Life Sciences, Vol. 43, pp. 991-998 Pergamon Press
Printed in the U.S.A.

HEPATIC GLUTATHIONE CONTENT IN PATIENTS WITH ALCOHOLIC AND NON ALCOHOLIC LIVER DISEASES

Emanuele Altomare, Gianluigi Vendemiale, Ottavio Albano

Istituto di Clinica Medica I, Universita' di Bari, Bari, Italy

(Received in final form August 1, 1988)

Summary

Reduced and oxidized hepatic glutathione was evaluated during alcoholic and non alcoholic liver injury. We studied 35 chronic alcoholics, 20 patients with non alcoholic liver diseases, 15 control subjects. Hepatic glutathione was measured in liver biopsies and correlated with histology and laboratory tests. Alcoholic and non alcoholic patients exhibited a significant decrease of hepatic glutathione compared to control subjects (controls: 4.14 $\pm$ 0.1 μmol/g liver; alcoholics: 2.55 $\pm$ 0.1, $p<0.001$; non alcoholics 2.77 $\pm$ 0.1, $p<0.001$). Oxidized glutathione was significantly higher in the two groups of patients compared to controls (controls: 4.4 $\pm$ 0.2% of total; alcoholics 8.2 $\pm$ 0.3, $p<0.001$; non alcoholics: 8.5 $\pm$ 0.8, $p<0.001$). The decreased hepatic glutathione levels in patients with alcoholic and non alcoholic liver diseases may represent a contributing factor of liver injury and may enhance the risk of toxicity in these patients.

The tripeptide glutathione (gamma-glutamyl-cysteinyl-glycine), considered to be the most important intracellular sulphydryl compound, is known to be involved in a number of structural and functional processes of the cell and to participate in several detoxification reactions (1). Reduced glutathione (GSH) exerts its protective role in the metabolism of several toxic agents, reacting either directly to form adducts with them or as a cosubstrate in conjugation and peroxidation systems (2,3). In addition, the ratio of reduced to oxidized glutathione (GSSG) seems to be critical in regulating protein and enzyme functions and protein synthesis, and the maintenance of the redox state (2). Furthermore, GSH depletion has been associated with enhanced toxicity of many compounds, including carbon tetrachloride, acetaminophen, bromobenzene, quinones, hydroperoxides and benzopyrene (4,5), and with lipid peroxidation (6). We have recently reported a decrease in hepatic GSH concentration in alcoholic patients compared to a selected group of control subjects (7). However, there is still some controversy concerning the hepatic glutathione levels in patients with liver diseases of mixed aetiology (8,9), since some investigators found decreased hepatic GSH levels in patients with abnormal liver function tests and histology (8), whereas others reported unaltered or

0024-3205/88 $3.00 + .00

elevated hepatic GSH concentrations in patients with liver diseases of mixed aetiology (9).

The aim of this study was to evaluate both reduced and oxidized hepatic glutathione content in patients with alcoholic and non alcoholic liver diseases and in a group of appropriate control subjects. We also correlated hepatic glutathione data with liver histology and blood tests of liver functions.

Methods

Seventy consenting patients were included in this study, and divided as follows: 35 chronic alcoholics; 20 patients with non alcoholic liver diseases; 15 control subjects.

Alcoholic Patients (all males, aged 23-55 years):
They conformed to the following criteria of inclusion:
a) history of alcohol abuse of at least 150 g/day, for at least 3 years until hospitalization in our ward;
b) good nutritional status, as assessed by dietary history, lack of weight loss, anthropometric data within normal limits (height, weight, skinfold thickness) normal serum total protein and albumin levels, normal creatinine clearance values;
c) no history of regular ingestion of other drugs during the 3 months prior to admission. Five patients were positive for one or more markers of the hepatitis B virus.

Patients with Non-Alcoholic Liver Disease (12 males, 8 females, aged 24-55 years):
All the following patients consumed only moderate amounts of alcohol (less than 40 g/day and than 3 times/week). Patients were included in this group according to clinical history, biochemical liver function tests and liver histology. The liver was judged histologically abnormal if it presented: steatosis, fibrosis, inflammatory cell infiltrates, cell necrosis or a combination of these. Patients were allocated to the following four diagnostic groups based on the results of microscopic examination: chronic active hepatitis: n=7; chronic persisting hepatitis: n=3; steatosis: n=2; cirrhosis: n=8. 15 patients were positive for one or more markers of the hepatitis B virus.

Control Patients (11 males, 4 females, aged 28-50 years):
This group was selected from patients admitted to the Surgery Department for uncomplicated abdominal procedures: cholecystectomy for stones confined to the gall-bladder (7 patients), excision of echinococcal hepatic cysts (3 patients), diagnostic laparotomy (5 patients). These patients conformed to all the following standards: infrequent and minor alcohol consumption (<40 g/day and < 3 times/week); no history of other regular drug consumption; good nutritional status as defined above; normal liver function tests and histology; no other major active pathologies of liver or other organs; two

patients were HBsAg positive.
All the patients included in the study showed a body weight within 10% of their respective ideal body weight. In the days preceding liver biopsy, patients were administered 25 Kcal/Kg of ideal body weight, divided as follows: 45% as carbohydrates, 30% as lipids, 25% as proteins.

