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EXTRA–HEPATIC GSH-DEPENDENT METABOLISM OF 1,2-DIBROMOETHANE (DBE) AND 1,2-DIBROMO-3-CHLOROPROPANE (DBCP) IN THE RAT AND MOUSE

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ABSTRACT

Although DBE is metabolized by both microsomal and cytosolic pathways, it is the latter, GSH–dependent route, that may lead to hepatic and extra–hepatic genotoxicity and mutagenicity. As both DBE and DBCP exhibit predominantly extra–hepatic toxicity, their in vitro GSH–dependent debromination was measured in cytosolic fractions prepared from liver, kidney, testes and stomachs of Sprague–Dawley rats and Swiss–Webster mice. There was a marked species difference between the rat and mouse, vith the rat metabolizing DBCP more rapidly than DBE, and the mouse metabolizing DBE more rapidly than DBCP. Hepatic rates exceeded those seen in extra–hepatic tissues in every case. Extra–hepatic rates of debromination represented as much as 84% of the hepatic rates, and generally followed the order: kidney > testes > stomach. Rates of metabolism for DBE and DBCP represented only a small fraction of the total cytosolic GSH S–transferase activity. These findings suggest significant levels of GSH–dependent metabolism may occur within those tissues associated with the in vivo toxicity of DBE and DBCP.

INTRODUCTION

1,2-Dibromoethane (DBE, ethylene dibromide, EDB) and 1,2-dibromo-3-chloropropane (DBCP) have both been widely used for

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agricultural purposes as nematocides in the control of roundworms. DBE is also used extensively as a lead scavenger component in leaded gasoline.[1] The toxicities of DBE and DBCP are predominantly extra-hepatic. DBCP and DBE have been shown to produce toxicity towards the male reproductive system in a number of species.[2,3,4] Nephrotoxicity has also been reported in a number of species following exposure to both DBE and DBCP.[5] In the case of DBCP, evidence appears to indicate the proximal convoluted tubule as the primary site of damage in the kidney.[3]

DBE and DBCP have both been shown to produce cancers in laboratory animals when administered in repeated doses. Squamous cell metaplasia, hyperplasia and cytomegaly of the respiratory nasal turbinals, as well as of the larynx, trachea, bronchi and bronchioles were reported in both rats and mice when exposed to these compounds via the inhalation route.[6] Chronic oral administration of either DBE or DBCP produced squamous cell carcinomas in the forestomach of both rats and mice.[7]

DBE and DBCP are mutagenic in bacterial test systems with the addition of some type of enzymatic activating system.[8] For DBE, mutagenicity has been shown to be NADPH-independent, and enhanced by the addition of exogenous reduced glutathione.[9]

Results from a number of studies have shown that DBE and the chlorinated analog 1.2-dichloroethane are conjugated with GSH via the glutathione S-transferases. The conjugate undergoes

intra-molecular rearrangement to form the highly reactive episulfonium ion, which preferentially binds to nucleic acids.[10,11] Therefore, biotransformation by glutathione transferases appears to result in the formation of a mutagenic/carcinogenic metabolite. Since GSH-transferase activities are often high in extra-hepatic tissues, the GSH-dependent biotransformation of DBE and DBCP using cytosol obtained from rat and mouse kidney, testes, stomach and liver was compared,

METHODS

Chemicals

1,2-Dibromoethane (Matheson, Coleman and Bell, Norwood, OH) was redistilled prior to use. 1,2-Dibromo-3-chloropropane was purchased from Pfaltz and Bauer, Inc., Stamford CN. Reduced glutathione, 1-chloro-2,4-dinitrobenzene (recrystallized from ethanol prior to use) and serum albumin stock solution were obtained from the Sigma Chemical Co., St. Louis, MO. Bromide standard solution was purchased from Orion Research Inc., Cambridge, MA.

Animals

Male Sprague-Dawley rats (250-350 g) housed 2-4 per cage were obtained from Hilltop Laboratories, Hilltop, PA. Male Swiss-Webster mice (25-40 g) were purchased from Charles River

Breeding Laboratories Inc., Wilmington, MA and housed 4-6 per cage. All animals were provided food (Wayne Lab Blox) and water ad libitum prior to termination.

