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Glutathione S-transferase in humans: development and tissue distribution

Gian Maria Pacifici[1], Marina Franchi[1], Cesare Colizzi[2], Lucio Giuliani[2], and Anders Rane[3]

Department of [1] General Pathology and [2] Surgical Pathology, Medical School, University of Pisa, Italy
[3] Division of clinical Pharmacology, University Hospital, Uppsala, Sweden

Abstract. Glutathione S-transferase (GST) was investigated with benzo(a)pyrene-4,5-oxide (BPO) as substrate in tissue specimens from 26 fetal and 27 adult livers and 27 placentas. The average (± SEM) of GST activity in the cytosol was 1.80±0.18 (fetal liver), 3.05±0.30 (adult liver) and 1.18±0.07 (placenta) nmol/min/mg. GST was also investigated in human fetal and adult lungs, kidneys and gut. In these tissues the average (± SEM) GST activity ranged between 0.71±0.12 (adult intestine) and 2.11±0.18 (fetal lungs) nmol/min/mg. Whereas in the fetal liver the conjugation of BPO was catalyzed at a rate of about two-thirds of the adult rate, similar or higher GST activities were found in the fetal non-hepatic tissues as compared to the adult organs. No correlation was found between the activity of the GST in fetal liver and placenta and the gestational age (11–25 weeks). GST develops before the 11th week of gestation and it does not undergo changes during the mid-gestation. No correlation was found between GST activity in adult liver and age (32–70 years).

Key words: Glutathione transferase – Benzo(a)pyrene-4,5-oxide – Human fetal and adult tissues

Introduction

Glutathione S-transferase (GST) is a family of enzymes with different physicochemical properties and substrate specificities (for review see Mannervik 1985). The composition of isoenzymes in GST is tissue dependent and changes with development. A multitude of GST isoenzymes, with different substrate specificities, have been found in the cytosolic fraction of the human adult liver (Kamisaka et al. 1975; Warholm et al. 1981a, 1983; Stockman et al. 1985; Vander Jagt 1985; Soma et al. 1986). In contrast, human fetal liver cytosol contains only two isoenzymes (Warholm et al. 1981b; Pacifici et al. 1986). The acidic isoenzyme is the major component of GST in human adult lung (Koskelo et al. 1981; Sherman et al. 1983; Partridge et al. 1984; Freyer et al. 1986). The intestinal mucosa contains only a basic form of GST (Sherman et al. 1983), whereas at least two isoenzymes were found in the human kidney cytosol (Sherman et al. 1983; Koskelo and Icen 1984). Only one isoenzyme, with similar physicochemical properties, was found in human placenta (Polidoro et al. 1980; Guthenberg and Mannervik 1981) and human fetal lungs, kidneys and gut (Pacifici et al. 1986). Such a complex picture makes it difficult to predict the activity of the cytosolic GST in different tissues.

Our previous investigations with styrene oxide, an alkene oxide, have shown that the activity of the cytosolic GST is comparable in the liver and in other tissues of the human fetus (Pacifici et al. 1982) in spite of the tissue dependent isoenzyme pattern. Little is known about the cytosolic GST in different tissues of adult subjects. However, similar GST activity was observed with 1-chloro-2,4-dinitrobenzene (Baars et al. 1981; Mukhtar et al. 1981) and styrene oxide (Pacifici et al. 1981a) in the liver and non-hepatic tissues of adult subjects.

Benzo(a)pyrene-4,5-oxide (BPO), an arene oxide, is generated by the metabolism of benzo(a)pyrene and it is one of the metabolites producing the toxic effects attributed to benzo(a)pyrene (Sims and Grover 1974). By perfusing the isolated rabbit lung it has been demonstrated that BPO undergoes conjugation with glutathione (Smith and Bend 1980). So far, no information is available on the developmental pattern of GST towards BPO in animals and humans. We report the results of an investigation on GST with BPO as substrate in different human fetal and adult tissues.

