PREMATURE DEVELOPMENT OF LIGANDIN (GSH TRANSFERASE B) IN MICE WITH AN INHERITED DEFECT IN ENDOPLASMIC RETICULUM-GOLGI STRUCTURE AND FUNCTION*

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SUMMARY

Radiation-induced albino mouse mutations have deficient microsomal enzyme activities and structurally abnormal endoplasmic reticulum-Golgi membranes in liver and kidney. Ligandin (GSH transferase B) is essential for nonoxidative detoxification. Cytochrome P-450, which is essential for oxidative detoxification, is virtually absent in mutant homozygous mice. The catalytic activity of ligandin in liver, kidney and small intestinal mucosa was double that of heterozygous littermates and newborn controls and was equivalent to enzyme activity in control adult mice. Early enzyme maturation in homozygous mutants probably results from accumulation of substrates which are normally metabolized by the cytochrome P-450 oxidative system.

INTRODUCTION

Previous studies of albino mutations in the mouse reveal enzyme deficiencies and structural defects in homozygous mutants which are not present in heterozygotes (1-7). The mutants are radiation-induced lethals which originated at Oak Ridge (c^{14CoS}, Dr. L. B. Russell) and Harwell (c^{3H}, Dr. Searle) (1). Deficient glucose-6-phosphatase activity results in hypoglycemia which accounts for death within several hours after birth (1). Other deficiencies include tyrosine transaminase (2), serine dehydratase (3), and UDP glucuronyl transferase (bilirubin) (4) activities and cytochrome P-450 (5). Arylesterase, NADPH cytochrome C reductase, 5'-nucleotidase, aspartate amino transferase and phosphopyruvate carboxylase activities are normal (2). Ultrastructural studies reveal severe abnormalities in the endoplasmic reticulum-Golgi membranes of liver and kidney cells in newborn mutants (6). Chemical and immunological studies did not reveal

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major differences between microsomal membrane proteins of mutants and normal mice which could account for the ultrastructural defects and accompanying enzyme deficiencies (7).

Ligandin, in abundant soluble organic anion binding protein found in mammalian liver, kidney and small intestinal mucosa binds bilirubin, heme, reduced glutathione (GSH), iodothyronines, corticosteroid metabolites and other ligands *in vivo* and *in vitro* (8). Ligandin also exhibits GSH transferase activity with several substrates and in rat liver is identical with GSH transferase B (0). GSH transferases are important in detoxification of many electrophiles and constitute the first step in nonoxidative detoxification. Because cytochrome P-450, which is essential for oxidative detoxification, is virtually absent in mutant homozygous mice, we studied ligandin to determine if compensatory changes in the nonoxidative system occur. Ligandin was measured catalytically rather than immunologically because available anti-rat ligandin IgG does not react with mouse ligandin (10).

MATERIALS AND METHODS

Newborn albino c^{14CoS}/c^{14CoS} and c^{3H}/c^{3H} mice and their colored heterozygous and homozygous chinchilla littermates were originally obtained from Oak Ridge and Harwell (1). Parents which were heterozygous for c^{ch} and c^{14CoS} were bred in order to obtain c^{14CoS}/c^{14CoS} mutants. (c^{ch}/c^{14CoS} x c^{ch}/c^{14CoS} yields c^{ch}/c^{ch}, c^{ch}/c^{14CoS} and c^{14CoS}/c^{14CoS}). Breeding of c^{ch}/c^{3H} and c^{ch}/c^{3H} parents yielded c^{ch}/c^{ch}, c^{ch}/c^{3H} and c^{3H}/c^{3H} mutants. Available albino strains of mice (muscular dysgenesis (mda) and adult cataract (act)), which are mutants at other loci but homozygous for c, served as controls. All mice were bred in Dr. S. Gluecksohn-Waelsch's laboratory at the Albert Einstein College of Medicine. Newborn mice were decapitated within 2-3 hours after birth.

