GLUTATHIONE REDUCTASE AND GLUTATHIONE PEROXIDASE ACTIVITIES IN HEPATOMOUS LIVERS OF RATS TREATED WITH DIETHYLNITROSAMINE

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1. Introduction

An increased concentration of acid-soluble thiols appear to be involved in cell division and in tumour formation [1]. This could be related with oxidation–reduction of glutathione which is probably a steady-state dependent, in part, on glutathione reductase and glutathione peroxidase activities [2–4].

The present communication compares the activities of these enzymes in rat hepatomous liver, induced by diethylnitrosamine [5], with control rat liver and shows that GSSG reductase is increased and GSH peroxidase is decreased in the liver of rats treated with diethylnitrosamine. The effect is sex independent.

2. Materials and methods

2.1. Materials

All the chemicals used were of the purest grade available. GSH, GSSG, NALPH and yeast glutathione reductase were obtained from C.F. Boehringer and Soehne GmbH, Mannheim, Germany. All the other reagents used were supplied by British Drug Houses, Ltd., Poole, Dorset, Great Britain.

2.2. Animals and diets

Wistar rats were from the Sheffield University Animal House colony and were fed ad libitum with Oxiod diet 86 (Herbert C. Styles Ltd., Bewdley, Worcs.).

2.3. Diethylnitrosamine induction of liver tumours

was carried out adding diethylnitrosamine to the drinking water (50 mg/ℓ) of 20 rats (10 males and 10 females) for about 4 months. After this time the animals started dying and for this reason the remaining treated rats and the control rats were killed at once for the experimental measurements. The livers were removed and after preparation of microscopic section for light microscopy they were kept in the deep freeze. In the livers of the rats treated with diethylnitrosamine there were large amounts of neoplastic foci and a few areas of necrosis which were absent in the controls. The average weight of the control rats was 390 ± 25 g for males and 252 ± 23 g for females and that of the treated rats was 280 ± 30 g and 180 ± 17 g, respectively. Thus the treated rats were on average about 70% of the weight of control rats.

2.4. Male rats weighing 470–510 g were starved

between 24 hr and 13 days which was about the limit of survival. Only one of the four rats starved for 13 days and for this reason only one experiment was possible with this long period of starvation.

2.5. Preparation of homogenates and measurement of enzyme activities

Glutathione peroxidase and glutathione reductase were assayed as earlier described [2]. Homogenates used for enzyme assays were prepared as in [2] but without liver perfusion.

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3. Results

3.1. Changes in glutathione reductase and glutathione peroxidase activities in the livers of rats treated with diethylnitrosamine (table 1)

The activity of GSSG reductase in hepatomous liver was 10.8 ± 2.0 and 10.6 ± 0.6 μmoles GSSG reduced/min/g fresh liver in the male and the female, respectively. These values were about 2.2-fold that found in normal rat liver and the increase was irrespective of sex.

GSH peroxidase activities in the hepatomous livers were 40 ± 9 and 72 ± 7 μmoles GSSG reduced/min/g fresh liver in the male and in the female, respectively. The 60% activities of the control values found maintains the normal [2] sex difference.

In hepatomous livers of the male rats the GSSG peroxidase/GSH reductase activities ratio was 3.7 ± 1.0 and in the female was 6.8 ± 1.8. These values are between one third and one quarter of those found in the normal rat liver.

3.2. Effect of starvation on glutathione reductase and on glutathione peroxidase activities in the liver of male rats (table 2)

The weight of the treated rats was lower than the controls. It was also observed that treated rats ate less food than non-treated rats. Therefore the difference in enzyme activities observed (table 2) in each case could be in part due to the partial starvation and loss of weight. Table 2 shows that the activity of GSSG reductase decreased about 15% in the liver of rats starved for 2 days and this activity was maintained for a time of starvation as long as 13 days. GSH peroxidase activity was maintained or increased till about 4 days of starvation. After this time the activity decreased till 13 days when enzyme activity was only about 30% of that of non-starved rats. Long starvation between the 7th and 12th days decreased GSH peroxidase activities to about 70% of the control values.

