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ACINAR DISTRIBUTION OF GLUTATHIONE-DEPENDENT DETOXIFYING ENZYMES

LOW GLUTATHIONE PEROXIDASE ACTIVITY IN PERIVENOUS HEPATOCYTES

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Abstract—The acinar distribution of glutathione *S*-transferase (GST), glutathione peroxidase (GPx), glutathione reductase (GR), and glucose-6-phosphate dehydrogenase (G-6-PDH) was examined by analyzing periportal (p.p.) and perivenous (p.v.) rat hepatocytes selectively isolated by the digitonin–collagenase perfusion. The cytosolic GST activity was higher in p.v. cells, but the microsomal GST and cytosolic GR were found to be evenly distributed in the acinus. In contrast, the activity of both the Se-dependent GPx and the microsomal (Se-independent) GPx, as well as G-6-PDH, was much lower in the p.v. than in the p.p. cells. The heterogeneous distribution of GST, GPx and G-6-PDH was confirmed by analyzing liver perfusion effluents collected after ante- or retrograde digitonin infusion. The relatively low activities of GPx and G-6-PDH in the p.v. cells could partly explain the susceptibility of this region to chemical injury.

Most xenobiotics preferentially damage the perivenous (p.v.) or centrilobular region of the liver acinus [1]. This may be related to an imbalance in this region between the rate of cytochrome P-450-linked production of reactive intermediates and the capacity for their detoxication [2].

Reduced glutathione has a pivotal role in the cellular protection against xenobiotics and peroxidative attacks [3], but its acinar distribution is not established [4–7]. More important than glutathione levels might be possible acinar differences in the glutathione-related detoxication pathways, of which only qualitative or semi-quantitative immunohistochemical or cytochemical information is available [2, 8–13].

We report here the acinar distribution of glutathione-coupled enzyme activities analyzed from intact hepatocytes isolated from the periportal or perivenous region by digitonin–collagenase perfusion [14].

MATERIALS AND METHODS

Male rats of the Alko mixed strain weighing 170–230 g for cell isolation and 220–310 g for analyses of effluents were provided tap water and a standard diet (Astra-Ewos AB, Sweden) *ad libitum*. The rats were anaesthetized with sodium pentobarbital (60 mg/kg, i.p.), and p.p. or p.v. hepatocytes were prepared by the digitonin–collagenase method [14]. After removing cell debris and non-parenchymal cells by low-speed centrifugation (25 $g \times 90$ sec, 2–3 times), the cell viability averaged 94%. The yield of intact hepatocytes by this procedure is 20–30% of that obtained by a complete conventional collagenase digestion.

The selectivity of the cell isolation technique was confirmed from the p.v. to p.p. ratios of the marker enzymes shown in Table 1. For enzyme activity assays cell suspensions were sonicated for 10 sec and treated by an equivalent volume of ice-cold 2% Triton X-100, followed by a centrifugation at 10,000 $g \times 20$ min, and the supernatant was used for enzyme assays.

For microsome isolation the hepatocytes were homogenized in ice-cold buffer containing 40 mM KH_2PO_4, 50 mM KCl, 130 mM sucrose, and 1 mM EDTA, pH 7.4. After centrifugation at 10,000 g for 20 min, the microsomes were pelleted at 105,000 g for 60 min, suspended in 50 mM K–Na–phosphate buffer containing 1 mM EDTA, pH 7.4, homogenized and finally sonicated for 10 sec.

The activities of the marker enzymes alanine aminotransferase, γ-glutamyltransferase and glutamate dehydrogenase were analyzed by standard enzymatic assays as described before [14]. Glutathione reductase (GR, EC 1.6.4.2) was assayed as described by Massey and Williams [15]. Activities of both Se-dependent glutathione peroxidase (Se-GPx, EC 1.11.1.9) with H_2O_2 and Se-independent glutathione peroxidase (non-Se-GPx) with cumene hydroperoxide were measured by the GR-coupled system [16]. Glutathione *S*-transferase (GST, EC 2.5.1.18) activities toward 1-chloro-2,4-dinitrobenzene (CDNB) and 4-nitropyridine-*N*-oxide (NPO) were measured according to the method of Habig *et al.* [17]. In the assay of the microsomal GST activity, a low CDNB concentration (0.05 mM) was used.

