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Decreased Activity of Liver Glutathione Peroxidase in Human Hepatocellular Carcinoma*

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Abstract—*Glutathione peroxidase (GSH-Px) activity, one of the scavenger enzymes of oxygen active radicals, has been measured in hepatocellular carcinoma (HCC) of 17 patients and the values compared with the activity of adjacent tumor-free tissue and with those of 30 histologically normal livers. The results demonstrate a reduced GSH-Px activity in neoplastic tissue (21.19 vs 33.74 U/g prot.; P < 0.001). However, the adjacent tumor-free liver also had a reduced activity when compared with normal tissue (23.15 vs 33.74 U/g prot.; P < 0.01), but this value did not differ from that of HCC tissue. These data suggest that HCC might develop in a GSH-Px-deficient condition.*

INTRODUCTION

DURING the evolutive process aerobiosis represents an advantage in terms of energy gain, but exposes the cell to the toxic effect of active radicals of oxygen. Therefore aerobiosis is necessarily accompanied by the development of enzymatic systems deputed to scavenge these radicals in order to protect the cell structures from oxidative injury. Among the critical sites that can be damaged by oxygen, there are lipids of cell membranes [1, 2] and nucleic acid [3]: therefore the result of this attack may be mutagenesis or oncogenesis. The enzymes involved in scavenging of oxygen radicals are: catalase, superoxide dismutase (SOD) and glutathione peroxidase. When the reactive radicals increase, like in senescence [4], the organism responds by increasing the GSH-Px activity [5].

The relationship between the production of active radicals and development of cancer as well as the usefulness of antioxidative factors against the neoplastic process are still debated. Several lines of study support indirect evidence for the relationship between active radicals and carcinogenesis. In experimental tumors, such as hepatoma-27, the activity of GSH-Px is low [6]. Selenium (Se), the catalytic trace element of GSH-Px, seems to have a protecting role against tumor: geographic areas with high Se content in soil have a low incidence of cancer and people with high Se in blood have a lower risk of cancer [7]; on the other hand, a reduced serum Se has been observed in neoplastic patients [8, 9]. Moreover, an interesting correlation has been noted between the Se level in blood and the extent of metastasis, rate of recurrencies and survival time of patients with cancer [10]. Two decades ago Se compounds were proposed as effective agents against experimental tumors [11].

More recently, reduced glutathione, the specific substrate of GSH-Px, caused regression of aflatoxin-induced liver tumor in rats [12].

Since liver is particularly exposed to oxygen-active radicals and consequently has the highest concentration of GSH-Px [13], it seems of particular interest to study the activity of this enzyme in human normal liver and hepatocellular carcinoma (HCC).

MATERIALS AND METHODS

Patients

Seventeen patients (ten males and seven females) with proven diagnosis of HCC were included in this study. The age of patients ranged from 20 to 76 yr (median 56 yr). In five cases HCC was associated with cirrhosis.

After diagnosis of liver tumor and exclusion of extrahepatic metastasis by standard methods, including computed tomography and/or angiography, all patients underwent hemihepatec-

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tomy. None received antiblastic chemotherapy before surgery.

As a control group we studied 30 patients, matched for sex and age, with functionally normal liver in whom a surgical wedge biopsy was performed for diagnostic purposes, after informed consent, during laparotomy for abdominal diseases (gall stones, peptic ulcer).

GSH-Px was assayed on a part of specimen used for histologic examination; in neoplastic patients the enzyme was assayed in both HCC tissue and in the adjacent tumor-free liver. Only patients with normal liver histology were included in the control group.

Enzyme assay

The specimens (100–200 mg), washed out from contaminating blood with ice-cold buffered saline, were suspended in phosphate-buffered saline added with digitonin, 3-aminocaproic acid (0.01 M) and EDTA (0.002 M), and homogenized in a Teflon-coated Potter-Elvehjem homogenizer.

GSH-Px activity was determined in supernatant of fresh liver homogenate (after 1 hr of centrifugation at 50,000 g) according to the method of Günzler *et al.* [14] partially modified using ter-butyl-hydroperoxide as substrate, as described elsewhere [5].