Preparation of Cellular Fractions

Hepatic and extra-hepatic cytosolic fractions were prepared From rats and mice by procedures similar to those described by Sipes, et al.[12] At termination, livers, kidneys, testes and stomachs were removed and immediately placed in ice-cold 0.05 M sucrose. Like-tissues were pooled, weighed and homogenized in 3 volumes of cold 0.05 M Tris-HCl, 1.15% KCl, at pH 7.4. Liver and testes were homogenized by hand using a Dounce tissue homogenizer (Vineland, NJ), while kidney and stomach tissues were homogenized at low speed for 30-60 sec in glass grinding tubes with power driven Teflon pestles (A.H. Thomas, Philadelphia, PA).

Cytosolic fractions were prepared by differential centrifugation at 4°C. The resulting 100,000 x g supernatant fractions were filtered through glass wool and dialyzed against 1.5 L of 0.05 M Tris-KCl buffer, pH 7.4, for 18 hr. During this time the buffer was changed three times.

Transferase Activities in Cytosolic Fractions

The rate of formation of the product of enzymatic reaction between GSH and the substrate 1-chloro-2,4-dinitrobenzene was measured by monitoring an increase in absorbance at 340 nm. The

reaction mixture consisted of the following: 1 mM glutathione, 0.001–0.1 mg/ml cytosolic protein, 1 mM chlorodinitrobenzene, in a 3 ml final volume of 0.1 M potassium phosphate buffer, pH 6.5, at 25°C. Reference cuvettes were identically prepared, except the GSH was omitted. Specific activities were calculated using an extinction coefficient value of 9.6 mM^{-1} cm^{-1}, as determined by Habig et al[13] for this substrate.

In Vitro Biotransformation of DBE and DBCP

Liberation of inorganic bromide was used to assess the degree of biotransformation of DBE and DBCP. Incubations were carried out in 12 ml screw–top culture tubes. Reaction mixtures consisted of the following: 1 mM substrate (DBE or DBCP dissolved in ethanol), 0.5–5.0 mg/ml cytosolic protein, 1 or 5 mM GSH, in a 2 ml total volume of 0.05 M Tris-HCl, 1.15% KCl buffer, pH 7.4. Experimental blanks were identical, except the GSH was omitted. Samples were incubated in a 37°C water bath for the various periods of time. Reactions were terminated by freezing in a methanol:dry ice bath.

Frozen samples from cytosolic incubations to be analyzed for the presence of free inorganic bromide were lyophilized to remove unreacted parent compound. Samples were rehydrated with distilled water, and the bromide ion concentration determined using the gas chromatography method of Maiorino et al.[14]

Statistics

The unpaired Student's t-test was used to evaluate differences between two means. For three or more groups, one-way analysis of variance, in conjunction with the least significant difference (LSD) test was employed. Groups were judged significantly different when a p value of less than 0.05 was obtained,

RESULTS

The various rates of glutathione S-transferase activity among hepatic and extra-hepatic tissues of the rat and mouse using 1-chloro-2,4-dinitrobenzene as a substrate are presented in Table 1. In the rat, the liver and testes had the highest activities. Activity in the kidney and stomach was considerably less. In the mouse, the liver also exhibited the highest equivalent activity, followed by the kidney, testes and stomach.

Optimization of In Vitro Conditions

In order to permit inter-species comparison in the rates of GSH-dependent metabolism of DBE and DBCP, effort was made to optimize the in vitro enzymatic conditions. Using hepatic cytosolic fractions, reactions were determined to be linear with respect to protein concentration over the range of 0.5 to 5.0 mg/ml. A concentration of 5 mM GSH was selected since it produced maximal enzymatic bromide release without producing a

TABLE 1

Hepatic and Extra-hepatic Metabolism of 1,2-Dibromoethane and 1,2-Dibromo-3-chloropropane in the Rat and Mouse Expressed as a Percentage of Overall Tissue Transferase Activity.

