Species Differences in Metabolic Activation and Inactivation of 1-Nitropyrene in the Liver

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ABSTRACT

To extrapolate from animal studies to humans the risk of 1-nitropyrene (1-NP), we determined the differences between human and experimental animals in oxidative activation of 1-NP to 1-NP oxides and inactivation of 1-NP oxides by epoxide hydration and glutathione conjugation in hepatic subcellular fractions from 6 species including humans. Species differences were found in both activation of 1-NP and inactivation of 1-NP oxides. 1-Nitro-4,5-dihydro-4,5-epoxypyrene-producing activity was highest in guinea pig and dog, followed by hamster, rat, human, and mouse. 1-Nitro-9,10-dihydro-9,10-epoxypyrene-producing activity was highest in hamster, followed in order by guinea pig, rat, dog, mouse, and human. The ratio of 1-nitro-4,5-dihydro-4,5-epoxypyrene to 1-nitro-9,10-dihydro-9,10-epoxypyrene also varied with the animal species. Hydration of 1-nitro-4,5-dihydro-4,5-epoxypyrene was highest in human, followed by dog, guinea pig, hamster, rat, and mouse. 1-nitro-9,10-dihydro-9,10-epoxypyrene was a poor substrate for epoxide hydrolase in all species. Glutathione conjugation of 1-NP oxides in rodents was higher than that in human and dog. In humans, hepatic microsomes produced the lowest level of 1-NP oxides but hydrolyzed them most efficiently, and glutathione conjugation activity of the cytosol was as low as in dogs, and there was a wide degree of interindividual variations in these activities. No single species studied was a good model for humans, and the balance of activation/inactivation tends toward detoxification in these adult animals.

INTRODUCTION

For extrapolation of carcinogenesis data to humans, it is necessary to understand the differences in metabolism between humans and animals. The extent of metabolic activation of carcinogens and the balance between their activation and inactivation are important factors affecting the toxicity of chemicals. In this paper, we describe the activation of 1-NP and inactivation of 1-NP oxides, which are oxidatively activated forms of 1-NP. 1-NP is a highly mutagenic (1–5) and carcinogenic (6–9) substance widely distributed in the environment. It clearly elicited positive response of DNA repair in human cells (6–9) substance widely distributed in the environment. It has been shown that 1-nitropyrene was metabolically activated to 1-nitro-4,5-dihydro-4,5-epoxypyrene (1-NP 4,5-oxide), 1-nitro-9,10-dihydro-9,10-epoxypyrene (1-NP 9,10-oxide), 1-nitro-9,10-dihydro-9,10-epoxypyrene (I-NP 9,10-oxide), and 1-nitro-9,10-dihydro-9,10-epoxypyrene (1-NP 9,10-oxide) in all species. Hydration of 1-NP oxides by epoxide hydrolase and glutathione S-transferase. In this study, we examined the extent of activation of 1-NP to 1-NP oxides and hydration or glutathione conjugation of 1-NP oxides in the liver of several species including humans. Human liver produced the lowest levels of 1-NP oxides and showed the highest hydrolyzing activity for 1-NP oxides. But no single species is a good model for humans.

