Induction and development of mouse liver glutathione S-transferase activity

D. Dale Shoemaker, D. D. Dietrick and R. L. Cysyk[1]

Laboratory of Chemical Pharmacology, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda (Maryland 20205, USA), 16 July 1980

Summary. Mouse liver glutathione S-transferase activity at birth was 1/10 that of adults, and increased steadily with each successive week of age until adult values were reached at 8 weeks. Activity was inducible with phenobarbital; however, the percentage increase in activity was dependent upon substrate. 2 distinct peaks of transferase activity were obtained on CM-cellulose chromatography. The ratios of transferase activity observed for each peak demonstrated that glutathione S-transferase activity in mouse liver is associated with at least 2 distinct proteins with differing substrate specificities.

The glutathione S-transferases (EC 2.5.1.18) are a group of soluble enzymes which function physiologically as detoxification agents[2-4]. The transferases are capable of catalyzing reactions between the nucleophilic thiol group of glutathione and the electrophilic site of a variety of compounds. Non-enzymatic functions of the transferases include acting as storage proteins (reversible binding), and as scavengers of highly electrophilic compounds (covalent binding)[4]. Although these enzymes have been well characterized in rats[4-8], little information is available for the mouse[9,10]. Mice are widely used in many routine screening procedures, such as the screening for antitumor or carcinogenic activity, where compounds of varied structure are tested. Because of the broad substrate specificity of the glutathione S-transferases[5-8], it would be expected that interaction with these compounds (either directly or following metabolic conversion) would be a likely occurrence. For such compounds, the amount of transferase activity would be an important determinant in the measured effect. We initiated the present study to determine the activity, postnatal development, and induction of mouse liver glutathione S-transferases.

Materials and methods. Female BALB/c mice pregnant with CDF_1 offspring (BALB/c × DBA/2) were received approximately 5 days before parturition and were maintained on a diet of Purina rat chow and water as were all mice used in this study. Groups of the CDF_1 offspring were sacrificed by cervical dislocation at approximately weekly intervals from birth through 14 weeks of age. The livers were rapidly excised, weighed, and homogenized in 4 vol. of ice-cold 0.1 M sodium phosphate buffer (pH 7.4) with a Polytron homogenizer. The homogenate was centrifuged at 200 × g for 30 min; the resulting supernatant fraction was centrifuged again at 100,000 × g for 60 min. The 100,000 × g supernatant served as the enzyme source and was assayed for transferase activity with 1-chloro-2,4-dinitrobenzene (DNCB) and 1,2-dichloro-4-nitrobenzene (DCNB) as described by Booth et al.[11] and Habig et al.[6].

For the induction studies male CDF_1 mice, 10 weeks old, were administered phenobarbital sodium (75 mg/kg, i.p.) daily for 7 days. Groups of 4 control mice (0.9% saline, i.p.) and 4 treated mice were killed daily from day 0 through day 16 and the 100,000 × g enzyme source was prepared and assayed for transferase activity as described above.

For purification, mouse livers were homogenized in 4 vol. of ice-cold 0.01 M Tris-HCl buffer (pH 8.0) and centrifuged as above. The 100,000 × g supernatant fraction was applied to a column of DEAE-cellulose (2.5 × 50 cm, Whatman DE-52) previously equilibrated with 0.01 M Tris-HCl (pH 8.0). The column was eluted with the equilibrating buffer until the eluate was devoid of transferase activity. The fractions (14.0 ml) which contained activity were combined and ammonium sulfate (660 g/l) was added. The preparation was centrifuged at 10,000 × g for 30 min. The resulting precipitate was dissolved in 30 ml of 0.01 M sodium phosphate buffer (pH 6.7) and dialyzed for 1 day against 3 changes of this buffer (1 l of buffer per change). The dialyzed preparation was applied to a CM-cellulose column (2.5 × 50 cm, Whatman CM-52) previously equilibrated with 0.01 M sodium phosphate buffer (pH 6.7). The column was washed with 250–300 ml of this buffer before applying a 550-ml linear salt gradient (0–75 mM NaCl) composed of 275 ml of 0.01 M sodium phosphate buffer (pH 6.7) and 275 ml of 0.01 M sodium phosphate buffer (pH 6.7) containing 75 mM NaCl. The fractions (7.5 ml)

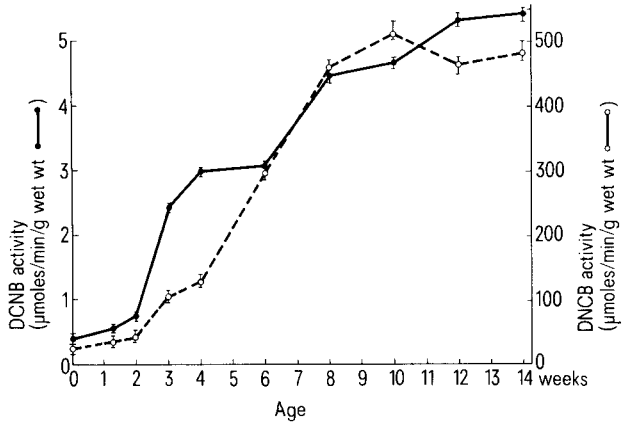

Fig. 1. Development of mouse liver glutathione S-transferase activity with age. The catalytic activity of the transferases for DCNB (●) and DNCB (○) was measured. Each value is the mean ± SE of the activities for 5 groups with pooled livers of 4 male mice per group and is reported as μmoles/min/g wet wt of liver.

