blocked the histamine release from mast cells induced by other secretagogues, such as compound 48/80, ionophore A23187, polymyxin B and ATP. 3-Deaza-SIBA may perturb membrane functions of basophilic leukemia cells and mast cells by inhibiting the biosynthesis of phosphatidylcholine via choline incorporation, the turnover of which is probably required for cellular degranulation and the release of histamine. Previous studies with 3-deaza-SIBA have been mainly interpreted in terms of its structural similarity to *S*-adenosylhomocysteine and its potential use as an inhibitor of methylation reactions. In light of our present findings, experiments utilizing 3-deaza-SIBA as a biochemical probe have to be carefully interpreted.

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Biochemical Pharmacology, Vol. 31, No. 11, pp. 2113–2116, 1982.
Printed in Great Britain.

0006-2952/82/112113-04 $03.00/0

Glutathione *S*-transferase and glutathione reductase activities in hepatic and extrahepatic tissues of female mice as a function of age

(*Received* 27 *June* 1981; *accepted* 17 *November* 1981)

Gluthatione (GSH) is the most abundant low molecular weight thiol-containing compound in living cells, and in the reduced form it functions as an antioxidant in protecting sulfhydryl groups of functional cellular macromolecules [1, 2]. GSH also participates in the detoxification of electrophilic intermediates of drugs and foreign chemicals produced by the mixed-function oxidases of the body [3]. Furthermore, GSH conjugation may provide the main protective mechanism against the formation of the carcinogenic forms of polycyclic aromatic hydrocarbons [4] and other tumorigenic chemicals.

The glutathione *S*-transferases (EC 2.5.1.18) form a group of enzymes which catalyze the conjugation of GSH with a wide vareity of xenobiotics [5, 6]. Several glutathione *S*-transferases (GST) have been identified in the rat liver cytoplasm and microsomal fractions, and these enzymes have been shown to have broad, and overlapping, substrate specificities [7, 8]. GST activities have been measured in other tissues as well as liver, including lung [9–12], intestine [13], and kidney [6, 11, 14].

When GSH serves as a reductant in oxidation–reduction processes, this function results in the formation of glutathione disulfide (GSSG; oxidized glutathione) [1]. The reduction of GSSG is consequently of fundamental importance for the metabolic function of glutathione, and this reaction is catalyzed by the enzyme glutathione reductase (EC 1.6.4.2) with the coenzyme NADPH. Glutathione reductase (GR) is believed to be as ubiquitous as GSH and has been studied in various tissues including liver [15–17], lung [16], kidney [18], and erythrocytes [19].

Stohs *et al.* [20] have shown that decreased glutathione levels occur in blood, liver, kidneys and intestinal mucosa of male CBF-1 mice as a function of age and have suggested that a decrease in cellular GSH with time may contribute

to the aging process as well as to the increased incidence of neoplastic diseases and the greater susceptibility to drugs and foreign chemicals with aging. Harman [21] has proposed that the aging process involves an increased susceptibility to free radicals. A number of antioxidants including vitamin E, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and ethoxyquin, when added to the diet, have increased the mean life-span of experimental animals [22–27]. These same antioxidants are effective inhibitors of 7,12-dimethylbenz[a]anthracene (DMBA)-induced neoplasms in rats and mice [28, 29]. Furthermore, BHA and ethoxyquin are inducers of hepatic GST in mice [30].

The ability of antioxidants to retard the aging process as well as chemical carcinogenesis suggests that GSH, a naturally occurring antioxidant, may exert an inhibitory effect on these processes. Since GST and GR play important roles in GSH metabolism and in detoxification processes, we have examined the GSH content of liver and the GST and GR activities of liver, lung and intestine of female Swiss–Webster mice.

Female mice of the Swiss–Webster strain were obtained from Sasco Inc., Omaha, NE. The animals were maintained at 20° on standard Purina laboratory chow and tap water *ad lib.*, with lighting from 6:00 a.m. to 6:00 p.m. daily. Ten animals each were utilized at 1, 3, 6, 9, 12, 15 and 18 months of age.