MATERIALS AND METHODS

Chemicals. The sources of materials used in this work were as follows: 1-NP from Aldrich Chemical Co., Milwaukee, WI; NADH, NADPH, and glucose 6-phosphate dehydrogenase from Oriental Yeast Co., Ltd., Tokyo, Japan; glucose 6-phosphate, TCPO, and glutathione (reduced form) from Sigma Chemical Co., St. Louis, MO; and all other chemicals from Wako Pure Chemical Industries, Ltd., Osaka, Japan. [3H]-1-Nitro-4,5-dihydro-4,5-epoxypyrene (1-NP 4,5-oxide), 1-nitro-9,10-dihydro-9,10-epoxypyrene (1-NP 9,10-oxide), 1-nitro-4,5-dihydro-9,10-epoxypyrene (1-NP 9,10-oxide), and 1-nitro-9,10-dihydro-9,10-epoxypyrene (1-NP 9,10-oxide) were synthesized by oxidation of [3H]-1-NP and with m-chloroperbenzoic acid following the method of Fifer et al. (21). 1-Nitro-4,5-dihydro-4,5-epoxypyrene (1-NP 4,5-oxide), 1-nitro-9,10-dihydro-9,10-epoxypyrene (1-NP 9,10-oxide), [3H]-1-NP 4,5-oxide, [3H]-1-NP 9,10-oxide, and [3H]-1-NP 9,10-oxide were purified to chemical purity of >97% by HPLC using system 1 as described below. [3H]-Labeled and cold 1-NP oxides were synthesized by oxidation of [3H]-1-NP in rats and mice. Glutathione S-transferase, in CHO cells (22). On the other hand, arene oxides are more mutagenic than 1-NP in the Ames test (18, 21) and in CHO cells (22). On the other hand, arene oxides are more mutagenic than 1-NP in the Ames test (18, 21) and in CHO cells (22). On the other hand, arene oxides are more mutagenic than 1-NP in the Ames test (18, 21) and in CHO cells (22).
per ml, 3 mM magnesium chloride, 1 mM TCPO, and 0.5 mg of liver microsomes per ml (26). The reaction was terminated by extraction with an equal volume of chloroform. After 2 additional extractions with chloroform, the extracts were combined, dried in vacuo, and redissolved in methanol. Metabolites were separated by HPLC system 3. Fractions of 0.5 ml were collected and the radioactivity was determined by a liquid scintillation counter using Tritosol (27). 1-NP oxide-producing activity was calculated from the radioactivity in fractions corresponding to authentic 1-NP 4,5- and 9,10-oxides. 1-NP oxide production was linear between 0 and 600 μg of rat liver microsomal protein for 30 min at 37°C.

Hydration of 1-NP Oxides by Hepatic Microsomes. The incubation mixture consisted of 50 mM sodium potassium phosphate buffer (pH 7.4), 20-800 μg of hepatic microsomes per ml, and 10 μM 1-NP 4,5- or 9,10-oxide. After incubation for 15 min at 37°C, an equal volume of methanol was added. After centrifugation at 12,000 rpm for 10 min, the supernatants were analyzed by HPLC system 4 or 5. For measurement of the amounts of 1-NP 4,5-dihydrodiol and 1-NP 9,10-dihydrodiol produced, [3H]1-NP 4,5-oxide (92.5 μCi/μmol, radiochemical purity 94.6%) or [3H]1-NP 9,10-oxide (120.8 μCi/μmol, radiochemical purity 85.5%) was incubated with rat liver microsomes, and the reaction mixtures were separated by HPLC. The peak area per nmol of 1-NP 4,5- or 9,10-dihydrodiol was calculated from the radioactivity in fractions corresponding to 1-NP 4,5- or 9,10-dihydrodiols. Amounts of nonradioactive 1-NP dihydrodiols produced in the other samples were calculated on the basis of these values. Nonenzymatic formation of 1-NP 4,5- and 9,10-dihydrodiol was 5.0 and 17.5 pmol, respectively, for 15 min. Net enzymatic rates were estimated by subtracting the nonenzymatic rate. Hydration of 1-NP 4,5-oxide was linear with respect to protein concentration (0-800 μg of microsome per ml in rat and mouse; 0-20 or 40 μg/ml in humans). Hydration of 1-NP 9,10-oxide was linear with respect to protein concentration (0.2-4 mg/ml) for 15 min and to time (up to 120 min) at 0.8 mg/ml in rat.

Glutathione Conjugation of 1-NP Oxides by Hepatic Cytosols. The incubation mixture consisted of 50 μM 1-NP 4,5- or 9,10-oxide, 100 mM sodium potassium phosphate buffer (pH 7.4), 1 mM glutathione, and 100 μg of dialyzed hepatic cytosolic protein per ml. Reactions were started by the addition of the substrates. After 10-min incubation at 37°C, the reaction mixtures were extracted 3 times with chloroform, and the aqueous layer containing glutathione conjugates was separated by HPLC system 6. To quantify the glutathione conjugates, [3H]-labeled 1-NP 4,5- or 9,10-dihydrodiol was used. The radioactivity of fractions corresponding to glutathione conjugates was measured by HPLC system 7. Glutathione conjugates and the major radioactive peaks that eluted after 40 min were identified as described by Kinouchi et al. (30).