Materials and methods

Chemicals. Unlabelled and radioactive (G-^{3}H, 4,5-dihydro-benzo(a)pyrene-4,5-oxide) BPO (specific activity 288 mCi/mmol) were obtained from the NCI Chemical Carcinogen Reference Standard Repository, NIH (Bethesda, MA, USA). The radiochemical purity of BPO was >99%.

Subjects. Specimens of human fetal liver, lungs, kidneys, gut and placenta were obtained at legal abortion performed on socio-medical indication. The age of the fetuses ranged between 11 and 25 weeks. The clinical data of the mothers are given in Table 1.

Liver specimens were obtained from adult patients of either sex (age between 34 and 70 years) that were subjected to laparotomy for cholecystectomy. The specimens were obtained as the residue of wedge biopsies for histological analysis. All biopsies had normal cell architecture. Lung specimens were obtained after lobectomy which was performed to remove a pulmonary carcinoma. Part of the

Offprint requests to: G. M. Pacifici, Department of General Pathology, Medical School, Via Roma 55, I-56100 Pisa, Italy

Table 1. Clinical data of the mothers

Fetus	Gestational age (weeks)	Maternal drug intake	Maternal smoking habits cig/day	Mode of abortion
1, 2	11	–	–	CS
3	13	–	–	CS
4	13	–	–	E
5, 6	15	–	–	E
7, 8, 9, 10	16	–	–	E
11	17	–	–	E
12	17	Cannabis abuser	–	E
13	18	Salicylic acid	20–40	CS
14, 15, 16, 17, 18	18	–	–	E
19	18	Thyroxin	15	E
20, 21, 22	18	–	–	E
23	19	–	–	CS
24, 25, 26	19	–	–	E
27	20	–	–	CS
28	20	–	–	PG
29	21	–	10	E
30	21	–	–	CS
31, 32	21	–	–	E
33	21	–	10	PG
34	21	–	–	PG
35	21	–	–	E
36	23	–	–	CS
37	23	–	–	E
38	23	Amphetamine abuser	20	PG
39, 40, 41	24	–	–	CS
42	25	–	–	CS

CS, Cesarean section; E, Instillation of ethacrinidine; PG, Administration of prostaglandins

resectate with normal appearance was made available for our study. Kidney specimens were obtained after nephrectomy for hydronephrosis (F 32) or to remove a renal carcinoma in the other subjects. The intestinal mucosa was obtained after resection of part of the organ for removal a cancer (adenocarcinoma). The mucosa was isolated from a normal part of the intestine next to the tumour. Sex and age of lungs, kidneys and gut donors are listed in Table 3.

Human fetal and adult tissues were immediately frozen and stored at −80° C. Cytosolic fraction was obtained after homogenization of tissue specimens in 3 volumes of 0.25 M sucrose (pH adjusted to 7.4 with tris base) by means of a Potter-Elvehjem glass-teflon apparatus. Homogenates were centrifuged at 9000 *g* for 15 min. The supernatants were centrifuged again at 105000 *g* for 1 h. The ensuing supernatant was investigated as the cytosolic fraction.

Assays. GST assay was performed essentially as described by Mukhtar and Bend (1977). In brief, samples (final volume 0.2 ml) contained 0.1 mmol/l BPO (100,000 cpm), 4 mmol/l glutathione, 108 mmol/l HEPES (pH 7.85) and an aliquot of cytosolic fraction to give a final protein concentration ranging between 0.44 and 0.61 mg/ml (adult liver), 0.62 and 0.92 mg/ml (fetal liver) and 0.83 and 1.46 mg/ml (other tissues). Reactions were started by adding BPO. They were carried out at 37° C for 8 min. Incubations were stopped by adding 0.7 ml ice-cold HEPES and 2.6 ml ethyl acetate and the tubes were immediately vortexed. The extraction was repeated twice. Aliquots (0.45 ml) of the aqueous phase residue were transferred into scintillation vials containing 10 ml cocktail [1 l toluene containing 5 g 2,5-diphenyloxazole (PPO) and 0.1 g 2,2-p-phenylene-*bis*-5-phenyloxazole (POPOP) was mixed with 0.5 l Triton X-100 (Turner 1969)]. The activity of the GST was measured on the basis of the specific radioactivity of BPO after correction for blanks. Each sample was assayed in duplicate. Each assay was provided with two blanks which were treated as the samples except that the active cytosol was replaced by the boiled specimen. Protein concentration was measured according to Lowry et al. (1951).

