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Comparison of the distribution of microsomal and cytosolic glutathione S-transferase activities in different organs of the rat

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The glutathione *S*-transferases are a ubiquitous class of enzymes which play a major role in the detoxication and excretion of xenobiotics (see Refs 1–3 for reviews). The individual members of this family of enzymes exhibit broad and overlapping substrate specificities towards a variety of mutagenic, carcinogenic and pharmacologically active substances, including many of the reactive intermediates formed by the metabolism of xenobiotics via the cytochrome P-450 system [4, 5]. Soluble glutathione *S*-transferases have been purified and characterized from a number of different sources [1].

Rat liver microsomes also demonstrate glutathione *S*-transferase activity, as shown in our own and others' laboratories [6–8]. On the basis of a variety of criteria—including activatability by sulfhydryl reagents, subcellular distribution, substrate specificity, response to inducers, minimum mol. wt and immunological properties—we have concluded that microsomal glutathione *S*-transferase activity is catalyzed by a protein which is unrelated to the cytosolic enzymes [6, 9].

In this present investigation we have examined the distribution of microsomal glutathione *S*-transferase in different organs of the male rat. As with other drug-metabolizing enzymes, it is important to know this distribution when attempting to explain differences in the susceptibility of different organs to the harmful effects of reactive chemicals and to assess the relative contributions of different organs to the total metabolism of various xenobiotics. At the same time, it should not be forgotten that many drug-metabolizing enzymes also recognize endogenous metabolites as substrates. It was hoped that the organ distribution of microsomal glutathione *S*-transferase might give some clue concerning possible endogenous functions of this enzyme. Finally, the distribution of the microsomal activity has been compared to that of the cytosolic activity—both in an effort to observe differences which might be of importance in drug metabolism and in order to gather evidence concerning the question as to whether the glutathione *S*-transferase activity of untreated microsomes is simply due to contamination by the cytosolic enzymes.

Methods and results. Male Sprague–Dawley rats weighing 250 g were starved overnight and then killed by decapitation. The different organs were removed, weighed and placed in a vol. of ice-cold 0.25 M sucrose equal in ml to 4 times the tissue wt in g. In order to obtain enough material for each experiment the organs from three rats were pooled. The organs were diced with scissors and then homogenized using four to six passes of a Teflon pestle in a Potter–Elvehjem homogenizer at 440 rpm. Thereafter microsomes were prepared using a standard centrifugation procedure [10], except that the microsomes were washed twice with 0.15 M Tris–HCl (pH 8) in order to remove cytosolic contamination [6]. The supernatant from the 100,000 *g* centrifugation was designated the cytosolic fraction.

Both microsomal and cytosolic glutathione *S*-transferase activity towards 1-chloro-2,4-dinitrobenzene as the second substrate were assayed spectrophotometrically [11]. In order to specifically determine microsomal glutathione *S*-transferase [6], the microsomal activity was also measured after activation with *N*-ethylmaleimide. This activation was achieved by preincubating the microsomes with 1 mM *N*-ethylmaleimide in 0.1 M potassium phosphate (pH 6.5) for 30 sec at room temp immediately before assay.

As can be seen from Table 1, the glutathione *S*-transferase activity of rat liver microsomes is activated 5.5-fold by treatment with *N*-ethylmaleimide. However, for no other organ was there a significant increase in microsomal glutathione *S*-transferase activity after such treatment. Indeed, in the case of brain and heart microsomes the activity is strongly inhibited by *N*-ethylmaleimide.

The S.E.M. for some of these determinations is rather

Table 1. The sp. act. of glutathione *S*-transferase in microsomal fractions from different organs of the rat

	Activity (nmoles/mg protein/min)		
Organ	Microsomes	Activated microsomes	$\frac{\text{Activated}}{\text{Microsomes}} \times 100$
Liver	126 ± 6 (100)	700 ± 107	556.0
Kidney	8.54 ± 3.98 (7)	8.44 ± 3.98	98.9
Lung	15.4 ± 4.0 (12)	17.8 ± 9.4	116.0
Intestine	60.2 ± 24.7 (48)	37.4 ± 11.4	62.0
Adrenal	51.9 ± 19.8 (41)	41.2 ± 24.3	79.3
Testicle	129 ± 28 (102)	129 ± 16	100.0
Spleen	9.01 ± 3.25 (7)	5.96 ± 2.99	66.1
Brain	7.95 ± 0.49 (6)	0.58 ± 0.29	7.3
Heart	7.23 ± 2.00 (6)	0.37 ± 0.37	5.1
Thymus	4.37 ± 2.51 (4)	6.90 ± 6.91	158.0

The microsomal fractions were prepared and activated and glutathione *S*-transferase activity measured as described in the text. Three rats were pooled for each preparation and three such preparations were performed. The figures shown are the means ± S.D. of the means for these three independent determinations. The figures in parentheses after the microsomal activity are percentages of the liver activity.

