DEPLETION OF GLUTATHIONE AND HEPATO-TOXICITY CAUSED BY VINYL ETHERS IN MICE

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ABSTRACT—4-Nitrophenyl vinyl ether (NPVE) and phenyl vinyl ether (PVE) administered i.p. in mice lowered hepatic non-protein sulfhydryl (NP-SH) content, but did not elevate the serum glutamate pyruvate transaminase (GPT) activity. n-Butyl vinyl ether (BVE) showed no significant effects either on the NP-SH content or on the serum GPT activity. Mice pretreated with buthionine sulfoximine were sensitive to the potential toxicity of NPVE. These results showed that aryl vinyl ethers, NPVE and PVE, are more toxic than the alkyl vinyl ether, BVE, and that glutathione plays an important role on the protection of hepatic injury by reactive metabolite(s) derived from vinyl ethers.

KEY WORDS : Vinyl ether, Epoxide, Glutathione, Hepatotoxicity.

Various alkyl vinyl ethers are widely used as raw materials of plastic and solvents in chemical industry. Also, fluoroethene, trifluoroethyl vinyl ether, had been used as an inhalation anesthetic. Though the metabolic formation of epoxide has been presumed, there are only a few reports on the metabolism and the toxicity of vinyl ethers (Murphy *et al.*, 1983 ; Kundomal and Baden, 1985 ; Ford *et al.*, 1992). We have carried out *in vitro* studies on the metabolism of 4-nitrophenyl vinyl ether (NPVE) as a model chemical of vinyl ethers. As the results, it was confirmed that NPVE was metabolized to glycolaldehyde and 4-nitrophenol via an unstable epoxide by hepatic microsomes in the presence of NADPH (Isobe *et al.*, 1988). In the previous paper, we reported that epoxides derived from aryl vinyl ethers are mutagenic in the Ames test and that the critical factor for the mutagenicity of vinyl ethers is the accumulation of reactive epoxide intermediates in the rat hepatic activation system (Sone *et al.*,

1989b). These results indicate that vinyl ethers inhaled or incorporated orally should be metabolically transformed to epoxides by hepatic monooxygenases, and potential hepatic damage may be caused. In the present study, *in vivo* tests were conducted to assess the hepatotoxicity of vinyl ethers and the protective role of intracellular reduced glutathione (GSH).

Male ddY mice weighing 35–40 g were given NPVE i.p. (1.8 mmol in 4 ml corn oil/kg body weight) between 9 : 00 and 10 : 00 AM. After the administration of NPVE, hepatic non-protein sulfhydryl (NP-SH) content decreased rapidly to 38% (30 min after) and 22% (1 hr after) of the control (Fig. 1). Then the NP-SH content in liver was recovered to the control level 8 hr after the administration of NPVE. The slight decrease of the NP-SH content observed in the daytime in control mice was within a deviation of the circadian periodicity. There were no significant differences in the serum glutamate pyruvate transaminase (GPT) activity throughout the experimental period between the NPVE-treated

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Fig. 1. Time course changes in mouse hepatic NP-SH content (A) and serum GPT activity (B) after the administration of NPVE. Male ddY mice received NPVE (1.8 mmol/kg, i.p., ●) or corn oil (○) were killed at the indicated time. The NP-SH content in hepatic homogenate was assayed with Ellman's reagent after deproteination with trichloroacetic acid (Sedlak and Lindsay, 1968). The GPT activity in serum was assayed by the method of Reitman and Frankel (1957). Each value represents the mean $\pm$ S.D. of five animals. Data analysis were performed by the use of Student's *t*-test.

** : Significantly different from the vehicle (corn oil) group at $P < 0.01$.

and control mice. In the urine collected 1 hr after the NPVE administration, 4-nitrophenol (NP) and its glucuronide were detected as the metabolites of NPVE. The rapid excretion of NP into urine showed the extensive metabolism of NPVE via the epoxide intermediate. Though the suspected formation of toxic epoxide, hepatic damage was not observed by NPVE. The hepatic GSH content enough to conjugate the toxic epoxide may be responsible for the observed nontoxicity.

Mice received NPVE, phenyl vinyl ether (PVE), butyl vinyl ether (BVE), or vehicle corn oil i.p. were sacrificed 1 hr after the administration, and the hepatic NP-SH content and the serum GPT activity were assayed. Though PVE caused marked decrease (54% of the vehicle) in the NP-SH content at the highest dosage (1.8 mmol/kg) as well as NPVE (17% of the vehicle), significant increase of the serum GPT activity was not observed (Table 1). BVE showed no effects either on the NP-SH content or GPT activity. Also it was confirmed that no significant increase of the serum GPT activity was observed 4, 6, and 8 hr after dosing of NPVE, PVE, or BVE. NPVE which is metabolized to a epoxide with a medium halflife in an aqueous medium (Sone *et*

al., 1989a) lowered the hepatic NP-SH content, but BVE which is metabolized to an unstable epoxide did not. In general, metabolically formed epoxides undergo enzymatic and/or nonenzymatic detoxication by hydrolysis reaction and GSH conjugation. Because highly unstable epoxide such as BVE epoxide formed in the catalytic pocket of cytochrome P-450 may be attacked by water and decomposed to the glycol, consumption of GSH is limited. On the other hand, epoxide with a medium halflife such as NPVE epoxide is susceptible to detoxication by GSH conjugation. Therefore the GSH conjugation of epoxide competes with the covalent binding of epoxide to biomacromolecules which is responsible for cell damage, tissue GSH level may be a critical factor for the hepatotoxicity of vinyl ethers. The effects of vinyl ethers on the hepatic NP-SH content are agreeable with the stability and mutagenicity of the epoxides (Sone *et al.*, 1989b).

