Comparison of the susceptibility of wild-type and CYP2E1 knockout mice to the hepatotoxic and pneumotoxic effects of styrene and styrene oxide

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Abstract

Styrene causes both liver and lung damage in non-Swiss albino, CD-1, and other strains of mice. This is considered to be due to the bioactivation of styrene to styrene oxide by cytochromes P450, principally CYP2E1 and CYP2F2. If so, one would expect CYP2E1 knockout mice to be less susceptible to styrene-induced toxicity than wild-type mice. However, previous in vitro and in vivo studies demonstrated little difference in the metabolism of styrene to styrene oxide between wild-type and CYP2E1 knockout mice. These findings would suggest that there should be no difference in the toxic responses to styrene between these two strains. To determine which of these possibilities was correct, styrene (600 mg/kg) or styrene oxide (300 mg/kg) was administered i.p. 24 h prior to measurement of serum sorbitol dehydrogenase as a biomarker of hepatotoxicity or lactate dehydrogenase activity, protein, and cells in bronchoalveolar lavage fluid as biomarkers for pneumotoxicity. Styrene was more hepatotoxic in the wild-type mice than in the knockout mice suggesting CYP2E1 activity is important. Strain differences were not observed with styrene oxide indicating no difference in intrinsic susceptibility. For lung, the response was similar in both strains to both styrene and styrene oxide supporting the idea that CYP2F2 is important in the bioactivation of styrene in this tissue and that there is no strain difference in susceptibility to the active metabolite.

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Keywords: Styrene; Hepatotoxicity; Pneumotoxicity; CYP2E1 knockout mice

1. Introduction

Styrene is a widely used chemical with the highest levels of exposure being found in workers in the reinforced plastics industry (Miller et al., 1994; Cohen et al., 2002). Styrene produces both liver and lung damage in mice (Morgan et al., 1993a, b; Gadberry et al., 1996; Cruzan et al., 1997). In order for styrene to exert its toxicity in liver and lung it is bioactivated to styrene oxide (Bond, 1989; Sumner and Fennell, 1994). The cytochromes P450 primarily responsible for the bioactivation have been identified as CYP2E1 and CYP2F2 (Nakajima et al., 1994a, b; Carlson, 1997b; Green et al., 2001) with the latter isozyme being of particular importance in lung.

The importance of CYP2E1 in the biotransformation of a number of chemicals, e.g. acetaminophen, acrylonitrile, methacrylonitrile, acrylamide and carbon tetrachloride, has been demonstrated using...
CYP2E1 knockout mice (Ghanayem et al., 2000). Sumner et al. (2001) reported that when wild-type and CYP2E1 knockout mice were exposed to a 250 ppm mixture of [13C8] styrene and [14C] styrene via nose-only inhalation for 6 h, the knockout mice excreted more total urinary metabolites and total expired [14C] styrene-equivalents than did the wild-type mice. The wild-type mice excreted a slightly higher percentage of metabolites derived from styrene oxide hydrolysis (28%) than did the knockout mice (16%). The authors concluded that CYP2E1 may not be a major isozyme involved in the in vivo metabolism of styrene to styrene oxide. Carlson (2003) compared the in vitro metabolism of styrene by hepatic and pulmonary microsomes from CYP2E1 knockout and wild-type mice. There was no difference in the rate of hepatic microsomal metabolism of styrene to styrene oxide between the two strains. Metabolism of styrene was slower in the lungs of the knockout mice than in the wild-type. The use of chemical inhibitors of styrene metabolism indicated that CYP2E1 and CYP2F2 may be important in wild-type mice, but they do not clearly indicate what cytochromes P450 are responsible for the metabolism in the knockout mice.

One would expect that if styrene toxicity is related to its bioactivation by CYP2E1, then the CYP2E1 knockout mice would be less able to bioactivate it and subsequently would be less susceptible to its toxicity compared to the wild-type mice. However, if there is really little or no difference between the wild-type and knockout mice with respect to the rate of metabolism of styrene as suggested by previous in vitro or in vivo studies (Sumner et al., 2001; Carlson, 2003), one would predict that there would be no difference in susceptibility to styrene-induced toxicity between the wild-type and knockout mice. The purpose of the current study was to examine these two possibilities by determining the hepatotoxicity and pneumotoxicity of styrene and its active metabolite styrene oxide in CYP2E1 knockout and wild-type mice.