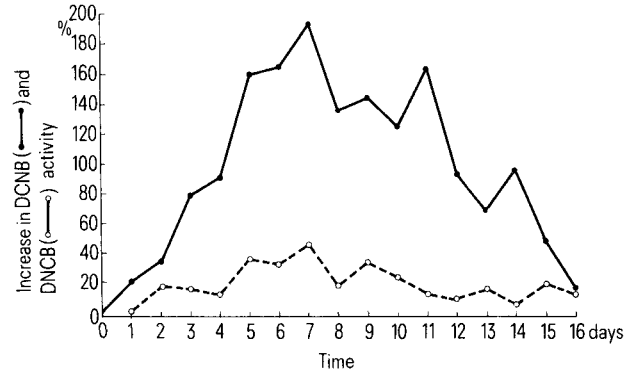

Fig. 2. Induction of mouse liver glutathione S-transferase activity. Phenobarbital was administered i.p. daily, days 1–7, and the catalytic activity of the transferase determined with DCNB (●) and DNCB (○) from days 0–16. Each value is the mean of the percent increase in activity as determined with enzyme prepared from the pooled livers of 4 control (saline) and 4 treated mice at each time point.

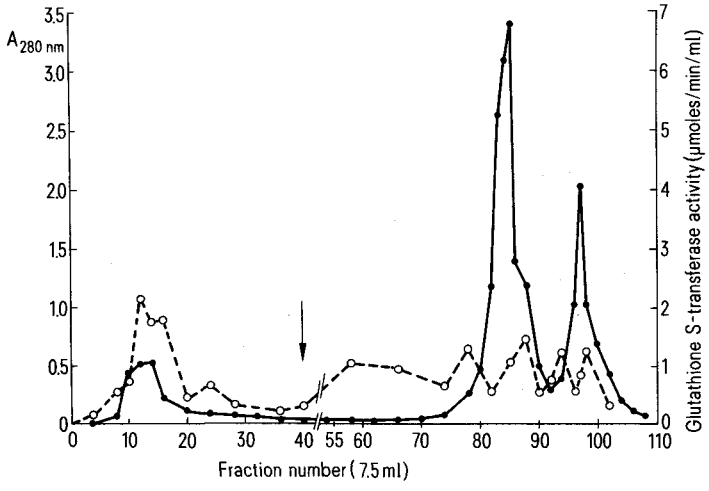

Fig. 3. Elution pattern of the glutathione S-transferases from a CM-cellulose column. The different transferases were separated by CM-cellulose chromatography. The values are respresentative of 5 such columns. The arrow indicates initiation of the gradient. DNCB activity (●) and absorbance at 280 nm (○).

were collected and assayed for transferase activity. The peaks containing activity were combined and the ratio of glutathione S-transferase activities determined with DNCB and DCNB.

Results and discussion. Liver transferase activity was determined at birth and at various times thereafter using DCNB and DNCB as substrates (figure 1). The activity at birth was approximately $^1/_{10}$ that found in adults and increased weekly until adult values were reached at approximately 8 weeks of age. The patterns of increase in transferase activity with DCNB and DNCB were comparable from birth until adult values, however, the transferase activity with DNCB was consistently greater than that with DCNB over the 14-week measurement period. The postnatal development of mouse hepatic transferase activity is similar to the developmental patterns reported previously for glutathione S-transferase B of rat liver[12] and for the sulfobromophthalein-glutathione conjugating enzyme system of mouse[9], rat[13], and guinea-pig[14] liver. More recently sulfobromophthalein has been shown to be a substrate for 3 of the glutathione S-transferases isolated from rat liver[6]. Using DCNB as substrate, the maturation period of hepatic transferase activity was 4 weeks for the guinea-pig and 10 weeks for the rabbit[15].

The increase in transferase activity following phenobarbital treatment (75 mg/kg, i.p., days 1–7) is shown in figure 2. Increased activity for both DCNB and DNCB could be detected as early as 24 h after the initial phenobarbital dose. The pattern of induction for DCNB paralleled that for DNCB; however, the increase in activity for DCNB was approximately 4-fold greater throughout the measurement period. The increase in activity peaked at day 7 and then gradually declined after the cessation of phenobarbital treatment. As an indicator of the effective action of phenobarbital, the percent increase in the liver weight to body weight ratio of the treated versus control mice was also measured and found to peak at approximately 35% on days 7–9. In a similar study in Sprague-Dawley rats[16], treatment with phenobarbital (80 mg/kg, i.p. for 7 days) resulted in an increase in activity on day 7 of approximately 80% with DCNB as substrate, much less than the almost 200% increase observed in this study. Accordingly, Kulkarni et al.[17] found that the hepatic transferases of mice were more responsive to induction by pesticides than those of rats. Thus, a species difference exists in the inducibility of hepatic glutathione S-transferase activity.

The elution pattern of glutathione S-transferase activity from a CM-cellulose column is shown in figure 3. 2 distinct peaks of transferase activity as determined with DNCB were eluted after initiation of the NaCl gradient. An additional smaller peak of activity was eluted with the wash as has been noted previously in the purification of rat[6] and mouse[10] hepatic transferases. The ratio of transferase activity determined with DNCB versus DCNB was 92.0 ± 11.8 for peak 1 and 12.2 ± 1.5 for peak 2 (mean ratio $\pm$ SE for 5 columns). Similarly, CM-cellulose chromatography of C3H mouse liver cytosol yielded 2 major peaks of transferase activity using DCNB and DNCB as substrates[18]. More recently, 2 peaks of activity were observed with DNCB after CM-cellulose chromatography of hepatic cytosol from ICR strain mice[19] and after isoelectric focusing of liver cytosol from CD1 mice[20].

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