High-Performance Liquid Chromatography. High-performance liquid chromatography was conducted with a Shimadzu LC-3A or LC-5A instrument equipped with a Shimadzu SPD-2A variable-wavelength UV detector. The chromatographic peak area was computed with a Shimadzu chromatopac CR3A. The column temperature was maintained at 50°C. At times, a Hewlett-Packard HP1040A high-speed spectrophotometric detector was used to obtain on-line UV spectra of the metabolites. All separations were conducted with a reversed-phase column by using one of the following systems: system 1, 70% acetonitrile (ACN) in 10 mM ammonium acetate buffer (pH 4.4), at 1.0 ml/min using a Chemcosorb 5-ODS-H column; system 2, 61% methanol at 3.0 ml/min using a Chemcosorb 10-ODS-H column; system 3, isocratic 60% methanol for 30 min at 1.0 ml/min through a Chemcosorb 5-ODS-H column followed by a 3%/min linear gradient to 100% methanol and then 100% methanol for an additional 20 min; system 4, 65% methanol at 1.0 ml/min using a Chemcosorb 5-ODS-H column; system 5, 55% methanol at 1.0 ml/min using a Chemcosorb 5-ODS-H column; system 6, 25% methanol in 10 mM ammonium acetate buffer (pH 4.4), at 1.0 ml/min using a Chemcosorb 5-ODS-H column; system 7, methanol/20 mM acetic acid (35/65, v/v) for 30 min at 1.0 ml/min through a Chemcosorb 5-ODS-H column followed by isocratic methanol/20 mM acetic acid (70/30, v/v) for 30 min.

Statistics. Enzymatic activity of the various species was compared by analysis of variance.

RESULTS

Formation of 1-NP Oxides by Hepatic Microsomes. When [3H]-labeled 1-NP was incubated aerobically with hepatic microsomes in the presence of TCPO, both 1-NP 4,5-oxide and 1-NP 9,10-oxide were detected in all species studied, but 1-aminopyrene was not detected in any species (detection limit, 0.27 pmol) 1-NP 4,5-oxide-producing activity was highest in hamsters, followed by guinea pigs, rats, dogs, mice, and humans (Table 1). 1-NP 9,10-oxide-producing activity was highest in hamsters, followed by guinea pigs, rats, dogs, mice, and hu-

Table 1 Species differences in the formation of 1-NP oxides by hepatic microsomes

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of animals</th>
<th>Amounts of oxide produced from 1-NP (pmol/min/mg protein)</th>
<th>Total oxides (pmol/min/mg protein)</th>
<th>Ratio of 1-NP 4,5-oxide to 1-NP 9,10-oxide (pmol/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>5</td>
<td>5.9 ± 5.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.4 ± 2.1</td>
<td>8.3 ± 5.5</td>
</tr>
<tr>
<td>ICR mouse</td>
<td>3</td>
<td>3.6 ± 1.8</td>
<td>3.1 ± 0.2</td>
<td>6.7 ± 2.0</td>
</tr>
<tr>
<td>Wistar rat</td>
<td>3</td>
<td>13.4 ± 6.2</td>
<td>12.0 ± 10.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.4 ± 11.2</td>
</tr>
<tr>
<td>Syrian golden hamster</td>
<td>3</td>
<td>15.6 ± 6.2</td>
<td>32.2 ± 8.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>47.8 ± 14.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hartley guinea pig</td>
<td>3</td>
<td>59.8 ± 19.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.7 ± 1.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>72.5 ± 20.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Beagle dog</td>
<td>2</td>
<td>64.2</td>
<td>4.6</td>
<td>68.8</td>
</tr>
<tr>
<td>Mongrel dog</td>
<td>1</td>
<td>65.0</td>
<td>6.3</td>
<td>71.3</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean ± SD

<sup>b</sup> Significantly different from human activity (P < 0.01).