GSH-Px activity was expressed as μmol of NADPH oxidized/min/g protein (U/g prot.). The protein concentration of supernatant was measured with the method of Lowry *et al.* [15].

Statistical analysis

The mean and standard deviation were calculated for each group and comparisons were performed by the analysis of variance. Differences of GSH-Px in tumor and in tumor-free tissues of the same patients were tested using Student's *t* test for paired data.

RESULTS

The mean GSH-Px activity in control liver was 33.74 ± 11.02 (S.D.) U/g prot.; no difference due to sex was noted.

The single values of GSH-Px activity in HCC tissue and in tumor-free liver of our 17 patients are shown in Table 1, together with some data of the patients. In HCC the mean GSH-Px was 21.19 ± 9.93 U/g prot.; the mean GSH-Px activity in tumor-free tissue of the same patients was 23.15 ± 13.10 U/g prot. The analysis of variance showed a statistically significant difference among the three groups ($F = 9.1$; $P < 0.001$). Both HCC tissue and the adjacent tumor-free liver had significantly lower activity than control group. Comparing the activity of GSH-Px in HCC and in tumor-free tissue of the same patient by *t* test for paired data, no significant difference was noted. The mean and standard deviation of the values of enzyme activity in various groups are represented in Fig. 1. In 9/17 supernatants the GSH-Px activity of tumor-free tissue was higher than the activity of the adjacent neoplastic tissue; in eight cases it was lower. Moreover, in both tumor and tumor-free tissues seven values of GSH-Px fell into the normal range of control group, whereas in ten cases the GSH-Px was lower (Table 1).

Table 1. Some clinical data of our patients and values of glutathioneperoxidase activity in hepatocellular carcinoma (HCC) and in adjacent tumor-free tissue

Patient	Sex	Age (yr)	Liver GSH-Px (U/g prot.) HCC	Tumor-free
C G	M	33	12.9	27.8
L A	M	64	16.8	21.3
R T	M	69	34.0	14.9
Z S	M	37	20.5	17.0
S G	M	60	6.3	3.7
T E*	M	71	14.5	6.8
T A*	M	57	15.3	49.9
D G*	M	49	38.7	28.8
C F*	M	66	33.4	16.0
S S*	M	59	8.5	9.5
M G	F	53	12.8	15.5
G M	F	51	37.2	20.3
C F	F	20	19.5	30.1
T O	F	55	23.0	35.8
B M	F	76	26.9	41.5
F A	F	64	24.5	40.4
R C	F	36	15.5	14.3
Mean			21.1	23.1
S.D.			9.9	13.1
Mean control group			33.7 ± 11.02 U/g prot.	
Analysis of variance:			$F = 9.1$	$P < 0.001$

*Patient was also cirrhotic.

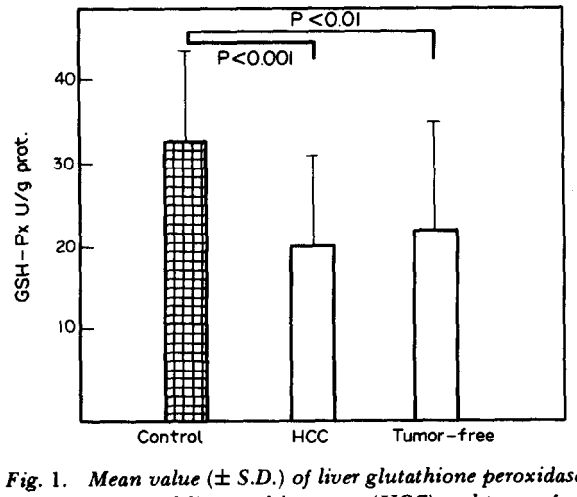

Fig. 1. Mean value (± S.D.) of liver glutathione peroxidase activity in control liver and in tumor (HCC) and tumor-free tissue of the same neoplastic patients.