2. Materials and methods

2.1. Chemicals

Styrene and styrene oxide were obtained from Aldrich Chemical Co. (Milwaukee, WI, USA). NADH, pyruvate, and Tris buffer were from Sigma Chemical Co. (St. Louis, MO, USA). Triethanolamine was from Mallinckrodt (Paris, KY, USA). Fructose was from Fisher Scientific (Fair Lawn, NJ, USA). All other chemicals were reagent grade or better.

2.2. Mice

Breeding colonies of CYP2E1 knockout mice (129/Sv-Cyp2e1 tmGonz; Jackson Laboratories, Bar Harbor, ME, USA) and wild-type controls (129S3/SvlmJ; Jackson Laboratories) were maintained in an AAALAC-accredited animal facility. Mice were housed in group cages kept in environmentally controlled rooms with a 12-h light/dark cycle. Rodent laboratory chow (number 5001, Purina Mills, Inc., St. Louis, MO, USA) and water were provided ad libitum.

2.3. Study design

To examine the toxicity of styrene and styrene oxide, groups of wild-type or CYP2E1 knockout mice (number of replicates presented in the tables) were administered 600 mg/kg of styrene or 300 mg/kg of styrene oxide in corn oil i.p. These doses and route were selected on the basis of previous studies by Gadberry et al. (1996). It was necessary to use a high dose to assure that if one of the strains was found to be less susceptible that sufficient toxicity would occur in the more susceptible strain to allow the detection of a difference. Equivalent doses of styrene and styrene oxide could not be used because of the greater potency of the styrene oxide which would have led to deaths at a higher dose. This same dose was used in studies that found differences in susceptibility among common strains of mice (Carlson, 1997a). Twenty-four hours after the styrene or styrene oxide administration, the mice were anesthetized with diethyl ether. The abdominal cavity was opened, and the diaphragm was cut. Blood was obtained by cardiac puncture. The blood was pooled from pairs of animals for measurement of sorbitol dehydrogenase (SDH).

For the pneumotoxicity studies, bronchoalveolar lavage fluid (BALF) was obtained 24 h after dosing. For the hepatotoxicity studies, the animals were anesthetized with diethyl ether, and the abdominal and thoracic cavities were opened. The incision was continued to the neck region to expose the trachea.
A nick was made in the trachea, and an oral feeding needle was inserted and tied in place. The lungs were perfused twice with 0.8 ml of lavage fluid for a total volume of 1.6 ml. This fluid consisted of NaCl (145 mM), KCl (5 mM), NaH₂PO₄ (1.9 mM), Na₂HPO₄ (9.4 mM) and glucose (5.5 mM) at a pH 7.4. These protocols and procedures were approved by the Purdue University Animal Care and Use Committee.

2.4. Biochemical analyses

Serum was prepared, and serum sorbitol dehydrogenase (SDH) activity was measured spectrophotometrically by the method of Gerlach (1983). Serum (0.2 ml) and NADH (12 mM) were incubated for 30 min at 30 °C in triethanolamine buffer (0.2 M, pH 7.4). The reaction was started by addition of 0.3 ml of 72% (w/v) fructose for a final reaction volume of 3.0 ml. SDH activity was measured by the decrease in absorbance of NADH at 366 nm for 2 min using a Shimadzu Model UV160U UV-Visible spectrophotometer. Results are expressed as μmol/min/l serum.

In the analysis of BALF, the number of cells in 100 μl of BALF was counted using a hemocytometer. The remaining BALF was centrifuged at low speed, and the amount of protein determined using the bicinchoninic acid method (Redinbaugh and Turley, 1986). Lactate dehydrogenase (LDH) activity was measured by the spectrophotometric method of Vassault (1983) in centrifuged BALF samples from individual mice. BALF fluid (0.1 ml), NADH (0.24 mM), and Tris (81 mM)/NaCl (203 mM) buffer (pH 7.2) were incubated for 15 min at 30 °C. The reaction was initiated by the addition of 0.5 ml pyruvate (9.8 mM) to make a total volume of 3.0 ml. The activity of LDH was measured 30 s after the addition of pyruvate by the decrease in absorbance of NADH at 339 nm for 2 min. Results are expressed as μmol/min/l BALF.