It is well known that administration of acetaminophen reduces the hepatic GSH level. After a toxic dose of acetaminophen, GSH was depleted to about 20% or less of the normal level and the covalent binding of radiolabelled acetaminophen to hepatic protein was extremely ele-

Table 1. Effects of vinyl ethers on hepatic NP-SH content and serum GPT activity in mice.

Treatment	Hepatic NP-SH content ($\mu\text{mol/g}$ liver)	Serum GPT activity (mU/ml serum)
Vehicle	7.77 ± 2.21	15.7 ± 6.5
BVE	6.14 ± 1.77	15.0 ± 4.5
PVE	$4.22 \pm 1.54^*$	9.1 ± 6.9
NPVE	$1.31 \pm 0.38^{**}$	12.9 ± 6.3

Mice received each vinyl ether (1.8 mmol/kg, i.p.) were killed 1 hr after the administration. Values represent the mean $\pm$ S.D. of five animals.

*, ** : Significantly different from the vehicle group at $P < 0.05$ and $P < 0.01$, respectively.

vated (Mitchell *et al.*, 1975). To investigate the protective role of GSH against the hepatotoxic effects of vinyl ethers, mice were treated with NPVE or 4-nitrophenyl ethyl ether (NPVE) in combination with buthionine sulfoximine (BSO), an inhibitor for GSH biosynthesis. In the combination experiment, two doses of BSO (4 mmol/kg body weight per dose) were given i.p. 1 hr before and 2 hr after the injection of the test chemical (Mizutani *et al.*, 1987). By the treatment with BSO, hepatic NP-SH as the marker of GSH content was depleted to about 30–40% of the control, but hepatic content of cytochrome P-450 which metabolizes NPVE to a reactive epoxide intermediate was not influenced. Though the administration of either NPVE or BSO caused no significant toxic effect, treatment of mice with NPVE in combination with BSO elevated the serum GPT activity remarkably (Table 2). After 6 hr of the NPVE administration, the NP-SH content in the liver of mice pretreated with BSO remained at 17–26% of the control. On the other hand, NPVE which forms a carbinol intermediate but not a epoxide in the major metabolic pathway did not induce the leakage of GPT into blood even though administered in combination with BSO.

Therefore BSO, a specific inhibitor of γ -glutamylcysteine synthetase and hence of GSH synthesis (Griffith and Meister, 1979), showed no significant effect on the content of hepatic microsomal cytochrome P-450 in this study, the metabolic formation of the epoxide from NPVE

Table 2. Effects of NPVE and BSO on serum GPT activity and hepatic cytochrome P-450 content in mice.

Treatment	Serum GPT activity (mU/ml serum)	Cyt. P-450 content (nmol/mg protein)
Vehicle	17.5 ± 6.3	1.06 ± 0.24
NPVE	17.0 ± 11.4	1.01 ± 0.31
BSO	15.2 ± 7.8	0.99 ± 0.11
BSO+NPVE	$203 \pm 160^*$	0.85 ± 0.31
BSO+NPEE	24.5 ± 4.9	n.e.

Male ddY mice treated with BSO (4 mmol/kg, i.p.) were killed 6 hr after i.p. dosing of NPVE (1.8 mmol/kg), NPVE (1.8 mmol/kg), or corn oil. The cytochrome P-450 content in the hepatic microsomes was assayed by the method of Omura and Sato (1964).

Values represent the mean $\pm$ S.D. of five animals.

* : Significantly different from the vehicle group at $P < 0.05$.

n.e. : not examined

should not be affected by the BSO treatment. The hepatotoxicity of NPVE appeared in combination with BSO is ascribed to the decreased detoxication metabolism by GSH conjugation and to the accumulation of the reactive epoxide in liver. The epoxide should bind covalently to cell components such as protein and DNA, and the cell damage as the end point may occur. The protective role of GSH is consistent with the *in vitro* observation that the bacterial mutagenesis induced by the epoxide of NPVE was strongly inhibited by GSH (Sone *et al.*, 1989b).

In the present study, it is concluded that an aryl vinyl ether, NPVE, cause potential hepatotoxic effect in mice when hepatic GSH has been depleted, whereas BVE, an alkyl vinyl ether, shows no significant hepatotoxicity, and that intracellular GSH plays a major role in the protection of hepatic injury by epoxides formed from aryl vinyl ethers.

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