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Hepatic and erythrocytic glutathione peroxidase activity in liver diseases

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Hepatic and erythrocytic glutathione peroxidase activity, together with malondialdehyde levels, were determined as indicators of peroxidation in 83 patients from whom liver biopsies had been taken for diagnostic purposes. On histological study, the patients were classified into groups as minimal changes (including normal liver), steatosis, alcoholic hepatitis, hepatic cirrhosis, light to moderately active chronic hepatitis, and severe chronic active hepatitis. The glutathione peroxidase activity in erythrocytes showed no significant changes in any liver disease group. In the hepatic study, an increased activity was observed in steatosis with respect to the minimal changes group, this increased activity induced by the toxic agent in the initial stages of the alcoholic hepatic disease declining as the hepatic damage progressed. There was a negative correlation between the levels of hepatic malondialdehyde and hepatic glutathione peroxidase in subjects with minimal changes. This suggested the existence of an oxidative equilibrium in this group. This equilibrium is broken in the liver disease groups as was manifest in a positive correlation between malondialdehyde and glutathione peroxidase activity.

Key words: Liver diseases, Glutathione peroxidase, Free radicals, Lipoperoxidation, Alcohol.

Free radicals have been implicated in the pathogenesis of cellular damage for multiple processes in different organs (4). In order to protect themselves against the aggression of free radicals, biological systems have certain antioxidant mechanisms at their disposal. Inside the cell, this defence is based around diverse antioxidizing enzymes, one of which is glutathione peroxidase (10).

Glutathione peroxidase has been found in animal tissues, but is not present in the higher plants or bacteria, although it has

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been discovered in some algae and fungi. Its substrate is glutathione, which is present in a reduced form (GSH). This enzyme contains one selenium atom, catalyzing the reduction of hydrogen peroxide originating from mitochondria, the endoplasmic reticulum, or soluble enzymes such as superoxide dismutase (9).

In patients with hepatic cirrhosis, the levels of glutathione peroxidase are diminished (12, 23), and it has been suggested that this could play a possible pathogenic role in the progress of the cirrhosis (22). An increase in hepatic concentration has been reported in animals after acute alcohol ingestion (11), and a decrease with chronic ingestion (15).

The aim of the present work was to establish the alterations produced in liver and erythrocyte levels of GSH-Px in different liver diseases, and their correlation with other tests of hepatic function.

Materials and Methods

The study was performed on 83 patients who had liver biopsies performed for diagnostic purposes in the Internal Medicine Service of the "Hospital Provincial San Sebastián" in Badajoz (Spain), in the period from 1987 to 1989. Indication for biopsy was performed by specialists in the service. In all cases, the patients gave their informed consent.

Sample collection and processing.– Liver tissue samples were taken from the right hepatic lobule by the usual technique of percutaneous needle biopsy with a Tru Cut needle under local anesthetic. Abdominal echography was performed on all patients in the hours previous to the biopsy to choose the point of puncture and to exclude the possibility of cysts, abscesses, or dilatations of intrahepatic bile ducts. The biopsy core was split into two. One fragment was weighed, homogenized in 0.1M pH 7 phosphate buffer and kept deep frozen at –70 °C until the glutathione peroxidase activity and malondialdehyde were determined. The second fragment was fixed in 10% formol for later histological study. Also, before the biopsy was taken, and with the subject fasted, 20 ml of blood was taken from the antecubital vein and divided into two aliquots. One was used for routine biochemical assays. The second was immediately centrifuged, separating the plasma from the erythrocytes which were washed three times with 0.9 % NaCl and hemolyzed with distilled water (1:4). The samples were deep frozen at –70 °C for the later determination of plasma malondialdehyde and erythrocyte glutathione peroxidase activity.