<sup>c</sup> Significantly different from human activity (P < 0.05).
of 1-NP 4,5-oxide (1.5-fold in rats, 6.2-fold in mice) and 1-NP protein) and the ratio of 1-NP 4,5-oxide to 1-NP 9,10-oxide was higher than that in humans and dogs (Table 3). When 1-NP 4,5-oxide was used as a substrate, mice showed the highest activity, which was 18 times higher than humans. Conjugation of 1-NP 9,10-oxide was highest in guinea pigs and lowest in humans and dogs. Aroclor 1254 treatment enhanced the conjugation of 1-NP 4,5-oxide (1.2-fold in rats and mice) and that of 1-NP 9,10-oxide (2.5-fold in rats, 1.5-fold in mice).

Hydration of 1-NP Oxides by Hepatic Microsomes. Hepatic microsomes hydrolyzed 1-NP 4,5- and 9,10-oxide to 1-NP 4,5-dihydrodiol and 1-NP 9,10-dihydrodiol, respectively. Products were identified as 1-NP dihydrodiols by comparison of their retention time on HPLC chromatograms and UV spectra with authentic 1-NP dihydrodiols. When 1-NP 4,5-oxide was used as a substrate, the highest hydrolyzing activity was present in hepatic microsomes from humans, followed in order by those from dogs, guinea pigs, hamsters, rats, and mice (Table 2). The hydrolysis of 1-NP 9,10-oxide was lower than that of 1-NP 4,5-oxide (1/130-1/10). Aroclor 1254 treatment enhanced the hydration of 1-NP 4,5-oxide (2.4-fold in rats, 2.0-fold in mice) and 1-NP 9,10-oxide (4.5-fold in rats, 2.4-fold in mice). Humans showed higher interindividual variation (about 3.5-fold) than the other species (less than 2-fold).

Glutathione Conjugation of 1-NP Oxides by Hepatic Cytosols. The glutathione conjugation of 1-NP oxides in rodents was higher than that in humans and dogs (Table 3). When 1-NP 4,5-oxide was used as a substrate, mice showed the highest activity, which was 18 times higher than humans. Conjugation of 1-NP 9,10-oxide was highest in guinea pigs and lowest in humans and dogs. Aroclor 1254 treatment enhanced the conjugation of 1-NP 4,5-oxide (1.2-fold in rats and mice) and that of 1-NP 9,10-oxide (2.5-fold in rats, 1.5-fold in mice).

HPLC Analysis of Biliary Metabolites in Rats Administered [3H]1-NP p.o. To determine the amount of 1-NP oxide derivatives produced in vivo, bile was collected from [3H]1-NP-treated rats and analyzed by HPLC. Fig. 1 shows the HPLC profile of 0- to 6-h bile specimens. H-labeled metabolites were eluted at the same retention times as glutathione conjugates of 1-NP 4,5- and 9,10-oxide (24.0-29.5 min and 14.5-19.0 min, respectively), which were enzymatically synthesized with rat liver glutathione S-transferase and identified as described previously (30, 31). The glutathione conjugates of 1-NP 4,5-oxide and 1-NP 9,10-oxide accounted for about 2% and 12%, respectively, of the total metabolites in 0- to 6-h bile. These glutathione conjugates were slowly degraded to cysteinylglycine conjugates or cysteine conjugates in the bile (30, 31). Since peak A disappeared after treatment of bile with β-glucuronidase and sulfatase and its UV spectrum was identical to that of 1-NP 4,5-dihydrodiol, peak A was tentatively identified as the glucuronide or sulfate conjugates of 1-NP 4,5-dihydrodiol, which is the same result as described by Kinouchi et al. (30). It contained 10% of the total metabolites in 0- to 6-h bile. 1-NP 9,10-dihydrodiol or its conjugates were not detected. 1-NP 4,5-oxide-derived metabolites accounted for 13% of the total metabolites in 0- to 6-h bile and 1-NP 9,10-oxide-derived metabolites for 15%.

