituric acid method (Buege and Aust, 1975). TRI metabolism was assessed by measuring the rate of chloral hydrate (CH) formation according to the method of Nakajima et al. 1990 using high-performance liquid chromatography. Protein assay was performed using a Bio-Rad Protein Kit (Bradford, 1976).

RESULTS AND DISCUSSION

Serum GPT activity showed peak values at 3 h and 6 h after administration of TRI (Figure 1). Hepatic MDA values at 6 h after administration of TRI increased, being 1.4 times the control value (Figure 1). In an in vitro assay with microsomes, TRI enhanced lipid peroxidation in a dose-dependent manner (Table 1). The final concentration of DMSO, the solvent of TRI, was 0.16% and this concentration of DMSO did not alter the MDA value. It is said that halogenated compounds induce lipid peroxidation by the process of

Table 2. Effect of trichloroethylene on microsomal lipid peroxidation and the metabolic rate of trichloroethylene

Compounds	MDA (nmol/mg protein)	Metabolic rate (nmol/mg protein/min)
DMSO	1.86 ± 0.03	0
TRI 0.1 mM	2.03 ± 0.02*	0.33 ± 0.06
TRI 1.0 mM	2.4 ± 0.07**	0.48 ± 0.02
TRI 10 mM	2.45 ± 0.07**	-

Each value represents the mean ± SE of 3 experiments. **$p < 0.01$, *$p < 0.05$

microsomal cytochrome P-450 or glutathione depletion (Fraga et al. 1987). The peroxidizability of tissues by halogenated compounds may depend on the polyunsaturated fatty acid and cytochrome P-450 content. The ability of the halogenated compound to induce lipid peroxidation is dependent on their capacity to form free radicals. This capacity is related to bond dissociation energy, solubility in membrane environments, the rate of metabolism, and the activity of cytochrome P-450 (Slater 1966). Compounds like benzene derivatives induce lipid peroxidation in spleen and testis without the requirement for cytochrome P-450 (Casini et al. 1985). As for TRI, microsomal lipid peroxidation is not enhanced by TRI according to Pessayre et al. (1982). However, the incubation mixture used by those authors contained NADPH, and not a NADPH-generating system. TRI is metabolized easily without $MgCl_2$, nicotinamide and a NADPH-generating system (Ikeda et al. 1980).[2] In our experimental system containing $MgCl_2$, nicotinamide and a NADPH-generating system, TRI was metabolized to CH (Table 2). The amount of CH formed in our experimental system was not changed in the preparation for high-performance liquid chromatographic assay. TRI is volatile and vials for incubation were open. Therefore, the concentration of TRI in the incubation mixture may decrease during the incubation time. The solubility of TRI in water is 7.6 mM (Irish 1963). It is not clear whether TRI at 10 mM dissolved in 0.16% DMSO is soluble in the incubation mixture or not. Generally, microsomal drug metabolism and lipid peroxidation are related "to" each other. Microsomal lipid peroxidation has been reported to be catalyzed by NADPH cytochrome c (P-450) reductase in association with chelated iron (Kamitaki et al. 1978). Electron transfer from NADPH to lipid occurs in NADPH cytochrome c (P-450) reductase-related lipid peroxidation (Hochstein and Ernster 1963). In the oxidation of xenobiotics, two electrons transferred from NADPH to cytochrome P-450 with the aid of cytochrome c (P-450) reductase. Microsomal lipid peroxidation induced by iron inhibited the metabolism of aminopyrine (Willis 1969). Inhibition of microsomal lipid peroxidation by α-tocopherol did not alter drug-metabolizing enzyme activity (Gram and Fouts 1966). Our results clarified that TRI induced lipid peroxidation in the liver. Moreover, elevation of serum GPT preceded the elevation of MDA values in the liver. Therefore, we suggest that lipid peroxidation is the result of other damage rather than its cause as shown by Kappus (1985).

However, the interaction of TRI and drug-metabolizing enzyme or the effect of TRI on glutathione content must be investigated in the future.

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