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Hepatic glutathione *S*-transferases: activities and cellular localization in rat, rhesus monkey, chimpanzee and man

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Glutathione *S*-transferases (EC 2.5.1.18) are a group of enzymes which catalyze the conjugation of a wide range of electrophilic agents with glutathione [1, 2]. They play an important role in the biotransformation of many xenobiotics, alkylating and carcinogenic compounds [3]. GSH* *S*-transferases represent some 10 and 3 per cent of the hepatic cytoplasmic proteins in rat and man, respectively [4, 5]. Their occurence in the membranes of the endoplasmic reticulum of rat liver has also been reported [6–8].

Since species differences in the activity of GSH *S*-transferases have been reported [9], the question of the relevance of the data for man is raised. In a comparative study we have, therefore, determined the hepatic activity and cellular localization of GSH *S*-transferases in the rat, rhesus monkey, chimpanzee and man. Activity of GSH *S*-transferases was measured toward the substrates chlorodinitrobenzene and dichloronitrobenzene in the 9000 × *g* and 100,000 × *g* supernatant of liver homogenate and in the microsomal fraction. The 9000 × *g* supernatant comprises the cytosolic and microsomal fraction, whereas the 100,000 × *g* supernatant represents only the cytosol.

Materials and Methods

1-Chloro-2,4-dinitrobenzene and 1,2-dichloro-4-nitrobenzene were purchased from Ega Chemie (Steinheim, West Germany). The latter was further purified by liquid chromatography in chloroform. Biochemicals were purchased from Boehringer (Mannheim, West Germany), and all other chemicals were from Merck (Darmstadt, West Germany).

Experiments with animals were performed with male SPF Wistar rats weighing 180–220 g, adult male and female rhesus monkeys (*Macaca mulatta*) weighing 9–15 kg, and juvenile and adult male and female chimpanzees, weighing 28–63 kg. All animals were maintained on standard laboratory diets (Altromin and Purina Monkey Chow, respectively) and received water *ad lib*. Additionally, the non-human primates received fruits twice a day.

Human liver biopsies were obtained from 25–70 year old individuals of both sexes suspected of having liver diseases, mainly chronic hepatitis and alcohol abuse. There was no significant correlation of the enzyme activities with sex, age or disease of the patients.

In contrast to the liver samples from rats and non-human primates which were processed immediately, the human samples were frozen immediately after biopsy and stored at −80° for up to 72 hr. In experiments with frozen rat samples, a freezing period of 11 days did not significantly influence the activity of GSH *S*-transferases (10).

Activity of GSH *S*-transferases was measured according to [11, 12]. Activities are expressed as nmoles conjugates formed per min and per mg protein of the respective liver fraction. For technical reasons protein recovery was only tested in samples of rat and man. Both species showed similar values which amounted to 80 ± 6, 46 ± 5 and 25 ± 3 mg protein/g liver in the S9, S100 and microsomal fraction, respectively (mean ± S.D., n = 4).

For the preparation of S9, S100 and microsomes, rat livers and biopsy samples were homogenized (Potter-Elvehjem and ultrasonication, respectively) in ice-cold phosphate buffer (0.1 M, pH 7.4) and centrifuged at 9000 × *g* and 100,000 × *g*, respectively. For the preparation of microsomes, the microsomal pellet was washed once with buffer and recentrifuged at 100,000 × *g* for one hour to remove contaminating cytoplasm. Dependent on the protein content of the samples, protein was determined by a biuret method or according to Lowry [13] using dried bovine serum albumin as the standard.

Results

Activity of GSH S-transferases toward the substrates CDNB and DCNB was present in the liver fractions of all species investigated. In the hepatic S9 fraction (Table 1) activity toward CDNB was 1–2.5 μmols/min × mg protein, being highest in the rhesus monkey. Similar values were found for rat and man. Activity toward DCNB showed greater variations and was only 0.2–2.2 per cent of that found with CDNB as substrate. It was highest in the rat and declined in the order of rat, rhesus monkey, and chimpanzee which was similar to man.