Results

Linear conditions of the reaction with respect to incubation time and protein concentration were first studied. Figure 1 shows the representative results from a fetal and adult liver and placenta cytosols. This figure shows also the pH dependence of the reaction.

All specimens investigated catalyzed the conjugation of BPO with glutathione at a significant rate. Figure 2 shows the GST activity in the cytosolic fraction form 26 human fetal livers, 27 placentas and 27 adult livers. No correlation between the age and the enzyme activity was found in any of these three tissues. GST activity was also measured in the lungs, kidneys and gut cytosols from fetuses (Table 2) and adult subjects (Table 3). The difference in GST activity between the liver and the other tissues was smaller in fetuses than in adult subjects. The fetal lung cytosol was the most active of the fetal tissues. GST in this specimen was 1.6 times higher than than in liver. Among the adult tissues, the lungs and the gut were the organs with the lowest GST activity.

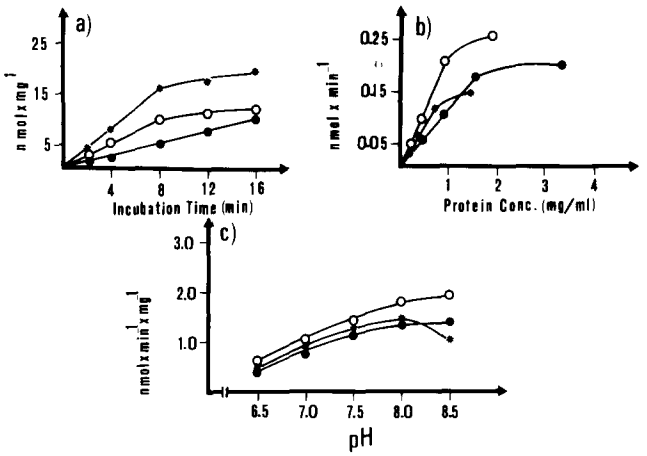

Fig. 1. Effect of **a** incubation time, **b** protein concentration and **c** pH on cytosolic GST activity. *Filled circles, unfilled circles* and *stars* refer to fetal liver (18 weeks old), placenta (16 weeks old) and adult liver (woman, 41 years old). **a** Protein concentration was 1.5 mg/ml (fetal liver), 0.7 mg/ml (placenta and adult liver). **b** Incubation time was 8 min. **a** and **b** pH was 7.85. **c** Protein concentration and incubation time were as in **a** and **b**, respectively