Mice were decapitated between 8:00 and 9:00 a.m. to eliminate possible effects due to diurnal variation. The tissues and enzymes were prepared and handled according to the general procedure of Benson *et al.* [31]. The tissues were perfused *in situ*. The portal vein was severed, and cold 0.15 M KCl containing 2 mM ethylenediaminetetraacetic acid (final pH 7.5) was perfused through the left ventricle. When necessary, the livers and lungs were perfused again after excision. The first 10 cm of the small intestine from each mouse was flushed with 50 ml of ice-cold normal saline and slit open longitudinally, and the upper villous layer of the mucosa was carefully removed by scraping with a glass slide. Tissues from two animals were pooled before homogenization, giving five groups for each tissue. Tissues were homogenized in 0.25 M sucrose (3 ml/g of tissue). After centrifugation at 9000 *g* for 15 min, the supernatant fractions were transferred to other tubes and 0.2 volume of 0.10 M $CaCl_2$ was added to each tube. The mixtures were then centrifuged at 9000 *g* for another 15 min to prepare the cytosol fractions.

Glutathione *S*-transferase activities of the cytosol fractions from liver, lung and intestine were determined in the presence of GSH with 1,2-dichloro-4-nitrobenzene (DCNB) (Eastman Organic Chemicals, Rochester, NY) as the substrate. The initial velocities of glutathione conjugate formation were measured spectrophotometrically according to the procedure of Habig *et al.* [7]. Assays were conducted in a thermostated cell compartment at 25° in 100 mM potassium phosphate, pH 7.5. The concentration of reduced glutathione (GSH) was 5.0 mM and the concentration of DCNB was 1.0 mM. The specific activity is expressed as nmoles product per min per mg protein.

The assay for GR activity was conducted according to the procedure of Carlberg and Mannervik [15]. The reaction system in a 1.0 ml volume contained: 1.0 mM GSSG, 0.10 mM NADPH, 0.50 mM EDTA, 0.10 M sodium phosphate buffer (pH 7.5), and a suitable amount of the glutathione reductase to give a change in absorbance of 0.05 to 0.30/min. The rate of oxidation of NADPH by GSSG at 30° was used as a standard measure of enzymatic activity. The oxidation of 1 μmole NADPH/min under these conditions was used as a unit of glutathione reductase activity. The specific activity is expressed as units/mg protein.

For the determination of reduced glutathione (GSH) levels, approximately 250 mg of liver was homogenized in an EDTA–phosphoric acid solution as previously described

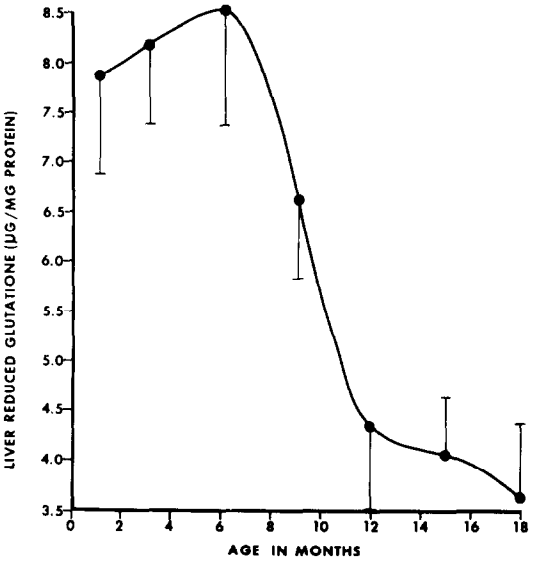

Fig. 1. Reduced glutathione (GSH) content of liver in mice as a function of age. Animals were killed at 1, 3, 6, 9, 12, 15, and 18 months of age. Each value is the mean ± S.D. of five determinations, using five pools of tissue with each pool being derived from two mice.

[20], and the homogenates were centrifuged at 4° at 100,000 *g* for 30 min to obtain the supernatant fractions for GSH assay. GSH was determined fluorometrically by the method of Hissin and Hilf [32] with the use of *O*-phthalaldehyde as the fluorescent reagent. Protein contents of the total tissue homogenates and cytosol fractions were determined by the method of Lowry *et al.* [33]. Each value represents the mean ± S.D. of five groups of two pooled animals. The data were subjected to one-way analysis of variance (ANOVA) using Scheffe's S method, and mean values with $P < 0.05$ were considered significantly different [34].