Histological study.– The routine staining techniques were performed of hematoxyline-eosine, PAS, PAS diastase, Masson trichromate, methenamine silver, Shikaba orceine, Perls stain, and IgA. In certain cases, Syrian red stain and immunohistochemical techniques were used for the demonstration of α-fetal protein, α-1-antitripsin, carcinoembryonic antigen, and lysozyme. The sections were studied under optical microscopy. Histological diagnosis and the degree of hepatic injury were determined according to standard criteria. For each sample, a quantitative analysis was made of 20 histological parameters, grading to what degree they were affected on a scale of 0 to 3. Additionally, six qualitative parameters were included referring to the architecture, inclusions or deposits, form of the deposits, and distribution or localization. After histological diagnosis, the patients were grouped as follows: Minimal changes (MIN, n = 10); Steatosis (STE, n = 14); Chronic hepatitis of light to moderate activity (CHM, n = 19); Chronic hepatitis

of severe activity (CHS, n = 10); Alcoholic hepatitis (ALH, n = 14); Hepatic cirrhosis (CIR, n = 16). In studying the liver, the reference group is the one of minimal changes (MIN) because of the methodology of this work, where the liver biopsy is performed on patients with a clinical history that points to a probable hepatic involvement or to other pathologies where a biopsy is indicated. Those patients who, on histological study, showed minimal alterations (diffuse sinusoidal ectasias, non-specific changes and normal liver) form therefore the reference group.

Determinations.– The assays of routine biochemical parameters, including ASAT, ALAT, γ-glutamyl-transpeptidase, cholinesterase, albumin, and gamma globulins, were made with a Hitachi 704 multichannel autoanalyzer.

The glutathione peroxidase activity was determined in erythrocytes and liver by means of the technique based on the spectrophotometric measurement (340 nm) of the disappearance of NADPH which is used up in reducing oxidized glutathione (6).

The levels of malondialdehyde were determined in plasma and hepatic tissue using high-performance liquid chromatography (5) with a 10 cm long, 4.6 mm internal diameter AS-MP Brownlee column, and, as mobile phase, 0.03 M Tris pH 7.4 (1:9) acetonitrile at a flow rate of 1 ml/min. Detection was performed at 270 nm.

The protein concentration in the liver samples was determined by the Coomassie blue method (19). The cyanmetahemoglobin method described by ZIJLSTRA and VAN KAMPEN (24) was used to assay the erythrocyte hemoglobin content.

The results are expressed as mean ± standard error of measurement. Student's *t* test for unpaired samples was applied in the statistical treatment of the data. For certain groups of data, a multivariate correlation analysis was carried out.

Table I. *Values of parameters used as indicators of hepatic damage and of chronic ingestion of alcohol.* Values of chronic alcohol consumption (C. C.), γ-glutamyl-transpeptidase (GGT), mean corpuscular volume (VCM), glutamic oxalacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), albumin (AL), cholinesterase (CHOLI), γ-globulin (GAM), IgA, IgG, and IgM, in subjects with minimal histological changes (MIN), and patients with steatosis (STE), alcoholic hepatitis (ALH), cirrhosis (CIR), chronic viral hepatitis of light to moderate activity (CHM) and of severe activity (CHS).

	MIN (N = 10)	STE (N = 14)	ALH (N = 14)	CIR (N = 16)	CHM (N = 19)	CHS (N = 10)
C.C. (g/24 h)	16±37	133±102 [c]	47±67 [a]	71±95 [c]	155±69	137±107
CGT (U/l)	83±87	122±128	331±369 [c]	169±170	106±117	234±316
VCM (fl)	92±5	96±7	106±9 [c]	101±7 [d]	93±5	96±2
GOT (U/l)	41±17	49±28	112±68 [d]	80±54	93±31 [a]	156±75 [c]
GPT (U/l)	65±37	49±26	81±56	63±43	164±85 [c]	192±126 [d]
AL (g/100 ml)	4.0±0.6	3.8±0.9	3.1±0.8 [d]	3.3±0.7 [d]	4.3±0.5	3.7±1.0
CHOLI (kU/l)	7.1±1.4	7.5±3.6	4.2±2.0 [c]	4.5±2.0 [b]	8.6±1.9	7.7±6.2
GAM (g/100 ml)	1.24±0.31	1.47±0.59	2.14±1.32	2.39±1.39 [d]	1.98±0.88 [d]	2.60±1.16 [d]
IgA (mg/100 ml)	216±122	302±186	614±313 [d]	678±307 [c]	297±165	366±311
IgG (mg/100 ml)	1439±329	1406±658	2314±1098	2256±976	1944±804	2670±1196
IgM (mg/100 ml)	285±293	213±222	282±182	242±172	226±137	523±526

(a) $p < 0.001$; (b) $p < 0.005$; (c) $p < 0.01$; (d) $p < 0.05$.

Results

Table I shows the results for the parameters normally used by clinicians as indicators of hepatic damage as well as other parameters of interest, such as the chronic ingestion of alcohol.