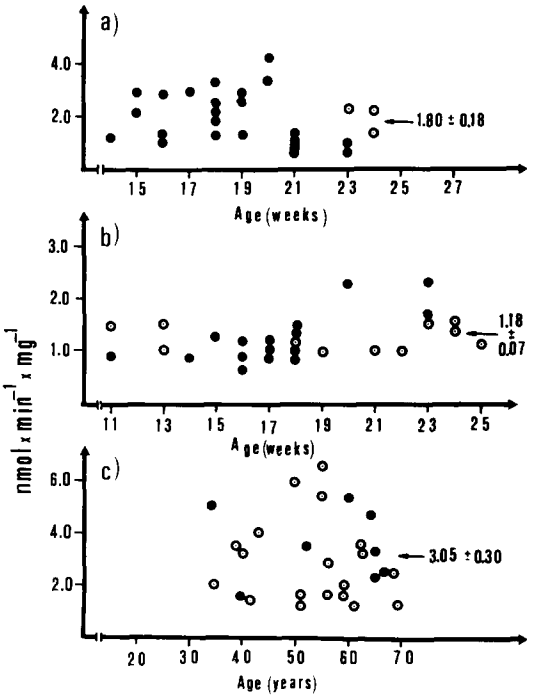

Fig. 2. GST activity in **a** fetal liver, **b** placenta and **c** adult liver cytosols. Panels show single values and mean ± SEM. **a** and **b** Symbols refer to the method of abortion. *Unfilled circles*, cesarean section. *Filled circles*, induction by prostaglandins or ethacridine. **c** Symbols refer to female (*unfilled circles*) and male (*filled circles*) patients

Discussion

Our results show that GST develops early in the human fetus. This enzyme has an ubiquitous distribution in fetal and adult tissues. Such a finding is in accordance with the results by Baars et al. (1981) and Mukhtar et al. (1981) obtained with 1-chloro-2,4-dinitrobenzene as substrate and with styrene oxide (Pacifici et al. 1981a; Pacifici and Rane 1982). Drugs and chemicals may cross the placenta and accumulate in fetal tissues. Conjugation with glutathione is often the first inactivation step of reactive molecules. The wide distribution of GST may be important from a detoxication point of view.

The average GST activity in human fetal liver was more than one half of that in the adult liver. The isoenzyme composition is different in these tissues (Mannervik 1985). In adult liver, the conjugation of BPO with glutathione is preferentially catalyzed by the near-neutral isoenzyme (μ form) (Warholm et al. 1981a). This enzyme is lacking in the human fetal liver (Warholm et al. 1981b; Pacifici et al. 1986). The conjugation of BPO with glutathione is thus catalyzed by different isoenzymes of GST.

The lack of studies of GST towards BPO in developing animals makes it difficult to compare the developmental pattern of GST in humans and animals. The effect of postnatal development on hepatic GST has been studied with different substrates. The developmental pattern is more marked in rodents than in humans. In near-term fetuses or newborn rats (Ryan et al. 1976; Hales and Neims 1976; Mukhtar and Bresnick 1976), mice (Shoemaker et al. 1981), guinea pig (Ryan et al. 1976; James et al. 1977) and rabbits (Pacifici et al. 1982) GST activity towards different substrates is about one tenth that in the adult specimens. Our previous studies on cytosolic GST towards styrene oxide have shown that the average activity in human fetal liver is 3.5 nmol/min/mg (Pacifici et al. 1981b), whereas in the adult liver it is 25 nmol/min/mg (Pacifici et al. 1981a). Using 1-chloro-2,4-dinitrobenzene as substrate, the average GST activity in human fetal (Mukhtar et al. 1981) and adult liver (Baars et al. 1981) cytosols was found to be 158

Table 2. Glutathione S-transferase activity in different human fetal tissues

Age (weeks)	nmol × min^{-1} × mg^{-1}					Lungs liver	Kidneys liver	Gut liver	Placenta liver
	Liver	Lungs	Kidneys	Gut	Placenta				
16	4.24	1.91	1.59	0.96	–	0.45	0.37	0.23	–
16	1.23	1.73	1.10	1.25	1.19	1.41	0.89	1.02	0.96
17	2.98	2.87	2.24	1.12	1.18	0.96	0.75	0.37	0.39
17	–	1.90	1.28	1.73	–	–	–	–	–
21	1.12	2.17	1.15	1.25	–	1.93	1.03	1.12	–
23	0.89	2.62	1.82	1.19	2.30	2.94	2.05	1.34	2.58
23	0.69	1.59	1.27	1.06	–	2.22	1.84	1.54	–
Mean	1.86	2.11	1.49	1.22	1.55	1.65	1.15	0.94	1.31
± SEM	0.58	0.18	0.16	0.09	0.37	0.36	0.26	0.22	0.66