Table 2. The sp. act. of glutathione *S*-transferase in the cytosol fractions from different organs of the rat and comparison with the unactivated microsomal activity

Organ	Cytosolic activity*	Maximal interference by blood† (%)	$\frac{\text{Cytosolic}}{\text{Microsomal}}$‡
Liver	1400 ± 30 (100)	0.0	11.1
Kidney	336 ± 21 (24)	0.1	39.3
Lung	78.8 ± 2.2 (6)	2.8	5.1
Intestine	429 ± 97 (31)	0.6	7.2
Adrenal	253 ± 21 (18)	0.7	4.9
Testicle	3850 ± 170 (275)	0.0	29.8
Spleen	55.7 ± 4.9 (4)	2.7	6.2
Brain	190 ± 37 (14)	0.1	23.4
Heart	92.6 ± 5.1 (7)	2.2	12.8
Thymus	46.3 ± 3.8 (3)	1.8	10.6

The cytosol fractions were prepared and glutathione *S*-transferase activity measured as described in the text.

* nmoles/mg protein/min. Three rats were pooled for each preparation and three such preparations were performed. The figures shown are the means ± S.D. of the means for these three independent determinations. The figures in parentheses are percentages of the liver activity.

† These figures were arrived at as follows: the amount of hemoglobin present in a blood sample and in the different cytosol fractions was determined by measuring the O.D. at 540 nm. The glutathione *S*-transferase activity of the blood sample was then measured and the contribution of contaminating blood to the activity of each cytosol fraction could thus be calculated.

‡ These figures were arrived at by dividing the first column in the table with the first column in Table 1.

large. One reason for this is that we used large amounts of protein in some of the measurements in order to detect low levels of activity and in a few cases we approached the limit of sensitivity of the assay procedure. In addition, at least some of the glutathione *S*-transferase activity of untreated microsomes is apparently due to contaminating cytosolic enzymes (see also later) and such contamination may vary from preparation to preparation. Under the conditions used here we could have easily detected a doubling of the microsomal activity upon treatment with *N*-ethylmaleimide and in certain cases (intestine, adrenal and testicle) an even smaller percentage increase. We can thus say that the specific *N*-ethylmaleimide-activatable glutathione *S*-transferase activity in microsomes from the kidney, lung, spleen, brain, heart and thymus is less than 1–2% of the corresponding activity in liver microsomes, whereas the activatable glutathione *S*-transferase in microsomes from the intestine, adrenal and testicle is less than 5–7% of the liver value.

As seen in Table 1, there are rather large differences in the glutathione *S*-transferase activities of untreated microsomes from different organs of the rat. The corresponding distribution of cytosolic glutathione *S*-transferase activity is shown in Table 2. As indicated in Table 2, the maximal interference by contaminating blood in these determinations is negligible. As can be seen, there are also large differences in the specific cytosolic glutathione *S*-transferase activities in the various organs examined. Most of the organs demonstrate a sp. act. which is between 3 and 31% of the liver value, whereas the activity of the testicle cytosol is almost three-fold greater than that of the liver.

The ratio of cytosolic to unactivated microsomal glutathione *S*-transferase activity is also shown in Table 2. There is an approximately eight-fold spread in these values (4.9 for the adrenal and 39.3 for the kidney). This suggests that either the unactivated microsomal activity is not entirely due to contamination by cytosol or else that the microsomes from different organs adsorb different amounts of the cytosolic transferases, even after washing twice with Tris buffer. Preliminary data from our laboratory suggest that only about half of the glutathione *S*-transferase activity of untreated rat liver microsomes is due to cytosolic "contamination".

In addition to the sp. act. of glutathione *S*-transferase in the microsomes and cytosol from different organs, the total activity is also of interest. The parameters used in calculating this total activity are shown in Table 3 and the results in Table 4. It is clear that the liver activities are much greater than those of any of the other organs. In addition, the total cytosolic activity is much greater than the microsomal activity in all of the organs (though it should be remembered that the microsomal activity might need to be multiplied by a factor of 3–10 in order to correct for incomplete recovery of fragments of the endoplasmic reticulum [12, 13]).

