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EFFECTS OF AGING ON HEPATIC AND PULMONARY GLUTATHIONE *S*-TRANSFERASE ACTIVITIES IN MALE AND FEMALE FISCHER 344 RATS*

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Abstract—The effects of aging on cytosolic glutathione *S*-transferase activities were evaluated with liver and lung cytosol from male and female Fischer 344 rats 3, 12, and 24 months of age. Age-related changes were tissue-, sex-, and substrate-specific. With liver and lung cytosol from both males and females, rates of metabolism of 1,2-epoxy-3-(*p*-nitrophenoxy)propane and *p*-nitrobenzyl chloride were lower in the old group than in the young group; however, patterns of decrease differed with tissue and sex. With 1,2-dichloro-4-nitrobenzene, metabolism was affected by aging only in liver and lung cytosol from males. Finally, with 1-chloro-2,4-dinitrobenzene, metabolic rates were altered during aging only with liver cytosol from females. However, the apparent K_m was higher with liver cytosol from old males; those values from lung cytosol of males and liver or lung cytosol from females were unchanged. These data indicate that changes in the cytosolic glutathione *S*-transferase isozymes occurred during aging.

The glutathione *S*-transferases (EC 2.5.1.18) are a group of cytosolic enzymes that catalyze the conjugation of the cellular nucleophile glutathione (GSH) with electrophiles such as epoxides [1–3], aryl compounds [4], nitrate esters [5], alkenes [4], and leukotriene A_4 [6]. Many of these electrophilic chemicals, such as epoxides, have been shown to be toxic or carcinogenic [7, 8]. In addition, these enzymes function as intracellular binding proteins [9], as a Δ^5-3-ketosteroid isomerase [10], and as selenium-independent glutathione peroxidases [11, 12]. The several isozymes of the glutathione *S*-transferases have been estimated to compose 5–10% of the total cytosolic protein of rat liver [13].

Two tissues in which the cytosolic glutathione *S*-transferases are of interest are liver, a quantitatively important organ of drug metabolism, and lung, a site of first exposure to many volatile chemicals which may consequently serve as a site of first metabolic action. The developmental patterns of glutathione *S*-transferase activities have been well characterized in liver and lung. As rabbits or rats develop from the fetus or neonate to young adulthood, these developmental patterns are organ-, sex-, and substrate-specific [14–17].

Changes in the glutathione *S*-transferase activities from young adults to senescent animals have not been studied as thoroughly. Birnbaum and Baird [18] reported that, with liver cytosol isolated from male CFN rats and C57BL/6J mice 3, 12, and 27 months of age, there were no significant age-related alterations in styrene oxide (STOX) conjugation with GSH. Stohs *et al.* [19] found that in liver and lung cytosol from aging female Swiss–Webster mice, 1-choro-2,4-dinitrobenzene (CDNB) reaches maximal specific acitivies in 9-month-old animals. In the liver, the specific activities at 18 months of age are significantly lower than those from the 3-month-old group. However, in the lung, specific activities in 18-month-old animals are significantly higher than in 3-month-old animals. Kitahara *et al.* [20] reported that, in livers from male Fischer 344 rats, specific activity with 1,2-dichloro-4-nitrobenzene (DCNB) is lower at 24 months than at 4.5 months of age. However, they found no age-related change in CDNB metabolism. We have demonstrated [21] that glutathione *S*-transferase specific activities toward six epoxides are lower in liver cytosol from 24-month-old male Fischer 344 rats than in those of 3- or 12-month-old animals, but that few such changes occur in lung cytosol.

These studies provide evidence for selective age-related, substrate-specific, and tissue-specific changes in hepatic and pulmonary glutathione *S*-transferases. To further extend these results, this report describes results obtained with liver and lung cytosol isolated from male and female Fischer 344 rats of three age groups: 3 months of age (sexually mature young adults), 12 months of age (approximately midway through a 29-month median lifespan [22]), and 24 months of age (chosen to avoid high mortality that might select a longer-living population).

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MATERIALS AND METHODS

General. Sources and treatment of Fischer 344 rats, preparation of lung and liver cytosol, measurement of protein concentrations, and statistical analyses were described previously [21].