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Table 1. Distribution of the activity of the marker enzymes alanine aminotransferase, γ-glutamyltransferase and glutamate dehydrogenase in hepatocytes isolated from the periportal (p.p.) or perivenous (p.v.) region

Enzyme	Enzyme activity (nmol/min per mg protein) p.p. (N = 12)	p.v. (N = 13)	p.v./p.p. ratio
Alanine aminotransferase	250 ± 70	156 ± 69*	0.62
γ-Glutamyltransferase	4.4 ± 1.6	1.7 ± 0.7†	0.39
Glutamate dehydrogenase	1060 ± 170	1400 ± 160†	1.32

Means ± SD. * $P < 0.01$, † $P < 0.001$.

Under these conditions, contamination by the cytosolic GST was minimal, because of the much higher apparent K_m value of the cytosolic isoenzyme [18]. GST, Se-GPx and G-6-PDH activities were assayed at 30° and microsomal GST and non-Se-GPx at 25° in presence of 2.5 mM GSH. Glucose-6-phosphate dehydrogenase (G-6-PDH, EC 1.1.1.49) activity was assayed at 37° by the method of Deutsch [19] but 1 mM maleimide was used to inhibit any further NADPH formation by 6-phosphogluconate dehydrogenase. Substrate was omitted from reference cuvettes. The reaction was linear for 5–6 min, provided that the ΔA_{340}/min was less than 0.01. The activity obtained was in good agreement with a previous study [20], where the G-6-PDH activity was estimated after correction for interference from the 6-phosphogluconate dehydrogenase activity. Protein was assayed by the fluorescamine method [21].

Statistical analyses were performed by using the Student's *t*-test.

RESULTS

Glutathione S-*transferase*

The total activity of GST, using CDNB as substrate, was 22% higher ($P < 0.05$) in the isolated p.v. cells than in p.p. cells (Table 2). A slight, statistically non-significant, p.v. dominance was seen also when NPO was used as substrate. The activity toward CDNB in effluents from the p.v. region was 60% higher than in the effluents from the p.p. region ($P < 0.05$, Table 3), suggesting that the GST activity in the most perivenous region may be several times higher than in the p.p. region. In contrast, the GST activity in the microsomal fractions isolated from the p.p. and p.v. cells was approximately the same (Table 2). The activation of the microsomal enzyme in the presence of *N*-ethylmaleimide [18] was about fourfold, but no p.p.–p.v. difference was discerned (data not shown).

Glutathione peroxidase

The total activity of Se-GPx in p.v. cells was only 64% of that in p.p. cells (Table 2) and in p.v. effluents only 54% of that in p.p. effluents (Table 3). In the microsomal fraction, the non-Se-GPx activity, which is identical with one or more isoenzymes of GST [16, 22, 23], was also significantly lower in p.v. than in p.p. cells (Table 2).

Glutathione reductase and glucose-6-phosphate dehydrogenase

No difference in the GR activity was found between p.p. and p.v. hepatocytes (Table 2). The

Table 2. Comparison of glutathione *S*-transferase, glutathione peroxidase, glutathione reductase and glucose-6-phosphate dehydrogenase activities in p.p. and p.v. hepatocytes or their microsomal fractions

	Enzyme activity (nmol/min per mg protein) p.p.	p.v.	p.v./p.p. ratio
Total activity			
GST (CDNB, 1 mM)	429 ± 36	525 ± 73*	1.22
(NPO, 0.2 mM)	8.99 ± 1.67	10.43 ± 1.05	1.16
Se-GPx (H_2O_2, 0.2 mM)	716 ± 111	461 ± 80**	0.64
GR	33.6 ± 3.4	33.9 ± 7.0	1.01
G-6-PDH	4.10 ± 0.71	3.13 ± 0.35*	0.76
Microsomal activity			
GST (CDNB, 0.05 mM)	53.7 ± 21.8	45.7 ± 10.3	0.85
Non-Se-GPx (cumene hydroperoxide, 0.07 mM)	28.1 ± 4.0	19.8 ± 2.8**	0.70

Means ± SD of 6–7 preparations. * $P < 0.05$; ** $P < 0.01$.