Table 2

Glutathione reductase and glutathione peroxidase activities in the livers of starved male rats

Days of starvation	GSH peroxidase (μmoles GSSG formed/min/g fresh liver)	GSSG reductase (μmoles GSSG reduced/min/g fresh liver)
0 (control)	66 ± 5	4.5 ± 0.7
1	70	4.2
2	67, 74	4.0, 3.7
3	70	3.9
5	55	3.7
7	49, 52	4.0
9	41, 45	3.0, 3.8
12	43	4.2
13	20	3.5

Enzyme activities of control rats are the means of 10 animals.

Table 1

Glutathione reductase and glutathione peroxidase activities and their ratio in rat hepatomous liver.

Sex		Glutathione peroxidase (μmoles GSSG formed/min/g fresh liver)	Glutathione reductase (μmoles GSSG reduced/min/g fresh liver)	Ratio peroxidase activity / reductase activity	No. of experiments
Male	Control rats	65 ± 5	4.6 ± 0.3	14.1 ± 1.8	5
	Treated rats	40 ± 9	10.8 ± 2.0	3.7 ± 1.0	7
Female	Control rats	118 ± 20	5.1 ± 0.5	23.0 ± 3.0	5
	Treated rats	72 ± 7	10.6 ± 0.6	6.8 ± 1.8	6

Rats were about 4 months old when drinking water containing 50 mg/l of diethylnitrosamine was first given. They were killed about 4 months later. For further details see Materials and methods section.

Therefore simple starvation and loss of weight cannot account for the increase of GSSG reductase activity in rats drinking water containing diethylnitrosamine. However, starvation or/and loss of weight may be in part resposible for the decrease in glutathione peroxidase activity in the liver of treated rats.

4. Discussion

Since the areas of necrosis in the hepatomous livers used were small (and would be expected to have low enzyme activities) and since the remaining normal liver cells can only 'dilute' the changes in activity, we assume the increase in enzyme activity must be associated with the tumour tissue.

The high GSSG reductase activity found in diethylnitrosamine induced hepatomous livers as compared with the normal liver agrees with the findings in hepatomas induced by other carcinogens [9]. Therefore it is probable that increase of GSSG reductase activity is a characteristic of hepatomas and possibly of tumours in general since a rise of activity has always been found when tested [6—8]. The activity of glucose-6-phosphate dehydrogenase is also increased in hepatomas [10] which appears therefore to be able to reduce more GSSG than normal liver.

The finding that GSH peroxidase activity is only about 60% of that in normal liver suggests that the rate of formation of GSSG might also be lower in hepatomas than in the normal liver. Peroxidation in Novikoff hepatoma is lower than in normal liver [11] and the activity of xanthine oxidase is decreased in hepatomas [10]. Lipid hydroperoxides are substrates for GSH peroxidase and are important for GSH oxidation [3,4,12,13]. Xanthine oxidase (via its hydrogen peroxide production) is probably an important enzyme for the catalysis of GSH oxidation [14,15]. The activity of catalase is also lower in hepatomas than in normal liver [16] and therefore some decrease in the competition between this enzyme and GSH peroxidase might occur [4]. However it would appear that the glutathione couple would be more reduced in hepatomas than in normal liver.

The foregoing suggests that the activities of enzymes involved in the formation and utilization of hydroperoxides in hepatomas may be decreased.

The lowered peroxidation associated with the high GSSG reductase and glucose-6-phosphate dehydrogenase activities would increase reduction of GSSG and a lower oxidation of GSH. The consequence of this might be a change of thiol disulphide ratio in the cell to a highly reduced state which might favour the increase of cell division. It appears that a rise in the concentration of any acid-soluble thiol is a pre-requisite for mitosis [1] and therefore oxidoreduction of glutathione and/or degradation of such a thiol [17,18] may be in part responsible for proliferation.

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