The GSH content of liver as a function of age is shown in Fig. 1. No significant difference existed in the GSH levels of female mice at ages 1, 3, 6, and 9 months. However, at 9 months of age the GSH content began to decrease, and significant decreases existed in animals that were 12, 15 and 18 months old as compared to the younger age groups that were examined. A 57% decrease in hepatic GSH

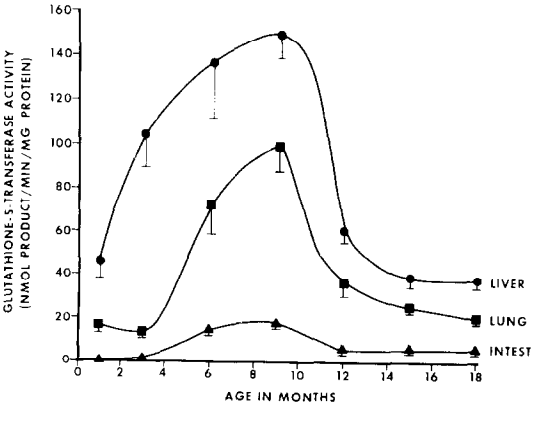

Fig. 2. Glutathione *S*-transferase (GST) activity of liver, lung, and intestinal mucosa of female mice as a function of age. 1, 2-Dichloro-4-nitrobenzene (DCNB) was used as the substrate. Each value is the mean ± S.D. of determinations from five pairs of pooled samples (ten animals).

content occurred when comparing 6- and 18-month-old mice.

The GST activities of liver, lung, and intestine as a function of age of female mice, using DCNB as the substrate, are presented in Fig. 2. Greatest GST activity at all ages occurred in the liver as compared to the other two tissues. For all three tissues the GST activity increased with age, reaching a maximum activity at approximately 9 months of age and decreasing sharply thereafter. The activities of liver, lung and intestinal GST decreased from 9 to 18 months by 75, 80 and 57%, respectively.

GR activities of liver, lung and intestinal mucosa of female Swiss–Webster mice from 1 to 18 months of age are presented in Fig. 3. GR activities increased with age for all three tissues, reaching maximum activities at approximately 9 months of age and decreasing sharply thereafter. The GR activities decreased from 9 to 18 months by 73, 67 and 50% for liver, lung and intestine, respectively. Greatest GR activity was present in the liver as compared to the other two tissues.

Significant changes occur in GST and GR activities of liver, lung and intestine with age. The activities of these two enzymes increased with age of the mice, reaching maximum activities at approximately 9 months and decreasing sharply thereafter. A basal level appeared to be reached for the two enzymes in mice 15–18 months old. No significant difference existed in hepatic GSH content between the ages of 1 and 9 months (Fig. 1). However, by 12 months of age a significant decrease in GSH content had occurred.

From these results it is apparent that not only the GSH content of tissues decreases with advanced age, but similar decreases in the activities of two enzymes which are intimately associated with glutathione metabolism also occur. We have shown previously that decreases in glutathione occur with advanced age in liver, blood, kidneys and intestinal mucosa of CBF-1 male mice [20]. A decrease in the synthesis and turnover of GSH in aged mice has been suggested by GSH depletion studies with acetaminophen [35]. Female Swiss–Webster mice at ages 3, 9, and 18 months were treated with 500 mg/kg acetaminophen (AAP), and only in 18-month-old mice did GSH fail to return to, or exceed, control values 24 hr after AAP administration. Mancini *et al.* [36] have demonstrated that young rats and mice are less vulnerable to AAP toxicity, compared to adult animals. Furthermore, liver damage appears to be more frequent in adults than children when exposed to high levels of AAP [37]. Therefore, the decrease in GSH content and the decreased ability to regenerate or synthesize GSH with advanced age may contribute to increased susceptibility to xenobiotics and the pathophysiology of aging.

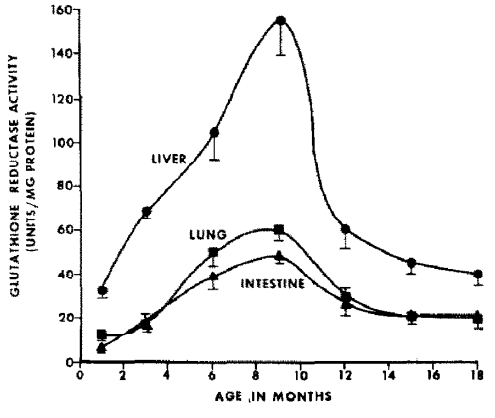

Fig. 3. Glutathione reductase (GR) activity of liver, lung and intestinal mucosa of female mice as a function of age. Each value is the mean ± S.D. of determination from five pairs of pooled samples (ten animals).