The determination of the erythrocyte GSH-Px activity showed no significant change in the different groups (STE: 24.4 ± 3.5, ALH: 22.3 ± 2.6, CIR: 19.9 ± 3.0, CHM: 22.3 ± 7.0, CHS: 18.8 ± 4.8) with respect to the control group (29.2 ± 3.5). In the liver, there was only a significant ($p < 0.05$) rise in the enzyme's activity in steatosis (0.94 ± 0.16). In the rest of the liver disease groups, the activity was high (ALH: 0.55 ± 0.25, CIR: 0.57 ± 0.22, CHM: 0.59 ± 0.17, CHS: 0.56 ± 0.31) with respect to the control group (0.24 ± 0.04) but not significantly (fig. 1). The values of hepatic glutathione peroxidase were correlated with those of the erythrocyte GSH-Px ($r = 0.6878$, $p < 0.05$) and with those of GPT ($r = 0.7808$, $p < 0.05$) in the patients with alcoholic hepatitis and chronic hepatitis of severe activity, and with GPT in the patients with high degrees of cellular lysis ($r = 0.9748$, $p < 0.001$).

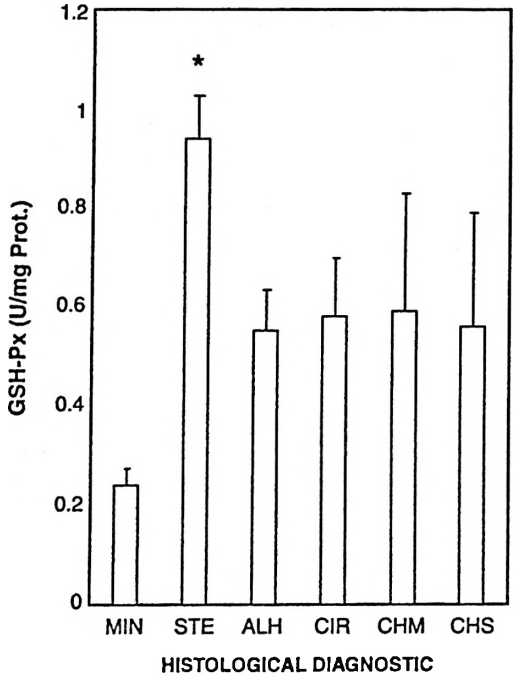

Fig. 1. *Hepatic glutathione peroxidase activity in patients with different liver diseases and in control subjects.*
* $p < 0.005$.

The plasma MDA values (μM) were: MIN; 34.5 ± 12.0, STE; 37.1 ± 5.7, ALH; 29.0 ± 2.8, CIR; 30.9 ± 2.7, CHM; 31.9 ± 2.7, CHS; 37.4 ± 4.5. The hepatic MDA levels (μg/g prot) were: MIN; 0.44 ± 0.19, STE; 1.83 ± 0.28, ALH; 1.79 ± 0.50, CIR; 1.05 ± 0.13, CHM; 1.86 ± 0.44, CHS; 1.65 ± 0.5.

The hepatic MDA levels were significantly positively correlated with the levels of hepatic GSH-Px in the steatosis ($r = 0.7719$, $p < 0.002$), CHM ($r = 0.7479$, $p < 0.002$), CHS ($r = 0.9705$, $p < 0.006$), and ALH ($r = 0.9470$, $p < 0.0001$) groups. No correlation was found in the cirrhosis group. In the case of the group with minimal alterations, the correlation between these two parameters was negative ($r = -0.9488$, $p < 0.0512$).

Discussion

Among the biological parameters of cytolysis, the highest degree of hypertransaminasemia is found in the group with chronic hepatitis of severe activity, followed by the moderate chronic hepatitis and alcoholic hepatitis groups. In cirrhosis, there was a moderate hypertransaminasaemia, and the movement of transaminases was minimal in patients diagnosed with steatosis and in those whose hepatic biopsy classified as normal. These findings concur with the usual descriptions (3, 16).

Regarding the degree of hepatocellular insufficiency, there was a cholinesterase in hepatic cirrhosis as well as in alcoholic hepatitis, corroborating the reported sensitivity of cholinesterase in hepatocellular insufficiency (18).