Activity of GSH *S*-transferases toward CDNB and DCNB is predominantly located in the soluble, cytosolic fraction of the liver. The ratios of the activities toward CDNB and DCNB were similar in S9 and S100 for all species investigated. However, a considerable fraction of the activity in S9 is present in the microsomes. Microsomal activity toward CDNB ranged from 43 to 176 nmols/min × mg protein in rat and rhesus monkey, respectively. Microsomal activity toward DCNB was similar in all species ranging from 0.7 to 2.9 nmols/min × mg protein in chimpanzee and rat, respectively.

Discussion

The livers of rat, rhesus monkey, chimpanzee and man catalyze the conjugation of GSH with CDNB and DCNB. However, considerable species differences are observed, particularly for the activity toward DCNB. Similar in man and chimpanzee, this activity in S9 and S100 was approximately 10 per cent of the activity in the rat. Similarly, the activity of GSH *S*-transferases toward several α–β unsaturated compounds in human liver was found to range from 2 to 33 per cent of the corresponding activity in rat [9].

In contrast to the lower activities in S9 and S100 of primates as compared to rat, the activity pattern in the microsomal fraction was different. Although the microsomal activity toward both substrates was roughly similar in all species tested, the ratio of microsomal to S9 activities was higher in primates than in the rat. The determination of GSH *S*-transferase activities in liver homogenates of different species, therefore, might not provide reliable information on the GSH-dependent detoxification of reactive intermediates which will be formed within microsomal membranes.

The nature of the microsomal enzymes is not yet known. Immunoprecipitation studies in the rat showed some similarities with the cytosolic enzymes [8]. However, different induction patterns [14] and different responses of cytosolic

* Abbreviations: CDNB, 1-chloro-2,4-dinitrobenzene; DCNB, 1,2-dichloro-4-nitrobenzene; GSH, reduced glutathione; S9, 9000 × *g* supernatant of liver homogenate; S100, 100,000 × *g* supernatant of liver homogenate.

Table 1. Activity of glutathione *S*-transferases in hepatic 9000 × *g*, 100,000 × *g* supernatant of liver homogenate and hepatic microsomal fraction of rat, rhesus monkey, chimpanzee and man*

Species	(*n*)	1-Chloro-2,4-dinitrobenzene	1,2-Dichloro-4-nitrobenzene
		nmols conjugate formed/min × mg protein	
9000 × *g* supernatant			
Rat	6	1097 ± 106	23.6 ± 2.5
Rhesus monkey	13	2439 ± 422	8.3 ± 2.0
Chimpanzee	32	1682 ± 289	3.7 ± 0.8
Man	6	981 ± 345	3.0 ± 1.3
100,000 × *g* supernatant			
Rat	11	1826 ± 179	51.4 ± 6.2
Rhesus monkey	†	6482 (5967–6996)	28.8 (25.5–32.1)
Chimpanzee	†	3196 (2501–3603)	9.2 (6.6–11.8)
Man	9	1638 ± 530	4.6 ± 1.7
Microsomes			
Rat	12	43 ± 4	2.9 ± 1.3
Rhesus monkey	†	176 (133–239)	1.6 (1.5–1.7)
Chimpanzee	†	143 (126–154)	0.7 (0.5–0.9)
Man	9	68 ± 20	1.9 ± 1.2

* Data represent mean ± S.D.

† Five samples were pooled. Data represent mean of two or three pools with range in parenthesis.

and microsomal GSH *S*-transferases to sulfhydryl reagents [7] probably reflect the presence of different enzymes in the microsomal fraction. As an important characteristic, GSH *S*-transferases localized within the membranes of the endoplasmic reticulum will affect the steady state level of lipophilic reactive intermediates close to their site of generation. Since the microsomal enzymes have been shown to be at least partially exposed on the cytoplasmic surface of the membranes [14], the availability of the cytoplasmic cofactor GSH is not likely to be rate-limiting.

In conclusion, our results point to quantitative species differences between primates and the rat in the GSH-dependent detoxification capacity of different liver compartments. The subcellular localization of GSH *S*-transferases in hepatic microsomes, first shown for the rat, has been confirmed in our experiments for rhesus monkey, chimpanzee and man. In all species investigated, the activity toward CDNB and DCNB was mainly located in the cytoplasmic fraction. Moreover, the microsomal membranes of all species showed distinct activities of GSH *S*-transferases which are likely to be involved in the metabolism of xenobiotics.

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