2.5. Statistical analysis

Values are expressed as mean ± S.E. In comparing the values, an ANOVA was utilized followed by Student Newman-Keuls test to detect differences among the groups. In each case the level of significance selected was \( P < 0.05 \). In some cases, because of differences in the variances, it was necessary to log transform the data.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Effect of styrene on sorbitol dehydrogenase activity in wild-type and CYP2E1 knockout mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain</td>
<td>Treatment</td>
</tr>
<tr>
<td>---------</td>
<td>----------------</td>
</tr>
<tr>
<td>Wild-type</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>Styrene $^b$</td>
</tr>
<tr>
<td>Knockout</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>Styrene $^b$</td>
</tr>
</tbody>
</table>

Values with different letters (c, d) are significantly different from each other \( (P < 0.05) \).

$^a$ Activity is in μmol/min/l.

$^b$ 600 mg/kg i.p., 24 h prior to sacrifice.

3. Results

As expected from previous studies in non-Swiss albino mice (Gadberry et al., 1996) and CD-1 mice (Carlson, 1997a), styrene at a dose of 600 mg/kg given i.p. caused hepatotoxicity in the wild-type mice as indicated by an increase in serum sorbitol dehydrogenase activity (Table 1). However, there was much less of an increase in the knockout mice. When styrene oxide, an active metabolite of styrene, was administered to the mice at a dose of 300 mg/kg i.p., there were increases in serum sorbitol dehydrogenase activity in both the wild-type and knockout mice (Table 2). There was no significant difference in the response between the two strains of mice.

Pneumotoxicity was assessed by measuring lactate dehydrogenase activity, cells, and protein in bronchoalveolar lavage fluid. Styrene (600 mg/kg i.p.) caused an increase in all three parameters in the wild-type mice (Table 3). A similar, perhaps even a

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Effect of styrene oxide on sorbitol dehydrogenase activity in wild-type and CYP2E1 knockout mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain</td>
<td>Treatment</td>
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<tr>
<td>---------</td>
<td>----------------</td>
</tr>
<tr>
<td>Wild-type</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>Styrene oxide $^b$</td>
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<tr>
<td>Knockout</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>Styrene oxide $^b$</td>
</tr>
</tbody>
</table>

Values with different letters (c, d) are significantly different from each other \( (P < 0.05) \).

$^a$ Activity is in μmol/min/l.

$^b$ 300 mg/kg i.p., 24 h prior to sacrifice.
Table 3
Effect of styrene on biomarkers of styrene-induced pneumotoxicity

<table>
<thead>
<tr>
<th>Strain</th>
<th>Treatment</th>
<th>N</th>
<th>Lactate dehydrogenase activity in BALF</th>
<th>Cells in BALF</th>
<th>Protein in BALF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>Control</td>
<td>4</td>
<td>93.6 ± 20.2^e</td>
<td>59 ± 12^e</td>
<td>415 ± 35^e</td>
</tr>
<tr>
<td></td>
<td>Styrene</td>
<td>4</td>
<td>193.3 ± 27.4^f</td>
<td>261 ± 44^f</td>
<td>638 ± 66^f</td>
</tr>
<tr>
<td>Knockout</td>
<td>Control</td>
<td>4</td>
<td>78.8 ± 21.9^e</td>
<td>53 ± 10^e</td>
<td>460 ± 73^e</td>
</tr>
<tr>
<td></td>
<td>Styrene</td>
<td>5</td>
<td>193.5 ± 16.1^f</td>
<td>418 ± 41^f</td>
<td>880 ± 47^f</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild-type</td>
<td>Control</td>
<td>4</td>
<td>40.0 ± 8.2^e</td>
<td>53 ± 13^e</td>
<td>288 ± 19^e</td>
</tr>
<tr>
<td></td>
<td>Styrene</td>
<td>3</td>
<td>119.2 ± 21.1^f</td>
<td>293 ± 52^f</td>
<td>543 ± 9^f</td>
</tr>
<tr>
<td>Knockout</td>
<td>Control</td>
<td>4</td>
<td>51.0 ± 11.3^e</td>
<td>78 ± 16^e</td>
<td>300 ± 25^e</td>
</tr>
<tr>
<td></td>
<td>Styrene</td>
<td>4</td>
<td>199.9 ± 16.4^f</td>
<td>669 ± 107^f</td>
<td>638 ± 54^f</td>
</tr>
</tbody>
</table>