Table 3. The wt and microsomal and cytosolic protein contents of different rat organs

Organ	Wt (g/100 g)	Cytosolic protein (mg/g)	Microsomal protein (mg/g)
Liver	4.08 ± 0.08	81.6 ± 1.6	17.7 ± 0.7
Kidney	1.15 ± 0.02	48.6 ± 1.1	9.83 ± 1.01
Lung	1.10 ± 0.06	64.7 ± 3.9	2.10 ± 0.02
Adrenal	0.04 ± 0.01	17.5 ± 1.1	3.02 ± 0.70
Testicle	1.40 ± 0.08	24.5 ± 1.0	5.89 ± 0.75
Spleen	0.33 ± 0.02	68.9 ± 8.7	13.9 ± 0.9
Brain	0.66 ± 0.02	19.9 ± 1.1	2.22 ± 0.25
Heart	0.50 ± 0.01	38.5 ± 0.8	1.53 ± 0.19
Thymus	0.36 ± 0.02	46.8 ± 5.4	1.38 ± 0.03

The microsomal and cytosolic fractions were prepared as described in the text. Both right and left kidneys, adrenals and testicles were used. Three rats were pooled for each preparation and three such preparations were performed. The values are the means ± S.D. of the means for these three independent determinations.

Table 4. The distribution of total glutathione S-transferase activity among various organs of the rat

Organ	Total activity (μmoles/organ/100 g rat/min) Unactivated microsomal	Cytosolic
Liver	9.100 (100.0)	466 (100.0)
Kidney	0.097 (1.0)	18.8 (4.0)
Lung	0.036 (0.4)	5.61 (1.0)
Adrenal	0.006 (0.0)	0.177 (0.0)
Testicle	1.060 (12.0)	132 (28.0)
Spleen	0.041 (0.5)	1.27 (0.3)
Brain	0.012 (0.1)	2.50 (0.5)
Heart	0.006 (0.0)	1.78 (0.4)
Thymus	0.002 (0.0)	0.78 (0.2)

The figures in parentheses are percentages of the liver value.

The total activated liver microsomal glutathione S-transferase activity can be calculated as 167 μmoles/liver/100 g rat/min [700 nmoles/mg microsomal protein × 17.7 mg microsomal protein/g liver × 4.08 g liver/100 g rat × 3 (to correct for the incomplete recovery of microsomes [12])]. Thus, if we assume that the microsomal glutathione S-transferase can be activated *in vivo* as it can *in vitro* (an assumption for which we as yet only have indirect evidence [14]), the largest capacity for conjugation of 1-chloro-2,4-dinitrobenzene and, presumably, of similar substrates with glutathione is found in the liver cytosol, the liver endoplasmic reticulum, and the testicle cytosol.

The most important observation presented here is that the N-ethylmaleimide-activatable microsomal glutathione S-transferase seems to be found only in the liver and is absent from the other organs examined. This is rather unusual, since most other drug-metabolizing enzymes are found in a variety of different tissues (see, for example, Table 2). Thus, if the N-ethylmaleimide-activatable microsomal glutathione S-transferase has some function other than conjugation of xenobiotics and/or their metabolites with glutathione, then this function must be localized mainly, if not exclusively, in the liver.

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Impairment by ruthenium red of anticancer drug cytotoxicity in CCRF-CEM cells

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It was shown recently that ruthenium red (RR), an inorganic dye, reduced both the cellular accumulation and the cytotoxicity of vinblastine (VLB) toward murine cells in culture [1]. It has been known for some time that RR binds to plasma membrane mucopolysaccharides and glycoproteins, and this property has been exploited in the staining of mucopolysaccharides for electron microscopy [2]. The dye is also known to inhibit both Ca^{2+} transport in mitochondria [3] and Ca^{2+}-ATPase activity in sarcoplasmic reticulum [4].

To account for the reversal of VLB cytotoxicity by RR in KB cells, it was proposed [1] that, besides the reduction in drug accumulation, the dye-induced inhibition of Ca^{2+}-ATPase could lead to an increase in intracellular Ca^{2+}, which in turn might eventually protect microtubules from binding by VLB.

The purpose of our present study was to analyze further this phenomenon of impaired drug toxicity by RR. Specifically, we wished to investigate the implied role of microtubules in this phenomenon and did so by examining