In five neoplastic tissues associated with cirrhosis GSH-Px activity was similar to that of 12 tumor tissues of patients without cirrhosis (22.09 ± 13.13 vs 20.82 ± 8.95 U/g prot.). Similarly, the enzyme activity in tumor-free liver of neoplastic patients was not influenced by the presence of cirrhosis (22.21 ± 17.84 U/g prot. in cirrhosis vs 24.96 ± 10.60 in non-cirrhotic liver).

DISCUSSION

Much evidence suggests that hepatitis B virus and aflatoxin may be involved in the etiology of liver carcinoma [16]. However, the pathogenetic mechanism of their carcinogenic action is not known. Probably many factors play a role in this process, including the host resistance; this, on the other hand, depends on the immunological, nutritional and biochemical status of the organism.

Our present findings show, for the first time, that GSH-Px activity is low in HCC (Fig. 1), confirming previous reports in experimental carcinogenesis [6, 17]. However,whether the decreased enzyme activity is a cause or a consequence of cancer transformation is impossible to assess from the present data. Dealing with this question, it is of some interest that in our neoplastic patients the mean activity of GSH-Px in adjacent tumor-free tissue is also lower than in normal liver (Table 1). This result can be interpreted in two different ways: (1) the neoplastic wasting might influence not only GSH-Px activity in tumor tissue, but also that of normal adjacent liver; or (2) the low GSH-Px activity might be a pre-existing condition of the liver which favours the neoplastic transformation, impairing the biological resistance of the cell against the oncogenic injury of oxygen radicals. However, while the comparison of the mean values of GSH-Px among the three groups of data is highly significant, so that the interpretation of the results seems relatively simple, the problem appears more complicated when we consider, one by one, the single values of GSH-Px: in seven patients the activity of GSH-Px in tumor-free tissue fell within the normal range; even in seven neoplastic tissues we found a normal activity of GSH-Px. Obviously in both these subgroups we cannot invoke the above hypothesis. Moreover, in nine cases, GSH-Px activity was higher in tumor-free tissue than in tumor, whereas in the remaining eight cases we observed the opposite situation. In other words, our data suggest that, while a low GSH-Px activity may be associated with and perhaps favours the cancer transformation, this is not a necessary condition in all cases, since some of our patients had normal GSH-Px activity in both tumor and tumor-free tissue.

It is otherwise improbable that low GSH-Px is simply a property of rapidly dividing cells, since we found low activity also in tumor-free tissue, while in cirrhosis not associated with HCC, characterized by increased cell regeneration, we found normal activity [18]. Similarly, Oberley *et al.* [19] reported that another scavenger enzyme, Mn-SOD, is decreased in tumor cells of rat liver but not in regenerating liver after partial hepatectomy.

As known, the activity of GSH-Px is due to two different proteins, one of which contains selenium (Se) and the other of which is identifiable with the glutathione transferase B [20]. The method we used measures total GSH-Px activity,using an organic peroxide as substrate. Since in rat and in human liver total GSH-Px is due in greater part to Se-dependent enzyme, we can suppose that a poor Se status is associated with low enzyme activity in the majority of our patients; a marked decrease in liver GSH-Px as a consequence of an Se-deficient diet was demonstrated in animals [20] and a close correlation was found in man between serum Se and erythrocyte GSH-Px [21-24]. On the other hand, an association between high Se in soil and low incidence of cancer has already been observed in man [8]. Recently it has been demonstrated, during chemical hepatocarcinogenesis in rats, that the low activity of GSH-Px is mainly due to the decrease of Se-dependent enzyme [17]. For all of these reasons we can speculate that an Se deficiency, due to previous nutritional factors or to cancer wasting, might be responsible for the low activity of GSH-Px observed in this study. However, since we did not assay Se in liver we cannot completely exclude an inborn enzyme deficiency.

In conclusion, the low GSH-Px activity noted in HCC patients and even in 59% of tumor-free tissue of our patients suggests that nutritional factors (deficit of Se?) may be responsible for the low activity of this enzyme, which may determine a situation of high risk of cancer.

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