Rat Tissue[a]	Total GSH S-transferase activity[b]	DBE Rate[c]	DBE %Total[d]	DBCP Rate[c]	DBCP %Total[d]
Liver	815±100	5.76	0.71	16.60	2.05
Kidney	151±45	3.14	2.08	8.11	5.36
Testes	615±72	0.84	0.14	7.24	1.17
Stomach	79±29	0.56	0.71	6.30	7.97
Mouse Tissue[a]	**Total GSH S-transferase activity[b]**	**DUE Rate[c]**	**DUE %Total[d]**	**DBCP Rate[c]**	**DBCP %Total[d]**
Liver	1891±401	19.14	1.01	1.60	0.09
Kidney	573±87	16.07	2.80	0.36	0.07
Testes	406±109	11.80	2.91	0.51	0.13
Stomach	281±97	7.80	0.17	0.27	0.10

[a] Testes and stomach supernatants were pooled. Liver and kidney were individual supernatants

[b] nmoles 1-chloro-2,4-dinitrobenzene consumed/mg cytosolic protein/min

[c] nmoles DBE- or DBCP-derived inorganic bromide/mg cytosolic protein/min

[d] DBE or DBCP rate divided by total transferase rate x100

significant non-enzymatic effect. Reactions were linear with respect to time for at least 5 min, with the exception of mouse liver cytosol using DBE as the substrate, where a decrease in linearity was noted between 2 and 5 min. Rates presented are based on linear regions of enzyme activity.

Hepatic and Extra-hepatic GSH-Dependent Metabolism of DBE and DBCP

Figure 1 compares the rates of GSH-dependent, enzymatic bromide release from DBE and DBCP when various cytosols from rats and mice were used. For DBE, the highest activities for bromide release were found in mouse liver cytosol, which was approximately three times more active than rat liver cytosol. The activities in mouse kidney, testes and stomach cytosols were also greater than those for the corresponding rat tissues.

A marked species difference in the rate of GSH-dependent debromination of DBCP was also observed. For this compound, the rat cytosols showed the greatest activities. Liver cytosol activities were roughly twice those obtained with the kidney, testes or stomach. For all tissues, the rat cytosols were approximately 10 times more active than the corresponding tissues of the mouse.

The rates of GSH-dependent debromination of DBE and DBCP by the various tissues of the rat and mouse represent only a small percentage of the total GSH S-transferase activity in that tissue (Table 1). For DBE, the kidney cytosols of both species and the

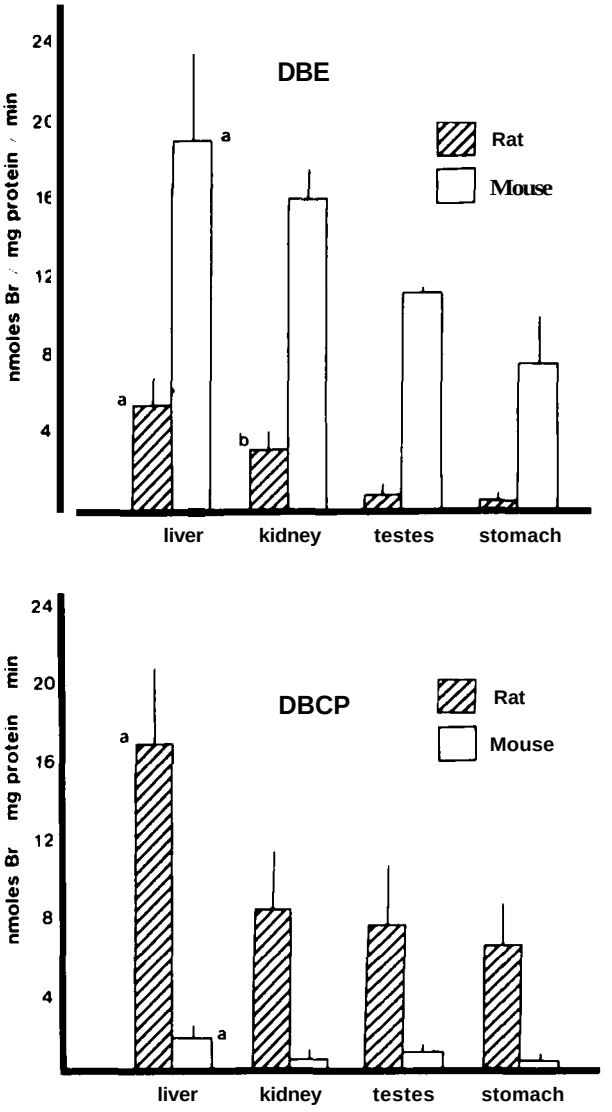

FIG. 1 GSH-Dependent Metabolism of DBE and DBCP by Hepatic and Extra-hepatic Cytosols of the Rat and Mouse. In Vitro conditions: 1 mg/ml cytosolic protein, 5 mM GSH, 1 mM DBE or DBCP.

[a]significant differences between hepatic and extra-hepatic rates ($p < 0.05$).