Table 2 Species differences in the formation of dihydrodiols by hepatic microsomes

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of animals</th>
<th>1-NP 4,5-dihydrodiol (nmol/min/mg protein)</th>
<th>1-NP 9,10-dihydrodiol (nmol/min/mg protein)</th>
<th>Ratio of 1-NP 4,5-dihydrodiol to 1-NP 9,10-dihydrodiol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>5</td>
<td>19.5 ± 12.3*</td>
<td>0.156 ± 0.07</td>
<td>130.4 ± 53.1*</td>
</tr>
<tr>
<td>ICR mouse</td>
<td>3</td>
<td>0.40 ± 0.07*</td>
<td>0.016 ± 0.0006*</td>
<td>24.2 ± 3.5</td>
</tr>
<tr>
<td>Wistar rat</td>
<td>3</td>
<td>0.88 ± 0.06*</td>
<td>0.039 ± 0.008*</td>
<td>23.4 ± 6.6</td>
</tr>
<tr>
<td>Syrian golden hamster</td>
<td>3</td>
<td>1.89 ± 1.56*</td>
<td>0.184 ± 0.009*</td>
<td>10.2 ± 2.7</td>
</tr>
<tr>
<td>Harlthy guinea pig</td>
<td>3</td>
<td>6.43 ± 1.46*</td>
<td>0.479 ± 0.014*</td>
<td>13.4 ± 2.9</td>
</tr>
<tr>
<td>Beagle dog</td>
<td>2</td>
<td>6.83</td>
<td>0.081</td>
<td>84.7</td>
</tr>
<tr>
<td>Mongrel dog</td>
<td>1</td>
<td>3.38</td>
<td>0.080</td>
<td>42.3</td>
</tr>
</tbody>
</table>

* Mean ± SD.

† Significantly different from human activity (P < 0.05).

‡ Significantly different from human activity (P < 0.01).

Table 3 Species differences in the formation of glutathione conjugates by hepatic cytosols

The incubation mixture consisted of 50 μM 1-NP 4,5- or 9,10-oxide, 100 mM sodium potassium phosphate buffer (pH 7.4), 0.1 mg glutathione, and 100 μg of dialyzed hepatic cytosols per ml. After 10-min incubation at 37°C, the reaction mixtures were extracted 3 times with chloroform and the aqueous layer containing glutathione conjugates was separated by HPLC.

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of animals</th>
<th>Amounts of glutathione conjugate produced from 1-NP oxide (nmol/min/mg protein)</th>
<th>Ratio of 1-NP 4,5-oxide-SG to 1-NP 9,10-oxide-SG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>6</td>
<td>1.16 ± 0.41*</td>
<td>1.83 ± 0.34*</td>
</tr>
<tr>
<td>ICR mouse</td>
<td>3</td>
<td>21.5 ± 0.56*</td>
<td>4.85 ± 0.40*</td>
</tr>
<tr>
<td>Wistar rat</td>
<td>3</td>
<td>18.6 ± 1.34*</td>
<td>4.85 ± 0.80*</td>
</tr>
<tr>
<td>Syrian golden hamster</td>
<td>3</td>
<td>14.4 ± 0.49*</td>
<td>6.55 ± 0.36*</td>
</tr>
<tr>
<td>Harlthy guinea pig</td>
<td>3</td>
<td>18.6 ± 1.03*</td>
<td>7.31 ± 0.24*</td>
</tr>
<tr>
<td>Beagle dog</td>
<td>2</td>
<td>1.83</td>
<td>0.55</td>
</tr>
<tr>
<td>Mongrel dog</td>
<td>1</td>
<td>1.54</td>
<td>0.59</td>
</tr>
</tbody>
</table>

* Mean ± SD.

† Significantly different from human activity (P < 0.01).