Chemicals. Reduced glutathione (GSH) and bovine serum albumin were purchased from the Sigma Chemical Co. (St. Louis, MO); CDNB, DCNB, and *p*-nitrobenzyl chloride (PNBC) were from the Aldrich Chemical Co. (Milwaukee, WI); and 1,2-epoxy-3-(*p*-nitrophenoxy)propane (EPNP) was from the Eastman Kodak Co. (Rochester, NY). Pentobarbital sodium (65 mg/ml) was purchased from the Butler Chemical Co. (Columbus, OH), and absolute ethanol from Florida Distillers (Lake Alfred, FL). All other chemicals were of reagent grade or better. All water was distilled once in glass from deionized water. CDNB and DCNB were recrystallized twice from ethanol/water before use. All other chemicals were used as purchased.

Glutathione S-transferase assays. Glutathione *S*-transferase activity was assayed by modifications of methods developed by Habig *et al.* [4, 23]. A Beckman model 5260 dual-beam u.v. spectrophotometer was used for all readings. Assays were conducted at room temperature. All incubations were conducted in 100 mM sodium phosphate (pH 6.5). CDNB, DCNB, and PNBC were used in 1 mM concentration, whereas EPNP and GSH were used in 5 mM concentration, except in studies of K_m and V_{max}, in which concentrations were varied. Extinction coefficients that were used were assumed to be the same as those reported by Habig *et al.* [4, 23]. All incubation volumes were 1 ml in semimicro quartz cells. Reactions were initiated by the addition of the cytosol, and reaction velocities were linear for 2 min.

RESULTS

The four substrates selected, CDNB, DCNB, PNBC, and EPNP, are differentially metabolized by individual glutathione *S*-transferase isozymes. The isozyme substrate specificities are shown in Table 1.

The results of studies with liver and lung cytosol from males of the three age groups are shown in Table 2. With both PNBC and EPNP, specific activities in the liver were significantly higher in middle-aged animals than in the young. These rates decreased by senescence to values that were significantly lower than those found in the young group. Specific activities with DCNB were significantly different only between young and senescent groups; no increase in activity by middle age was seen. Specific activities with CDNB were unchanged throughout the lifespan.

In the lung, a somewhat different pattern of age-related change was revealed. Effects of aging on metabolism of CDNB and DCNB were comparable to those found in the liver. However, with both PNBC and EPNP, the increase in activity found in the liver cytosol of middle-aged animals was absent in lung cytosol. Instead, a pattern of decline in specific activity was measured with the lung cytosol; this decline was significant by middle age with EPNP and by senescence with PNBC. In addition, the ratios of specific activities in liver to those in lung were about

Table 1. Specific activities of purified glutathione *S*-transferase isozymes*

	Specific activity				
Substrate	AA	A	B	C	E
CDNB	14	62	11	10	0.01
DCNB	0.008	4.3	0.003	2.0	<0.0001
PNBC	0.09	11.4	0.1	10.2	4.1
EPNP	NR	0.1	<0.006	<0.1	6.7

* Values are expressed as μmoles per min per mg of protein. Values not reported are indicated by NR. Taken from Refs. 4 and 23.

Table 2. Specific activities of cytosol from males*

		Specific activity		
Organ	Substrate	3 Months old	12 Months old	24 Months old
Liver	CDNB	1320 ± 100 (A)	1390 ± 94 (A)	1260 ± 94 (A)
	DCNB	37.7 ± 1.8 (A)	33.3 ± 1.7 (A, B)	32.6 ± 1.8 (B)
	PNBC	268 ± 12 (A)	339 ± 17 (B)	221 ± 20 (C)
	EPNP	92 ± 2 (A)	119 ± 6 (B)	76 ± 8 (C)
Lung	CDNB	115 ± 8 (A)	109 ± 6 (A)	103 ± 7 (A)
	DCNB	4.0 ± 0.2 (A)	3.7 ± 0.6 (A)	2.7 ± 0.3 (B)
	PNBC	22.5 ± 0.4 (A)	21.7 ± 0.5 (A)	18.1 ± 0.6 (B)
	EPNP	23.8 ± 1.2 (A)	17.8 ± 2.0 (B)	16.7 ± 2.2 (B)

* Data represent means ± S.E. Means in a horizontal row with the same letter are not significantly different at $P < 0.05$. N = 17 (3 months old), 16 (12 months old), and 15 (24 months old). Specific activities are expressed as nmoles per min per mg of cytosolic protein.

