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 sulhydryl (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, fluroxene, 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 glycoaldehyde 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
sulhydryl (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 signifi-
cant differences in the serum glutamate pyruvate transaminase (GPT) activity throughout the ex-
perimental period between the NPVE-treated

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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.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hepatic NP-SH content (µmol/g liver)</th>
<th>Serum GPT activity (mU/ml serum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>7.77±2.21</td>
<td>15.7±6.5</td>
</tr>
<tr>
<td>BVE</td>
<td>6.14±1.77</td>
<td>15.0±4.5</td>
</tr>
<tr>
<td>PVE</td>
<td>4.22±1.54*</td>
<td>9.1±6.9</td>
</tr>
<tr>
<td>NPVE</td>
<td>1.31±0.38**</td>
<td>12.9±6.3</td>
</tr>
</tbody>
</table>

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

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

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Serum GPT activity (mU/ml serum)</th>
<th>Cyt. P-450 content (nmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>17.5±6.3</td>
<td>1.06±0.24</td>
</tr>
<tr>
<td>NPVE</td>
<td>17.0±11.4</td>
<td>1.01±0.31</td>
</tr>
<tr>
<td>BSO</td>
<td>15.2±7.8</td>
<td>0.99±0.11</td>
</tr>
<tr>
<td>BSO+NPVE</td>
<td>203±160*</td>
<td>0.85±0.31</td>
</tr>
<tr>
<td>BSO+NPEE</td>
<td>24.5±4.9</td>
<td>n.e.</td>
</tr>
</tbody>
</table>

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), NPEE (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±S.D. of five animals.

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

n.e. : not examined

Hepatotoxicity of vinyl ether. should not be affected by the BSO treatment. The hepatotoxicity of NPVE appeared in combination with BSO 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.

REFERENCES


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