For each experiment, values with different letters (e–g) are significantly different from each other (P < 0.05).

a μmol/min/ml.

b Cells per μl.
c Micrograms protein per ml.
d 600 mg/kg i.p., 24 h prior to sacrifice.

Table 4
Effect of styrene oxide on biomarkers of styrene oxide-induced pneumotoxicity

<table>
<thead>
<tr>
<th>Strain</th>
<th>Treatment</th>
<th>N</th>
<th>Lactate dehydrogenase activity in BALF</th>
<th>Cells in BALF</th>
<th>Protein in BALF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>Control</td>
<td>4</td>
<td>64.8 ± 19.9^e</td>
<td>64 ± 35^e</td>
<td>358 ± 47^e</td>
</tr>
<tr>
<td></td>
<td>Styrene oxide</td>
<td>3</td>
<td>180.3 ± 27.10^f</td>
<td>215 ± 37^f</td>
<td>697 ± 141^f</td>
</tr>
<tr>
<td>Knockout</td>
<td>Control</td>
<td>4</td>
<td>56.1 ± 14.3^e</td>
<td>42 ± 6^e</td>
<td>313 ± 43^e</td>
</tr>
<tr>
<td></td>
<td>Styrene oxide</td>
<td>3</td>
<td>277.7 ± 105.2^f</td>
<td>248 ± 93^f</td>
<td>787 ± 192^f</td>
</tr>
</tbody>
</table>

Values with different letters (e, f) are significantly different from each other (P < 0.05).
a μmol/min/ml.
b Cells per μl.
c Micrograms protein per ml.
d 300 mg/kg i.p., 24 h prior to sacrifice.

slightly greater response, was observed in the knockout mice. A replication of the experiment verified these findings.

4. Discussion

Styrene and its active metabolite styrene oxide have both been shown to cause hepatotoxicity and pneumotoxicity in mice using the same measurements as utilized in the present experiments (Gadberry et al., 1996; Carlson, 1997a). While the response in the liver of the wild-type mouse was as expected, the knockout mouse proved much less susceptible. The two strains responded in a similar fashion to the administration of the active metabolite styrene oxide but instead that the strain difference in response to styrene is linked to its bioactivation by CYP2E1 to styrene oxide.

The finding of a strain-dependent difference in susceptibility to styrene-induced hepatotoxicity which is apparently related to CYP2E1 bioactivation of styrene is interesting in light of previous findings from both in vivo (Sumner et al., 2001) and in vitro (Carlson, 2003) studies that there is little or no difference in the
rate of metabolism of styrene between the wild-type and knockout mice. The reason for this disconnect is not clear. It may possibly be related to kinetic factors associated with styrene metabolism within the liver itself in the intact animal.

The wild-type and knockout mice responded in a similar fashion to the pneumotoxic effects of the active metabolite styrene oxide again indicating no difference in intrinsic susceptibility. In contrast to what was observed in the liver, in the lung there was no difference between the two strains with respect to susceptibility to styrene-induced toxicity. This is probably related to the importance of styrene metabolism by CYP2F2 in lung (Nakajima et al., 1994a; Carlson, 1997b; Hynes et al., 1999). Therefore, this lack of a difference was not unexpected.

In summary, the difference in styrene-induced hepatotoxicity between the wild-type and knockout mice demonstrates the importance of CYP2E1 to the bioactivation of styrene in that tissue in the intact animal whereas the lack of a strain difference in response to the pneumotoxic effects appears to reflect the importance of CYP2F2 in that tissue in both strains.

Acknowledgements

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