Table 3. Specific activities of cytosol from females*

Organ	Substrate	Specific activity		
		3 Months old	12 Months old	24 Months old
Liver	CDNB	1010 ± 77 (A)	1190 ± 65 (A)	1510 ± 97 (B)
	DCNB	31.5 ± 0.9 (A)	30.8 ± 2.1 (A)	32.2 ± 1.1 (A)
	PNBC	268 ± 26 (A)	205 ± 25 (A, B)	160 ± 14 (B)
	EPNP	68 ± 2 (A)	78 ± 1 (A)	50 ± 2 (B)
Lung	CDNB	136 ± 12 (A)	134 ± 10 (A)	129 ± 8 (A)
	DCNB	3.7 ± 0.5 (A)	3.1 ± 0.3 (A)	2.6 ± 0.1 (A)
	PNBC	29.2 ± 1.7 (A)	23.6 ± 1.8 (A, B)	21.0 ± 0.9 (B)
	EPNP	17.6 ± 2.3 (A)	13.4 ± 0.7 (A, B)	10.7 ± 0.6 (B)

* Data represent means ± S.E. Means in a horizontal row with the same letter are not significantly different at $P < 0.05$. N = 4 for all determinations. Specific activities are expressed as nmoles per min per mg of cytosolic protein.

10:1 for most substrates except EPNP, for which the ratio was closer to 4:1–5:1.

The results of studies with liver and lung cytosol from females of three age groups are shown in Table 3. With both PNBC and EPNP, specific activities in liver cytosol from the senescent group were lower than those found with the young group, similar to results with liver cytosol from males. However, unlike the results with liver cytosol of males, there were no significant increases in specific activities in liver cytosol from the middle-aged group. There was no significant decrease in DCNB metabolism throughout the lifespan, unlike the significant decrease in liver cytosol of males found by senescence. However, there was a significant increase in CDNB metabolism by senescence, relative to the young and middle-aged groups. This change in specific activity with CDNB by senescence was also unlike results found with liver cytosol of males.

With lung cytosol from females, the metabolism of both PNBC and EPNP was decreased significantly by senescence, relative to the young group, similar to lung cytosol of males and liver cytosol of females. These changes were not significant by middle age, however. No age-related change was found in DCNB metabolism, similar to liver cytosol of females, but unlike lung cytosol of males. In addition, no change during aging was found in CDNB metabolism, as with lung cytosol of males but unlike liver cytosol of females. Similar to the liver and lung cytosol of males, ratios of specific activities of CDNB, DCNB, and PNBC in liver to those in lung were about 10:1 in the female; the ratio of EPNP in the liver to that in the lung was closer to 4:1–5:1.

Michaelis–Menten kinetic parameters were evaluated at a constant concentration of GSH. The Lineweaver–Burk plots were linear over a 50-fold concentration range of CDNB that spanned K_m. The results for liver and lung cytosol are summarized in Table 4. Previous work has shown that glutathione *S*-transferase isozymes A and C have the lowest K_m with CDNB (0.06 and 0.1 mM respectively), whereas isozymes AA and B have higher K_m values (0.4 and 0.8 mM respectively) [4, 23]. The K_m of isozyme E with CDNB has not been measured because of very low specific activities of isozyme E with CDNB [4]. The V_{max} is much higher with isozyme A (3000 moles/min/mole of enzyme) than with AA, B, or C (920 860, and 500 moles per min per mole of enzyme respectively) [4, 23]. The results in Table 4 demonstrate that a significant increase in apparent K_m in liver cytosol of males occurred by senescence, when compared with young or middle-aged groups. No corresponding change in apparent K_m was measured with liver cytosol from females or with lung cytosol from males or females. With the liver cytosol from

Table 4. Apparent K_m and V_{max} of liver and lung with CDNB*

Parameter	3 Months old	12 Months old	24 Months old
Male			
K_m (liver)	0.095 ± 0.007 (A)	0.090 ± 0.011 (A)	0.122 ± 0.006 (B)
V_{max} (liver)	1190 ± 29 (A)	1410 ± 68 (B)	1370 ± 23 (B)
K_m (lung)	0.25 ± 0.021 (A)	0.25 ± 0.090 (A)	0.26 ± 0.050 (A)
V_{max} (lung)	136 ± 4 (A)	165 ± 22 (A)	139 ± 10 (A)
Female			
K_m (liver)	0.096 ± 0.014 (A)	0.103 ± 0.024 (A)	0.129 ± 0.017 (A)
V_{max} (liver)	1090 ± 50 (A)	1390 ± 61 (B)	1130 ± 79 (A)
K_m (lung)	0.134 ± 0.065 (A)	0.267 ± 0.059 (A)	0.220 ± 0.048 (A)
V_{max} (lung)	142 ± 22 (A)	167 ± 14 (A)	152 ± 13 (A)

* K_m values are expressed as mM and V_{max} as nmoles per min per mg of cytosolic protein ± S.E. Means with the same letter in a horizontal row are not significantly different at $P < 0.05$. GSH concentration was 5 mM throughout, and CDNB concentrations ranged from 0.02 to 1 mM. N = 4 for all determinations.

both males and females, there was a significant increase in V_{max} in the middle-aged groups relative to the young groups. With liver cytosol of males, this value remained elevated through senescence, whereas with liver cytosol of females the values decreased to those found in the young group. There were no other significant age-related changes in K_m or V_{max} with liver or lung tissue from males or females.