Table 3. Enzyme activities in the liver perfusion effluents after destruction of p.p. or p.v. cells by antegrade or retrograde digitonin infusion

Enzyme (substrate)	Enzyme activity (nmol/min per mg protein) p.p. (N = 7)	p.v. (N = 6)	p.v./p.p. ratio
GST (CDNB, 1 mM)	688 ± 145	1102 ± 291*	1.60
Se-GPO (H_2O_2, 0.2 mM)	950 ± 137	516 ± 141**	0.54
G-6-PDH	11.64 ± 2.86	5.30 ± 1.56**	0.46

Means ± SD. * $P < 0.05$; ** $P < 0.01$. The duration of the infusion of 7 mM digitonin to destroy the p.p. or p.v. region was 18–35 sec and 25–47 sec, respectively.

activity of G-6-PDH was, on the other hand, 24% lower in p.v. than in p.p. cells (Table 2). The p.p. dominance was even more pronounced when assayed in perfusion effluents (Table 3).

DISCUSSION

Acinar heterogeneity

In this study, the enzyme activity was directly assayed either in the intact hepatocytes isolated from the p.p. and p.v. regions or in the p.p. and p.v. effluents, and "p.p." and "p.v." refer respectively to the cells surrounding the afferent and efferent halves of the sinusoid. Evidence presented in [14] suggests that the contamination from the opposite region is small and that the preparations represent the average of either "half". Assuming a linear acinar gradient for a heterogeneously distributed enzyme, a moderate difference between the p.p. and p.v. preparations reflects larger a difference between the most distant p.p. and p.v. cells. This concept was supported by analyzing the effluents collected after selectively rupturing either the most afferent or efferent sinusoidal regions with a short digitonin infusion. Indeed, by this method the p.p.–p.v. differences in activities of GST, Se-GPx and G-6-PDH in effluent samples were much more pronounced.

Acinar distribution of glutathione-coupled detoxifying enzymes

The two main pathways of glutathione-related detoxication are conjugation mechanisms mediated by GST and reduction of peroxides mediated by GPx [3]. We found the total activity of GST to be enriched in p.v. cells. Since most of the activity of total GST is found in the cytosol [22], this enzyme must be responsible for the higher p.v. activity previously reported by immunohistochemical studies [8, 9]. The higher activity of cytosolic GST in p.v. cells may overcome the possible accumulation of reactive intermediates produced by the cytochrome P-450-linked mono-oxygenases [24], which show higher activity in this region [2]. Since, however, these reactions occur in the endoplasmic reticulum, and the GST activity in this compartment seems to be evenly distributed within the acinus (Table 4), it is conceivable that there exists a relative deficiency of the capacity for glutathione conjugation in the endoplasmic reticulum of the p.v. cells.

Glutathione peroxidases and glutathione reductase form the glutathione redox cycle, and G-6-PDH catalyzes the rate-limiting step of the pentose phosphate pathway, that is the main source of NADPH for the cycle under increased NADPH consumption [24, 25]. Although high GPx activity efficiently protects against acute peroxidative attack, the long-term defence capacity depends on the function of the whole glutathione redox cycle [25].

A lower activity of Se-GPx in p.v. cells, as observed in this study, is in concordance with a previous immunohistochemical study [10]. In addition to the cytosolic Se-GPx, which dominates the total GPx activity in rat liver [16, 23], a significantly higher activity of non-Se-GPx was also found in the microsomal fraction from p.p. cells. This enzyme may be important in the detoxication of membrane lipoperoxides [23, 26]. The Se-GPx, on the other hand, exhibits a higher activity and has a broad substrate specificity. Therefore, for efficient elimination of different kinds of hydroperoxides both GPx activities are required and their heterogeneous acinar distribution may be crucial.

The p.v./p.p. ratio of G-6-PDH was much lower in the effluents than the isolated cells. The relatively high G-6-PDH activity of the non-parenchymal cells [2, 12] may explain the higher G-6-PDH activity in the effluents. Thus the more marked p.p. dominance in effluents as compared to cells may reflect not only a better p.p.–p.v. selectivity but a heterogeneous G-6-PDH distribution also in non-parenchymal cells. Our data are at variance with previous studies suggesting either even acinar distribution in parenchymal cells [12] or higher activity in the p.v. region [11, 13]. These studies may have been confounded by the presence of non-parenchymal activity or by use of an unspecific G-6-PDH assay. We noted that unless maleimide was added, a much higher rate of unspecific NADPH formation occurred, due to activity of 6-phosphogluconate dehydrogenase [19]. This enzyme activity has been reported to be higher in the p.v. region [2].

In conclusion, considering the perivenous dominance of cytochrome P-450-mediated chemical activation, the present results indicate that in this region an imbalance between the enzymatic capacities for production of peroxides and for detoxication contributes to the greater susceptibility of the p.v. hepatocytes to xenobiotic damage.

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