An increase in the susceptibility to drugs and foreign chemicals is known to occur in conjunction with the aging process and a decrease in the ability to metabolize drugs may, in part, account for this phenomenon. Recent investigations have shown that decreases in mixed-function oxidase activities also occur with aging in mice. Hepatic activities of AHH [36], aniline hydroxylase [38], 7-ethoxycoumarin *O*-deethylase [38, 39], benzphetamine-*N*-demethylase [39, 40], biphenyl-4-hydroxylase [41], zoxazolamine hydroxylase [38], and ethylmorphine-*N*-demethylase [40, 42] decrease with advanced age in rats and mice.

GST also plays an important role in xenobiotic metabolism and detoxification [3]. Jernström *et al.* [4] have demonstrated a requirement for GSH and GST to inhibit formation of DNA-bound metabolites of 7,8-dihydro-7,8-dihydroxybenzo[*a*]pyrene, a major proximate carcinogenic form of benzo[*a*]pyrene (BP), by rat liver nuclei, suggesting that enzyme-mediated GSH conjugation may be a major protective mechanism against chemical carcinogenesis. Therefore, age-related decreases in tissue GSH content and GST activity increase the likelihood of interactions between DNA and reactive electrophiles and thus may, in part, explain age-related increases in many neoplastic diseases.

Benson *et al.* [30] have shown that BHA and ethoxyquin in the diet of mice decrease the levels of mutagenic metabolites of BP and increase hepatic GST activity. Furthermore, Sparnins and Wattenberg [43] observed that inhibitors of BP-induced neoplasia in mouse forestomach enhanced GST activity in this tissue, suggesting that inhibition of the action of carcinogens may involve detoxification by GST.

The results support the hypothesis that a decrease in GSH content and GSH-metabolizing enzymes may contribute to changes associated with aging as well as the increased susceptibility to disease, drugs and carcinogens which occurs with advanced age.

Acknowledgements—This study was supported in part by Grant 1 R01 AG01513 from the National Institute on Aging. The authors thank Mr. Ronald Heinicke and Mrs. Judy Williams for their technical assistance.

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Biochemical Pharmacology, Vol. 31, No. 11, pp. 2116–2119, 1982.
Printed in Great Britain.

0006-2952/82/112116-04 $03.00/0

Temperature dependence and effect of membrane lipid alteration on melphalan transport in L1210 murine leukemia cells

(*Received* 21 *September* 1981; *accepted* 1 *December* 1981)

Melphalan (L-phenylalanine mustard) is an aromatic alkylating agent derived from phenylalanine. It is an important drug for the treatment of human malignancies. There has been renewed basic interest in the drug because of evidence that its transport into tumor cells is mediated by two different carrier-mediated systems [1–3]. The major objective of our study was to examine the response of melphalan transport to a change in the ordered-fluid transition state of the membrane. This was done by measuring the initial rate of transport as a function of temperature. In addition, we have utilized a method previously reported by us for altering the type of lipids in the membranes of L1210 leukemia cells, using experimental diets fed to the host animal [4]. This alteration in membrane lipids results in a change in fluidity as measured by electron spin resonance; therefore, changes in transport rate could be related to this phase transition. We have reported previously that the kinetic parameters of transport of the anti-leukemic drug, methotrexate, are affected by these lipid alterations [4]. Since melphalan transport, like that of methotrexate, is carrier-mediated, we anticipated there would be a similar effect of membrane lipid alteration on melphalan transport. Moreover, this approach offered an opportunity to study the effect of lipid modification on the two separate carrier components of the melphalan transport system.

Materials and methods

Male DBA/2 mice (Jackson Laboratories, Bar Harbor, ME) were fed either a predominantly saturated-fat diet (basal fat-deficient mixture supplemented with 16% coconut oil), a diet rich in polyunsaturates (basal fat-deficient mixture plus 16% sunflower oil) or a rodent chow (Ralston Purina Co., St. Louis, MO). The fat-deficient base was purchased from Teklad Test Diets, Madison, WI. The coconut oil was purchased from the Ruger Chemical Company, Inc., Hillside, NJ, and the sunflower oil from Cargill Industries, Minneapolis, MN. The exact fatty acid composition of these diets has been reported [5]; briefly, the sunflower oil contains 88% unsaturates, (mostly linoleic acid), whereas the coconut oil contains 90% saturated fatty