Table 3. Glutathione S-transferase in different human adult tissues

Tissue	Sex	Age (years)	Cigarette smoking habits (cig/day)	nmol/min/mg
Renal cortex	F	32	–	1.35
Renal cortex	F	40	–	1.80
Renal cortex	M	64	20–30	1.56
Renal cortex	M	75	40	2.06
Mean ± SEM				1.69 ± 0.15
Renal medulla	F	32	–	3.61
Renal medulla	F	40	–	1.60
Renal medulla	M	64	20–30	1.10
Renal medulla	M	75	40	2.25
Mean ± SEM				2.14 ± 0.54
Lungs	M	57	10	0.83
Lungs	M	59	15	0.88
Lungs	M	60	10	0.62
Lungs	M	61	10	0.73
Mean ± SEM				0.76 ± 0.06
Ileum	M	59	20	0.91
Ileum	M	69	–	1.21
Ileum	M	72	–	0.67
Ascending colon	F	54	–	0.50
Ascending colon	M	59	20	0.37
Transverse colon	M	72	–	0.98
Sigmoid colon	M	69	–	0.36
Mean ± SEM				0.71 ± 0.12

and 119 nmol/min/mg, respectively. Therefore the developmental pattern of GST is substrate dependent at least in human liver.

GST activity in fetal lungs was found higher than in the adult specimen. The lung was the only organ showing this developmental pattern. Our finding is in accordance with the results by Fryer et al. (1986). These authors have observed that the rate of conjugation of glutathione with 1-chloro-2,4-dinitrobenzene was higher in lung cytosol of mid-gestational human fetuses than in newborn infants and adult subjects. The isoenzyme pattern of GST is not influenced by development in human lungs, since more than 90% of the activity resides in the acidic form (Koskelo et al. 1981; Sherman et al. 1983; Partidge et al. 1984; Fryer et al. 1986). Our observation can-not thus be explained on the basis of a change in the composition in GST isoenzymes. The function of this rather high GST activity in fetal lungs remain to be explained.

No relationship between GST activity and gestational age was observed in fetal liver and in placenta. The enzyme thus develops before the 11th week of gestation and it does not undergo rapid changes, at least not during the second trimester. Consistent results have been observed with styrene oxide (Pacifici and Rane 1981; Pacifici et al. 1981b). Interestingly, aging too does not influence the activity of GST, at least not in the liver.

The placenta is the largest tissue perfused by the fetal blood. This organ is perfused by the whole volume of the fetal blood. Considering that the activity of GST towards BPO is close to that of the fetal liver, the placental GST may contribute greatly to the fetal detoxication capacity. Such a conclusion has earlier been drawn for styrene oxide (Pacifici and Rane 1981, 1982).

Part of the carcinogenic effects of smoking is ascribed to the intermediate epoxide metabolites produced by the conversion of aromatic hydrocarbons, including benzo(a)pyrene. Human adult lung cytosol catalyzes the conjugation of BPO with glutathione at a rate several times lower than liver. Consistent results were obtained with an alkene epoxide, styrene oxide (McManus et al. 1980). Thus, low pulmonary capacity to inactivate epoxides by GST has been shown for different substrates. In adult subjects, the renal and intestinal rates of GST activity are lower than that in liver. The liver is thus the most important organ for the metabolism of the epoxides. However, the contribution of the non-hepatic to over-all GST is considerable because of the wide distribution of this enzyme.

Another finding that deserves to be discussed is the variability of the GST activity in adult liver. Such a variability may depend on several factors including environmental and genetic ones. Among the genetic factors, the occurrence of the transferase μ in half of the adult individuals deserves to be highlighted, since GST μ is the most active one towards BPO (Warholm et al. 1981a). This transferase is not found in any of the extrahepatic organs investigated from adult subjects (Mannervik 1985). The non-hepatic GST may contribute to a larger extent to the detoxication of the epoxides in those individuals whose liver lacks the transferase μ.

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