DISCUSSION

These data confirm and extend the findings of Kitahara *et al.* [20] regarding age-related changes in male Fischer 344 hepatic metabolism of CDNB and DCNB. The substrate EPNP is metabolized almost exclusively by glutathione *S*-transferase E (Table 1). The conjugation of EPNP thus gives an indication of the status of the activity or level of isozyme E. Similarly, DCNB is metabolized predominantly by isozymes A and C (although specific activities of isozyme A are about double those of isozyme C); PNBC is metabolized by isozymes A and C equally well and by isozyme E about half as efficiently; CDNB is metabolized by isozymes AA, A, B, and C but not E (Table 1).

With liver and lung cytosol from males, no changes were found in the rate of CDNB conjugation with increasing age, implying that no change in the AA–A–B–C isozyme group occurred. However, results with EPNP, DCNB, and PNBC and kinetic studies with CDNB indicate that selective changes indeed occurred. These changes were tissue-specific, as found with metabolic rates for EPNP and PNBC in liver and lung cytosol. They indicate that, in liver cytosol, activities or levels of isozyme E were lowest in the senescent group. Additionally, they suggest that either isozyme A or C, or perhaps both, may be also lowest in the senescent group. The kinetic studies also suggest that there was a shift from the high affinity isozymes (A and C) to the low affinity isozymes (AA and B) and that there was an increase in the high specific activity isozymes (A) with a decrease in the low specific activity isozymes (AA, B, and C). Clearly, isozyme-specific changes occurred with aging. Similarly, in lung cytosol from males, results with EPNP, DCNB, and PNBC indicate that isozyme E activites or levels were lowest in the senescent group and that an overall decrease in the A–C isozyme group also occurred, although an increase in one isozyme with a decrease in the other could have occurred. The lack of significant differences in K_m or V_{max}, as measured with CDNB, indicates that the relative concentrations of isozymes A, B, and C were not altered significantly during aging, unlike the case of liver cytosol.

Specific activities, measured with CDNB, of liver cytosol, but not lung cytosol, from aging females indicate that changes in the AA–A–B–C isozymes occurred with aging. Similarly to results found with liver and lung cytosol from males, activities or levels of isozyme E were lowest in liver and lung cytosol from senescent females. With liver cytosol from aging females, the overall rates of the A–C isozyme group were apparently not affected by aging: rates of metabolism of DCNB were unchanged. However, a decrease in one isozyme with a corresponding increase in the other may have occurred. Indeed, changes in PNBC metabolism were found which indicate that a decrease in the A–C–E group occurred. Similar results were found with DCNB and PNBC in lung cytosol from females. The lack of many significant changes in CDNB metabolism in kinetic studies with liver or lung cytosol from females indicates that few overall shifts from high or low affinity or activity forms occurred during aging.

These results support several conclusions. First, age-related changes in glutathione *S*-transferase isozymes occurred. These changes were tissue-specific (comparing liver and lung cytosol in males), sex-specific (comparing liver cytosol in males and females), and substrate-specific (comparing CDNB, DCNB, and EPNP metabolism in liver cytosol from males). Second, they provide clear indications that isozyme E activities or levels were lowest in both liver and lung cytosol from senescent males and females. Some epoxides, such as EPNP, are metabolized predominantly by this isozyme, although another epoxide, benzo[*a*]pyrene 7,8-oxide, has been shown to be a substrate also for isozymes A, B and C [24]. Third, they suggest that changes in the A–C, A–C–E, or AA–A–B–C isozyme groups occurred with aging. These data thus support and substantially extend earlier findings [20]. Finally, they provide important initial indications that isozyme-specific changes occurred during aging and that risks of toxic results from exposure to electrophiles may be greater with increasing age. We believe that this study has provided important indications that further investigation into the molecular nature of these changes will be fruitful in elucidating the age-related changes in electrophile metabolism by the cytosolic glutathione *S*-transferases. Further studies in which changes in transferase isozymes are quantified will be published elsewhere.

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