Microsome-mediated Covalent Binding of 1,2-Dichloroethane to Lung Microsomal Protein and Salmon Sperm DNA

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ABSTRACT

In order to determine whether the covalent binding of the carcinogen 1,2-dichloroethane to macromolecules is dependent on microsomes or cytosol, microsomes and cytosol from lungs of C57BL/6 × C3H/He F1 (hereafter called B6C3F1) mice and Osborne-Mendel rats were incubated with [1,2-¹⁴C]dichloroethane and salmon sperm DNA. 1,2-Dichloroethane binds covalently to microsomal protein and DNA only in the presence of microsomes, whereas cytosol has insignificant metabolic activation. The binding to macromolecules was significantly higher in the presence of native microsomes than denatured microsomes. The interaction of 1,2-dichloroethane with DNA was enhanced following pretreatment of the animals with phénobarbital and 3-methylcholanthrene. On the other hand, glutathione reduced the binding. The binding of 1,2-dichloroethane to lung microsomal protein of B6C3F1 mice and to DNA was significantly higher following pretreatment of the animals with 3-methylcholanthrene; PB, sodium phénobarbital; GSH, glutathione; DBE, 1,2-dibromoethane.

INTRODUCTION

DCE, also known as ethylene dichloride, is primarily used in the manufacture of vinyl chloride (80%), trichloroethylene (10%), and other chlorinated solvents. It is also used as a lead scavenger in gasoline and as a fumigant for grain, carpets, and upholstery. The annual production of DCE in 1978 was 10.5 million pounds in this country (6). DCE is used to cause liver and kidney damage (8). DCE has been reported to induce hepatocellular carcinomas, lung adenoma, and adenocarcinomas in mice (6). DCE is known to cause lung tumors as well as skin papillomas and carcinomas in ICR/Ha Swiss mice (17). DCE is mutagenic in bacterial systems (6, 13).

We had demonstrated previously that DCE binds covalently to hepatic microsomal proteins of B6C3F1 mice and to salmon sperm DNA and that the binding is dependent on metabolic activation (1, 3). Furthermore, the binding of DCE to liver protein of B6C3F1 mice and to DNA was significantly lower than in similar binding studies using Osborne-Mendel rats (3). Since lung is a target organ for DCE-induced tumorigenesis in mice, we have studied the interaction of this compound with protein and salmon sperm DNA in the presence of a lung microsomal system. We have compared differences in DCE binding between B6C3F1 mice and Osborne-Mendel rats.

MATERIALS AND METHODS

Chemicals. [1,2-¹⁴C]DCE (specific activity, 5 mCi/mmol) and [¹⁴C]toluene were purchased from New England Nuclear, Boston, Mass. The radiochemical purity of DCE was determined by gas chromatography-mass spectrometry by the supplier and was reported to be > 99%. Salmon sperm DNA and bovine pancreas RNase were obtained from Calbiochem, San Diego, Calif. 3-MC was from Aldrich Chemical Co., Milwaukee, Wis. and was recrystallized from toluene in this laboratory. PB was obtained from Elkins-Sinn, Inc., Cherry Hill, N. J., and NADP⁺, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase were purchased from Sigma Chemical Co., St. Louis, Mo.

Animals. B6C3F1 mice were purchased by the National Cancer Institute, Bethesda, Md. Osborne-Mendel rats, originally supplied by the National Cancer Institute, were bred in our animal facility.

Preparation of Rodent Lung Microsomes. Male B6C3F1 mice and Osborne-Mendel rats, 7 to 10 weeks old, were sacrificed by cervical dislocation, and the lungs were removed and immediately immersed in ice-cold 0.05 M Tris-HCl buffer, pH 7.4, containing 0.15 M KCl. Both lobes of the lung were placed in filter paper, and connective tissues were removed. Primarily, our difficulty was to remove hemoglobin from the microsomal preparation. We found 0.05 M Tris-HCl buffer with 0.15 M KCl was the best buffer system to make hemoglobin-free microsomes. The cleaned lung tissue was reimmersed in fresh buffer, and the process was repeated until the buffer solution was clear. It was wiped on filter paper, and the wet weight was recorded. On the average, 0.6 g of lung tissue was obtained from 5 mice while 3 rats yielded 3.9 g. The tissue was chopped and homogenized in the above buffer using a glass-Teflon homogenizer. To insure uniform homogenization of all preparations, equal numbers of homogenizing strokes were used. The homogenate was then subjected to differential centrifugation to prepare microsomes as described earlier (2, 15). The lung microsomes were characterized as reported previously for liver microsomes (2, 16). The washed microsomes were suspended in 0.05 M potassium phosphate buffer, pH 7.4, at a protein concentration of 15 mg/ml. The protein content was determined by the Biuret method (7) with Crystalline Bovine Serum Albumin Fraction 5 as standard. The microsomes were used immediately.