Fig. 1. HPLC of biliary metabolites in 0- to 6-h bile specimens from rats administered [3H]1-NP p.o. Bile was separated by HPLC system 7. Fractions of 0.5 ml were collected and the radioactivity was determined by a liquid scintillation counter using Triton X. Bars, radioactivity in the fractions; line graph, A250.
DISCUSSION

In this study, we investigated the oxidative activation and metabolic inactivation of 1-NP, and found species differences in 1-NP oxide-producing activity, microsomal epoxide hydrolase, and cytosolic glutathione S-transferase (Fig. 2). In humans 1-NP oxide-producing activity was as low as that in mice, and inactivation of 1-NP oxides was similar to that in dogs. But no single species of the 5 species studied was a good model of human. The balance of activation/inactivation tends toward detoxification in adult animals of all species.

Since 1-NP oxide-producing activity in hepatic microsomes was induced by Aroclor 1254 treatment in mice and rats, P-450-dependent mixed-function oxidases must have catalyzed the production of 1-NP oxides. Humans showed the lowest activity, which was at the same level as mice. But interindividual variation was very large: the greatest increase was 6.3-fold in 1-NP 9,10-oxide formation compared with the lowest level. It is interesting that the ratio of 1-NP 4,5-oxide to 9,10-oxide differed between the species. Different isozymes might oxidize the 4,5-region and 9,10-region of 1-NP. Ioannides et al. (32) showed that sterically hindered positions of xenobiotics were effectively oxidized by cytochrome P-448, which is a 3-methylcholanthrene-inducible form of P-450. Hamster liver may contain more P-448 than that of other species because the hindered 9,10-position was effectively oxidized to epoxide. Aroclor 1254 treatment of rats lowered this ratio from 1.1 (untreated) to 0.73 (treated). Therefore, Aroclor 1254 possibly induces more P-448, which oxidizes the 9,10-position efficiently. In the human sample, this ratio also varied widely between individuals, probably because of the polymorphism of P-450, which is regulated by genetic factors and affected by nutrition, drug intake, exposure to pollutants, and cigarette smoking as reported by Eichelbaum (33).

Epoxide hydrolase activity was highest in human hepatic microsomes. The ability to hydrolyze 1-NP 4,5-oxide was higher in large mammals than in rodents as shown in previous data (34-36). 1-NP 9,10-oxide was a very poor substrate for epoxide hydrolase in all species tested. This may be due to steric hindrance by a nitro group and the lipid environment near the active site of the enzyme as indicated by Seidegard and DePierre (34) and Lu and Miwa (37). The lipid environment near the enzyme would be expected to influence the catalytic properties of the enzyme or the local concentration of substrates near the active site of the enzyme. The properties of the enzyme are affected by differences in the ratio of phospholipid to enzyme (38). Differences in lipid environment among the species tested might affect the differences in the ratio of 1-NP 4,5-dihydriodiol to 9,10-dihydriodiol.

Aroclor 1254 treatment enhanced the ability to hydrolyze 1-NP oxides 2.0- to 4.5-fold, the same levels as reported previously (34, 36). The wide degree of interindividual variation in humans may be due to the different levels of induction by various xenobiotics in each individual. A number of compounds induce microsomal epoxide hydrolase to various extents (34, 39).

Since epoxide hydrolase is also found in cytosols and its activity is high in the mouse liver (40), we determined the epoxide hydrolase activity of liver cytosols from mice. With 1-NP oxides used as substrate, mouse liver cytosols showed very low activity. Cytosolic epoxide hydrolase may contribute little to the hydration of 1-NP oxides because of its difference in specificity for substrates and its cellular location far away from the endoplasmic reticulum where 1-NP oxides are produced, as indicated by Oesch and Golan (41).

Human cytosols showed less ability to conjugate glutathione than those of the other animals, in agreement with previous findings (42, 43). This is probably due to the small amount of isozymes that catalyze the conjugation of 1-NP oxides. Nemoto et al. (44) suggested that the human liver contains a lower level of glutathione S-transferase than the rat liver. The low activity and interindividual variation of glutathione conjugation in humans were partially due to the loss of isozyme \( \mu \), which has shown high activity toward benzo[a]pyrene 4,5-oxide but has been lost in 40% of adult humans (45).