Liver, kidney and small intestine were removed, frozen on dry ice and kept at -20°C until assays were performed within 1-2 days. Tissues were homogenized at 4°C using a motor-driven Potter-Elvehjem teflon-glass homogenizer with 0.25M sucrose in 0.01M phosphate buffer, pH 7.4, and centrifuged at 104,000 x g for 2 hours. Supernatant GSH transferase activity was assayed using 1 mM 1-chloro-2, 4-dinitrobenzene as substrate by measuring changes in absorbance at 340 mμ (10). A complete assay mixture without enzyme was used as a control. Assays were performed in duplicate at 25°C in 0.1M potassium phosphate, pH 6.5 with 1 mM GSH. The rate of conjugation of GSH with 1-chloro-2, 4-dinitrobenzene was expressed as ΔE min per mgm protein. When an amount of enzyme was utilized which resulted in absorbance change of less than 0.04 per min, enzyme activity was linear with respect to protein concentration and time for at least 5 minutes. Protein was measured by the method of Lowry, *et al.* (11) with bovine serum albumin as standard.

TABLE I

GSH-transferase B activity (ligandin) in tissues from normal and mutant newborn and adult mice. Enzyme activity represents the rate of conjugation of GSH with 1-chloro, 2,4-dinitrobenzene expressed as ΔE/min/mg protein. Numbers in parenthesis refer to number of animals tested.

		LIVER	KIDNEY	SMALL INTESTINE
I	Normal adult mice of various strains including 2 heterozygotes for lethal albino alleles	11.66 ± 1.63 (6)	3.73 ± 0.24 (6)	3.07 ± 0.37 (6)
II	Normal albino newborn			
	1) c/c from mdg strain	3.33 ± 0.19 (6)	0.67 ± 0.09 (3)	1.02 ± 0.08 (6)
	2) c/c from act strain	4.99 ± 0.60 (6)	1.14 ± 0.08 (3)	1.42 ± 0.05 (6)
III	c^{14CoS} newborn			
	1) lethal albino c^{14CoS}/c^{14CoS}	11.61 ± 1.65 (18)	2.07 ± 0.50 (6)	3.11 ± 0.04 (6)
	2) controls c^{ch}/c^{ch} and c^{ch}/c^{14CoS}	5.66 ± 0.83 (22)	0.85 ± 0.25 (5)	1.25 ± 0.04 (5)
IV	c^{3H} newborn			
	1) lethal albino c^{3H}/c^{3H}	13.48 ± 1.83 (8)	1.83 ± 0.04 (4)	2.19 ± 0.61 (4)
	2) heterozygous controls c^{ch}/c^{3H}	4.37 ± 0.85 (11)	0.91 ± 0.11 (5)	1.17 ± 0.35 (5)
	3) homozygous controls c^{ch}/c^{ch}	3.43 ± 0.20 (3)	0.99 ± 0.08 (3)	1.06 ± 0.10 (3)
	4) mixed controls c^{ch}/c^{ch} and c^{ch}/c^{3H}	4.32 ± 0.68 (5)	------	------

RESULTS

Mean GSH transferase activity of supernatant fractions from homogenates of liver, kidney and small intestine from newborn homozygous mutants, heterozygous littermates and corresponding tissues from normal adult and newborn mice from several strains is shown in Table I. GSH transferase activity in each of the three organs from c^{14CoS} and c^{3H} newborn homozygous mutant mice was more than twice that of heterozygous littermates and normal newborn controls and comparable to the activity in adults.

DISCUSSION

Studies of hepatic ligandin in newborn guinea pig, rat, monkey and man reveal deficiency of the protein in fetal and newborns, increased levels after birth, and maturation to adult levels within the first week of life (11-14). GSH transferase B (ligandin) activity in liver, kidney and small intestine of newborn homozygous mouse mutants was the same as in comparable tissues from adult mice. This impressive increase in enzyme activity may represent early maturation of the enzyme following accumulation of substrates which are normally metabolized by the cytochrome P-450 oxidative system. In adult rats, ligandin is induced by many drugs and chemicals which also induce hepatic microsomal protein, particularly cytochrome P-450 (15,16).

The almost identical increase in enzyme activity in the three tissues in homozygous mutant mice suggests that GSH transferase is produced at approximately the same time and rate in these tissues. The catalytic activity of ligandin in liver, kidney and small intestine from heterozygous mice did not differ from that in normal newborn mice suggesting that the increase in homozygous mutants is probably a secondary effect of other phenotypic abnormalities.

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