Enzymatic Binding of ¹⁴C]DCE to DNA and Microsomal
Protein. Lung microsomes prepared from B6C3F1 mice or from Osborne-Mendel rats were incubated with 1.04 μmol of [14C]DCE, 8.75 mg of DNA, 10.0 μmol of glucose-6-phosphate, 2.5 units of glucose-6-phosphate dehydrogenase, 2.0 μmol of NADP, 5.0 μmol of MgCl₂, 1.6 to 2.0 mg of lung microsomal protein, and 12.5 μmol of potassium phosphate buffer, pH 7.4, as described before (3). The reaction was terminated by chilling the incubation tube at 0°, and microsomes were immediately separated by centrifugation (2).

The microsomal proteins were precipitated with 5% trichloroacetic acid and purified, and radioactivity was determined (15). The DCE-bound DNA obtained from the microsomal supernatant was purified, and radioactivity was determined as described earlier (2, 3).

Nonenzymatic Binding of [14C]DCE to Microsomal Protein and Salmon Sperm DNA. In order to evaluate whether the binding of [14C]DCE is dependent on microsomally mediated metabolism, incubations were done in the presence of microsomes devoid of enzymatic activity simultaneously with enzymatic incubations for both protein and DNA. Microsomes were denatured by boiling for 20 min, cooled to room temperature, and incubated with 1.04 μmol of [14C]DCE in the absence of NADPH for 60 min (3). After incubation, the proteins were collected and purified, and radioactivity was determined (15). DNA was incubated with 1.04 μmol of [14C]DCE without microsomes and NADPH. After incubation, DNA was isolated and purified, and radioactivity was determined (2, 3).

Preparation of Lung Cytosol. Cytosol fraction was prepared from the supernatant obtained after 105,000 × g centrifugation in the preparation of lung microsomes of B6C3F1 mice. This supernatant was recentrifuged at 135,000 × g using a SW 27.1 rotor in a Beckman LS-65 ultracentrifuge for 60 min. The resultant supernatant represented the cytosol. The protein content was then determined (7) and used immediately in the incubation.

Binding of [14C]DCE to Cytosol. In a Dubnoff shaking bath, 1.04 μmol of [14C]DCE were incubated with cytosol and 8.75 mg of DNA in the absence of microsomes and NADPH for 60 min at 37°. After the incubation, the reaction mixture was stirred with water-saturated phenol for 30 min. The resulting emulsion was separated into aqueous and phenol layers by centrifuging at 10,000 × g for 10 min. DNA was precipitated by adding an equal volume of ethanol. The precipitate was then redissolved in 0.5 ml of ethanol and centrifuged at 10,000 × g for 10 min. DNA was precipitated again, and the supernatant was recentrifuged at 135,000 × g using a SW 27.1 rotor in a Beckman LS-65 ultracentrifuge for 60 min. The supernatant was purified, and radioactivity was determined (2, 3).

Enzymatic binding using native microsomes; there was no detectable binding using denatured microsomes isolated from both mice and rats. On the contrary, binding of DCE to denatured microsomal protein was negligible compared to the binding using native microsomes; there was no detectable radioactivity found with DNA when it was incubated in the vitro addition of microsomal and cytosol preparations to DNA in the presence of microsomes or cytosol of B6C3F1 mice was the same. On the contrary, binding of DCE to denatured microsomal protein was negligible compared to the binding using native microsomes; there was no detectable radioactivity found with DNA when it was incubated in the vitro addition of microsomal and cytosol preparations to DNA in the presence of microsomes or cytosol of B6C3F1 mice.