1-NP 9,10-oxide was a poorer substrate for cytosolic glutathione S-transferase than 1-NP 4,5-oxide, probably owing to steric hindrance by a nitro group. Hayakawa et al. (46) indicated that a sterically hindered arene epoxide isomer is relatively inactive, while the other isomer is active.

Recently microsomal glutathione S-transferase, which could conjugate carcinogenic substances such as benzo[a]pyrene 4,5-oxide and hexachlorobutadiene, was found to be present in all mammalian species including humans (47). As 1-NP oxides were produced and would accumulate in a membraneous site, we examined hepatic microsomes from rats and mice for the ability to conjugate glutathione. But hepatic microsomes from both animals showed about 13-fold lower ability to conjugate 1-NP 9,10-oxide, and 50-fold lower ability to conjugate 1-NP 4,5-oxide than hepatic cytosols. Microsomal glutathione S-transferase contributes little to conjugation of 1-NP oxides as reported by Glatt and Oesch (48).

Biliary metabolites produced from 1-NP 9,10-oxide were 15% as glutathione conjugates or its metabolites. 1-NP 4,5-oxide was also 15% as glutathione conjugates or its metabolites.

Table 2. Species differences in oxidative activation and metabolic inactivation of 1-NP. Values under open arrows, 1-NP oxide-producing activity (pmol/min/mg protein); values under dark arrows, hydrolysis or glutathione conjugation of 1-NP oxides (nmol/min/mg protein). A, 1-NP; B, 1-NP 4,5-oxide; C, 1-NP 9,10-oxide; D, 1-NP 4,5-dihydridiol; E, glutathione conjugates of 1-NP 4,5-oxide; F, 1-NP 9,10-dihydridiol; G, glutathione conjugates of 1-NP 9,10-oxide.

* K. Kataoka, T. Kinouchi, and Y. Ohnishi, unpublished observations.

Fig. 2. Species differences in oxidative activation and metabolic inactivation of 1-NP. Values under open arrows, 1-NP oxide-producing activity (pmol/min/mg protein); values under dark arrows, hydrolysis or glutathione conjugation of 1-NP oxides (nmol/min/mg protein). A, Human; B, Hamster; C, Mouse; D, Guinea pig; E, Rat; F, Beagle dog; G, Human.
oxido-degraded metabolites accounted for 14% of the total biliary metabolites: 4% as glutathione conjugates or its metabolites and 10% as conjugates of 1-NP 4,5-dihydirodiol. The ratio (1.1) of 1-NP 4,5-oxide to 9,10-oxide produced in vitro reflects the in vivo data well.

Djuric et al. (49) have analyzed 0- to 4-h bile from 1-NP-dosed rats. They reported the occurrence of glutathione conjugates in the bile and indicated that the preferential hydrolysis and/or glutathione conjugation of the particular oxides. In our experimernt, lower amounts of glutathione conjugates (25% of biliary metabolites) were detected in 0- to 6-h bile. This is probably due to the difference of the route and dose of administration of 1-NP. Further metabolism of 1-NP oxides in rat liver was similar to the results of Djuric et al. (49).

The specific activity of cytosolic glutathione conjugation was higher than that of microsomal epoxide hydrolase in the in vitro assay, but in rat bile, the amounts of 1-NP 4,5-oxide-glutathione conjugates were less than those of conjugates of 1-NP 4,5-dihydirodiol. This is due to the difference in the subcellular location of these enzymes. As indicated by Glatt and Oesch (48), epoxide hydrolase is localized in microsomes, where reactive metabolites are usually generated by P-450-dependent mixed-function oxidases in the microsomes, and where lipo philic metabolites would accumulate. Therefore, in the case of 1-NP 4,5-oxide, hydron of epoxide occurred in preference to glutathione conjugation.

ACKNOWLEDGMENTS

We are grateful to Dr. R. Kato for giving us the human hepatic microsomes and cytosols.

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