The immunological syndrome characterizing chronic hepatitis was manifest in the increase in the gamma fraction of the proteinogram in chronic active hepatitis as well as in alcoholic hepatitis and hepatic cirrhosis. The greatest IgG and IgM increases were produced in chronic active hepatitis, and that of IgA in alcoholic hepatitis and alcoholic cirrhosis (18). The highest values of gamma-glutamyltranspeptidase were obtained in patients with alcoholic hepatitis, although major increases were also found in the chronic active hepatitis (CHS) and non-alcoholic cirrhosis groups. A characteristic of this enzyme is its lack of specificity, since it may be elevated in cholestasis as well as in different hepatic and extra-hepatic diseases, and may be induced by ethanol and various drugs (8). The increase in mean corpuscular volume, that is seen in alcoholic hepatic diseases, has been used as an indicator of alcohol ingestion, but it also lacks of specificity, suffering alterations in other liver diseases, in folate deficiency, and in thyroid diseases, thus reducing its capacity to detect small alcohol ingestion rates.

The biochemical results, considered as biological parameters of cell lysis (ASAT, ALAT), hepatocellular insufficiency (albumin, cholinesterase), immunological alterations, and indicators of alcohol ingestion, fit those usually described for each of the nosological entities (3, 16, 18) lending support to the validity of the separation into groups that is herein presented.

In the present study, the levels of hepatic GSH-Px were lower in subjects with minimal changes than in the other groups. This fact could not be considered as a sign of oxidative damage, however, since the negative correlation between hepatic MDA and hepatic GSH-Px in this group points to a maintenance of oxidative equilibrium, altered in the other groups.

In steatosis, a major significant rise was detected in glutathione peroxidase activity. This rise seemed to reflect a response of the hepatic cells to oxidative damage, and the values of this enzyme were strongly correlated with those of malondialdehyde ($r = 0.7719$, $p < 0.005$). The rise in malondialdehyde could not be attributed to any consequence of cell mortality since only two patients in the histological study showed a minimal degree of cell lysis. In the rest of the liver disease groups, a descent was observed in the activity with respect to steatosis, although it was not significant. This fact, together with the augmented lipid peroxidation in these groups (data not shown), could be explained by a selenium deficiency such as has been reported in alcoholic hepatic diseases (1, 20) and in other hepatic diseases (17). Only one earlier study on the liver of animals following acute alcohol ingestion showed increased activity of this enzyme (11), while in the other cases no modifiactions were noted (21). In studies of chronic ingestion, the results are again contradictory. Thus, AYKAC *et al.* describe a rise (2), while MISRA *et al.* observe a fall in the activity of the said enzyme in periportal and pericentral regions of the hepatic lobule (14).

With respect to the erythrocyte GSH-Px activity, we observed a non-significant decline, in the activity in the patients with alcoholic hepatic disease as the disease progressed. Likewise, GSH-Px activity in chronic hepatitis of severe activity (CHS) was less than the values found in chronic hepatitis of light to moderate activity. These declines have been

reported in alcoholic liver disease and chronic active hepatitis (7, 13).

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R. CORDERO, A. ORTIZ, R. HERNÁNDEZ, V. LÓPEZ, M. M. G, GÓMEZ y P. MENA. *Actividad glutation peroxidasa hepática y eritrocitaria en enfermedades hepáticas.* J. Physiol. Biochem. (Rev. esp. Fisiol.), 52 (3), 167-172, 1996.

Se determina la actividad glutation peroxidasa hepática y eritrocitaria y los niveles de malondialdehído, como indicador de peroxidación, en 83 pacientes a los que se les practicó biopsia hepática. Tras el estudio histológico los pacientes se clasifican en cambios mínimos (incluyendo hígado normal), esteatosis, hepatitis alcohólica, cirrosis, hepatitis crónica de actividad leve moderada y de actividad severa. La actividad glutation peroxidasa en eritrocitos no muestra cambios significativos en ningún grupo. En tejido hepático se observa un incremento de la actividad en el grupo de la esteatosis ya en estadios iniciales de la enfermedad hepática alcohólica que disminuye a medida que progresa la lesión hepática. La correlación negativa observada entre los niveles de malondialdehído y glutation peroxidasa hepáticos en sujetos con cambios mínimos sugiere la existencia de un equilibrio oxidativo alterable en las hepatopatías manifestado en una correlación positiva entre el malondialdehído y la actividad glutation peroxidasa.

Palabras clave: Hepatopatías, Glutation peroxidasa, Radicales libres, Lipoperoxidación, Alcohol.

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