RESULTS

Enzymatic and Nonenzymatic Binding of [14C]DCE to Lung Microsomal Protein of B6C3F1 Mice and Osborne-Mendel Rats and to Salmon Sperm DNA. Table 1 shows the extent of binding of DCE to lung microsomes of male B6C3F1 mice and Osborne-Mendel rats and to exogenous DNA in the presence of either native or denatured lung microsomes. The binding of DCE to protein of denatured lung microsomes from both mice and rats was only 1% of that obtained with native microsomes. Similarly, the amount of DCE bound to DNA in the presence of native microsomes isolated from both mice and rats was significantly higher than that obtained after incubation with denatured lung microsomes and/or in the absence of microsomes. Negligible radioactivity was associated with DNA when incubated with [14C]DCE in the absence of microsomes. This difference between the enzymatic and nonenzymatic binding is statistically significant. In subsequent tables, the results have been corrected for nonenzymatic binding.

Table 1

<table>
<thead>
<tr>
<th>Species</th>
<th>Microsome*</th>
<th>[14C]DCE bound to macromolecules*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mice</td>
<td>Native</td>
<td>4.4 ± 0.22</td>
</tr>
<tr>
<td></td>
<td>Denatured</td>
<td>0.04 ± 0.03</td>
</tr>
<tr>
<td>Rats</td>
<td>Native</td>
<td>1.2 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>Denatured</td>
<td>0.04 ± 0.01</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentage of change compared to microsomes.

Table 2

<table>
<thead>
<tr>
<th>In vitro addition</th>
<th>[14C]DCE bound to macromolecules*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microsomes</td>
<td>4.1 ± 0.2</td>
</tr>
<tr>
<td>Denatured microsomes</td>
<td>0.05 ± 0.03</td>
</tr>
<tr>
<td>Microsomes + GSH (5 μM)</td>
<td>0.34 ± 0.06 (−92)</td>
</tr>
<tr>
<td>Cytosol</td>
<td>0.33 ± 0.01 (−92)</td>
</tr>
<tr>
<td>Denatured cytosol</td>
<td>0.003 ± 0.001</td>
</tr>
<tr>
<td>Denatured cytosol +</td>
<td>0.02 ± 0.001</td>
</tr>
<tr>
<td>Microsomes</td>
<td>0.03 ± 0.01</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentage of change compared to microsomes.

REFERENCES


presence of denatured microsomes. The addition of microsomes to the incubation system containing either native or denatured cytosol, DCE, and DNA enhanced considerably the binding to DNA.

On addition of GSH to the incubation system containing DCE, DNA, and microsomes, the binding to microsomal protein and DNA was significantly inhibited by 92 and 67%, respectively.

Effect of PB and 3-MC Induction on the Macromolecular Binding of DCE to Lung Microsomal Protein and DNA. Table 3 illustrates the effects of in vivo administration of PB and 3-MC on the binding of DCE to protein and DNA. The covalent binding of [14C]DCE to DNA was enhanced by PB and 3-MC by 67 and 54%, respectively. There was, however, no change in the binding of DCE to lung microsomal protein prepared from either PB-treated or 3-MC-treated animals compared with respective control groups.

Binding of DCE to Lung and Liver Microsomal Protein of B6C3F1, Mice and Osborne-Mendel Rats and to DNA. The results in Table 4 demonstrate a significant difference in the binding of DCE to exogenous DNA and lung microsomal protein of B6C3F1, mice and Osborne-Mendel rats. The covalent binding of DCE to microsomal protein of B6C3F1, mice was 3 times more than was the binding to microsomal protein of Osborne-Mendel rats. The variation in the binding to DNA in the presence of microsomes isolated from B6C3F1, mice and Osborne-Mendel rats is more prominent than that in protein binding. DCE was bound 5 times higher to DNA in the presence of lung microsomes of mice than those from rats. For the sake of comparison, we have included our previous data reporting binding of DCE to liver microsomal protein of B6C3F1, mice and DNA in this table. DCE binds 85% and 5 times more to lung microsomal protein than to liver microsomal protein of mice (p < 0.001) and rats (p < 0.01), respectively. It interacts 100% higher with DNA in the presence of lung microsomes than with DNA incubated with liver microsomes for both mice (p < 0.01) and rats (p < 0.05). The difference between the binding to DNA in the presence of lung and liver microsomes of rats is not statistically significant.

DISCUSSION

Earlier studies from this laboratory have shown that both DCE and DBE are metabolized in vitro by hepatic and forestomach microsomes of rodents and interact covalently with microsomal proteins and DNA (1, 3). Results from the present report provide evidence of the lung microsomal-dependent activation of DCE and subsequent covalent binding to salmon sperm DNA and lung microsomal proteins of B6C3F1, mice and Osborne-Mendel rats. There was no detectable binding of DCE to RNA and negligible binding to protein in the presence of denatured microsomes.