[b]significant differences between kidney and other extra-hepatic rates ($p < 0.05$).

cytosol of mouse testes exhibited the greatest specific activity relative to total glutathione S-transferase activity. Glutathione S-transferase activity towards DBE appears to represent a somewhat lower proportion of the overall activity in the liver and stomach tissues of both species. The glutathione S-transferases with significant levels of activity towards DBCP were most concentrated in the stomach and kidney of the rat. In the mouse, these activities represented only 0.1% of the activity.

DISCUSSION

A marked species difference between the rat and mouse was observed for the GSH-dependent debromination of DBE and DBCP in all tissues examined. In the rat, rates of metabolism of DBCP were significantly greater than those observed for DBE in each of the four tissue cytosols. The reverse situation was seen for the mouse cytosols, where DBE underwent much greater rates of debromination than DBCP.

If the GSH-dependent route is central to the formation of the toxic species formed from these two compounds, a positive relationship between GSH-dependent enzymatic rates and *in vivo* toxicity would be expected. DBCP, which is more readily metabolized along a GSH-dependent route by the rat than the mouse, appears to be a much more potent toxicant in the rat. Chronic studies in which DBCP was administered orally to rats and

mice indicate that much lower doses were required in rats compared to mice, to produce comparable toxicity.[7,15] In one such study, the maximally tolerated doses of DBCP when administered by gavage were 30 mg/kg in the rat, versus 260 mg/kg in the mouse.[15] A clear–cut difference in species–specific potency does not appear to exist however, for DBE in rats and mice. Clearly, a number of complicating factors present in the whole animal model limit the degree to which the in vitro data can be interpreted.

Although rates of debromination vary between compounds and species, the activities of the four tissues relative to one another are similar, with the liver exhibiting the highest rate in every case. A similar pattern among liver and extra–hepatic rat tissue cytosols using DBE as a substrate has been reported by Hill.[16] This pattern is again seen using the four tissue cytosols from the rat and mouse, and the standard colorimetric substrate 1-chloro-2,4-dinitrobenzene (Table 1). The only exception is seen in the rat, where a greater activity was seen for the testes compared to the kidney.

The distribution of cytosolic, as well as microsomal, transferase activity in the rat has previousoly been examined using chlorodinitrobenzene.[17] Rates of cytosolic metabolism were comparable to those seen in the current study, but differences were observed in the relative activities of the various tissues. In particular, very high rates were seen for

the testes, exceeding even those seen using hepatic cytosol. These inconsistencies may be the result of differences in the methods used in the preparation of subcellular fractions.

Both DBE and DBCP are readily metabolized using hepatic and extra-hepatic cytosols. When expressed as percentages of the overall transferase activity in each tissue, however, these rates represent only a fraction of the overall activity (Table 1). Expression of the data in this manner may indicate the relative predominance of those transferase forms involved in the metabolism of short-chain organohalogens. In general, these results indicate a greater proportion of this type of transferase activity in extra-hepatic tissues compared to liver. Such a situation would increase the likelihood of an extra-hepatic toxic response, especially if the GSH-dependent pathway predominates over cytochrome P-450 mediated biotransformation of these compounds in extra-hepatic tissues.

Although hepatic rates of debromination exceed those seen in the other tissues for DBE and DBCP in the rat and mouse, neither compound is characterized as being predominantly hepatotoxic. A possible explanation may lie in the relative proportion of the parent compound being metabolized along a GSH-dependent route, versus that amount initially entering into a cytochrome P450-mediated oxidative pathway. Using tetradeutero-DBE, Van Bladeren et al[18] estimated that the predominant *in vivo* biotransformation was mediated by the microsomal *P-450* pathway, accounting for approximately 80% of the total biotransformation.

These estimates were, however, based on levels of urinary metabolites and, as such, may not reflect the relative significance of the two pathways within specific tissues or organs.

Evidence for a unique mechanism of GSH–dependent bioactivation for DBE, coupled with an observed pattern of extra–hepatic toxicity for both DBE and DBCP, may indicate a role for extra–hepatic metabolism in the development of toxicity. Findings in the current study show both compounds to be readily metabolized using cytosolic preparations from rat and mouse tissues. Further studies comparing the relative rates of P450-mediated Phase I metabolism to GSH conjugation within the various tissues may provide more information concerning the role of the GSH–dependent pathway in the toxicity of DBE and DBCP.

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