The mutagenic activity of DCE has been reported to be due to activation via conjugation with GSH catalyzed by cytosol and also is NADPH independent (12). Our results, on the other hand, demonstrate negligible binding of DCE to macromolecules in the presence of cytosol indicating that cytosol is devoid of any activity which would induce DCE to bind to macromolecules. When GSH was added to the microsomal incubation system, DCE binding to macromolecules was inhibited which is in accordance with our earlier report (3) for DBE, the brominated analog of DCE and a potent carcinogen (18). The inhibition of binding by GSH would be expected if the activated metabolic intermediate of DCE was electrophilic. The binding of DCE to macromolecules was also NADPH dependent.

The binding of DCE to DNA in the presence of lung microsomes was enhanced by both PB and 3-MC, inducers of cytochrome P-450 and P-448, respectively, both present in microsomes (10). These results show that DCE is dependent on metabolic activation mediated by microsomes in order to bind to DNA. The binding to protein, however, remained unchanged by either PB or 3-MC treatment indicating that different metabolite(s) may be involved in the interaction between DCE and protein as opposed to its interaction with DNA.

<table>
<thead>
<tr>
<th>Table 3</th>
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<tbody>
<tr>
<td>Effect of PB and 3-MC induction on the covalent binding of [14C]DCE to macromolecules</td>
</tr>
<tr>
<td>Species</td>
</tr>
<tr>
<td>Lung microsomes, nmol/mg protein</td>
</tr>
<tr>
<td>Mean ± S.D.</td>
</tr>
<tr>
<td>PB-treated</td>
</tr>
<tr>
<td>0.15 m NaCl</td>
</tr>
<tr>
<td>Triocanoin control</td>
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<tr>
<td>3-MC in triocanoin</td>
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<tr>
<th>Table 4</th>
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<tr>
<td>Binding of [14C]DCE to salmon sperm DNA and to lung and liver microsomal protein of B6C3F1, mice and Osborne-Mendel rats</td>
</tr>
<tr>
<td>Species</td>
</tr>
<tr>
<td>B6C3F1, mice</td>
</tr>
<tr>
<td>Osborne-Mendel rats</td>
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</tbody>
</table>

a Mean ± S.D. of 3 groups of male mice consisting of 8 mice for lung and liver per group of mice, and mean ± S.D. of 9 male rats for lung and liver per group of rats. For the binding to protein of both lung and liver between mice and rats, p < 0.001, and for the binding to DNA of lung and liver between mice and rats, p < 0.001, respectively.
A number of potential activated intermediates of DCE have been postulated (3). Among them, chloroacetaldehyde, chloroethanol, and chloroethylene oxide (3) are all electrophilic in nature and, therefore, capable of reacting readily with nucleophilic macromolecules. Chloroacetaldehyde is highly mutagenic (11) and also binds to nucleosides (5,14) although it has not been found to be carcinogenic by skin application in mice (17). A recent report from this laboratory has demonstrated that bromoacetaldehyde, a metabolite of DBE (9), and 2-bromoethanol, a likely metabolite, bind covalently to macromolecules without metabolic activation (4).

Our results also demonstrate that DCE binds significantly higher to DNA and protein in the presence of lung microsomes from B6C3F1 mice, a species susceptible to DCE-induced pulmonary tumorigenesis, than in the presence of those from Osborne-Mendel rats, which are resistant to lung tumorigenesis by DCE. This correlation between species susceptibility and microsomally mediated binding of DCE to macromolecules is in agreement with our previous report which indicated a similar correlation in the case of DCE and trichloroethylene binding to liver microsomal protein and DNA (2,3). A comparison of the extent of binding of DCE to DNA and to microsomal protein in lung and liver of mice indicates that DCE interacts more with protein and DNA in the presence of lung microsomes than in the presence of liver microsomes. DCE has been found to cause lung tumors by feeding (18) as well as by skin application in mice (17).

In conclusion, DCE binds to macromolecules in the presence of a lung microsomal oxidase system. Lung cytosol, on the other hand, does not activate DCE binding. There is preliminary evidence of a correlation between the microsomally mediated binding and species and organ susceptibility to DCE-induced tumorigenesis. Also, different activated metabolic intermediates are involved in DNA compared to protein binding.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the valuable comments provided by Dr. S. A. Kline during the preparation of the manuscript.

REFERENCES

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