Liver Biopsy:

All the patients with liver disease (alcoholics and non-alcoholics) underwent Menghini biopsy (during laparoscopy or guided by ultrasonography) for diagnostic or control purposes, 3-4 days after admission. The procedure was performed at mid-morning after an overnight fast. Surgical patients representing the control group, underwent mid-morning abdominal surgery after overnight fasting. Anaesthesia procedure was standardized and included administration of diazepam, perphenazine, droperidol, fentanyl, succinyl choline and nitrous oxide. Small wedge liver biopsies (<150 mg) were obtained as soon as the peritoneal cavity was opened, in order to minimize the possible effects of anaesthesia and surgical trauma, which have been reported to increase with the duration of surgery (10). All liver samples were immediately divided into 2 parts, one for histological study, the other for glutathione estimation.

Hepatic Glutathione and Protein Determination:

Liver samples were washed for a few seconds in isotonic saline solution to remove blood and rapidly placed in ice-cold Krebs-Henseleit buffer (pH=7.4). Samples were either processed immediately or stored at -20°C.
In agreement with other observations (9), we have previously confirmed that GSH and GSSG concentration is not affected by a short-term freezing (7 days) or sample size (7).
The liver tissue was homogenized in ice-cold Krebs buffer and the homogenate was treated with 5% v/v concentrated perchloric acid for protein precipitation. The sample was centrifuged at 10000 x g for 10 minutes at 2 C and the supernatant retained. Hepatic GSH and GSSG were determined by high performance liquid chromatography (HPLC) according to the method of Reed et al.(11) using a Beckman 342 gradient liquid chromatograph equipped with an ultrasil-NH2 column. Results were calculated relative to peak areas of freshly prepared standards. The recovery of GSH and GSSG standards added to liver homogenate was 97 $\pm$ 6% and 98 $\pm$ 5% respectively. Protein content of the homogenates was assessed by the Lowry method (12).

Blood Tests of Liver Function:

Biochemical blood tests were performed by the clinical laboratory using standard assay kits (Boehringer Mannnheim GmbH).

Statistics:

Data were analyzed by the student group T-test or by regression coefficient.

Results are expressed as mean ± S.E.M..

Results

The total hepatic glutathione content of patients with alcoholic and non-alcoholic liver disease was found to be significantly decreased in respect to a selected group of controls (controls: 4.14 ± 0.1 μmol/g liver; alcoholics: 2.55 ± 0.1, $p<0.001$; non-alcoholics: 2.77 ± 0.1, $p<0.001$) whereas no difference was observed between the two groups of patients with liver disease (Table 1). The same significant difference in GSH levels between patients and controls was observed when glutathione was expressed as μmol/g liver protein (controls: 24.2 ± 1.1; alcoholics: 14.5 + 0.9, $p<0.001$; non-alcoholics: 14.1 ± 1.5, $p<0.001$).
Although GSSG concentration was comparable in the three groups, alcoholic and non-alcoholic patients showed higher GSSG values, compared to controls, when expressed as percentage of total GSH (Table 1).

TABLE I

Hepatic Values of Total (GSH) and Oxidized Glutathione (GSSG) in the Three Groups of Patients

	GSH (μmol/g liver)	GSSG (μmol/g liver)	GSSG (% of total)
CONTROLS (15)	4.14 ± 0.1	0.18 ± .01	4.4 ± 0.2
ALCOHOLICS (35)	2.55 ± 0.1*	0.20 ± .01	8.2 ± 0.3*
NON ALCOHOLICS (20)	2.77 ± 0.1*	0.23 ± .02	8.5 ± 0.8*

GSH and GSSG were measured by HPLC as described in "Methods". The number of patients in each group is shown in parentheses. Values are expressed as mean ± SEM
* $p < 0.001$ when comparated to control patients.

No significant correlations were found between total hepatic GSH concentration and any of the blood tests indicative of liver function in both alcoholic and non alcoholic patients. The glutathione content was found to be unaltered also

among the patients positive for the hepatitis B virus, relative to their own groups.

Since in the alcohol group many patients assessed to be free of advanced histological alterations or abnormal liver function tests, the relationship between GSH content and the histological or biochemical pattern was investigated by dividing patients into 4 subgroups on the basis of the degree of microscopic alterations and the presence/absence of biochemical tests abnormalities .

The four subgroups were arranged as follows:

a) Based on the histology report: 12 patients with no changes or steatosis only; 23 patients with advanced lesions (hepatitis, fibrosis, cirrhosis);

b) Based on blood tests values: 11 patients with normal biochemical tests; 24 patients with abnormal biochemical tests.

No significant difference was found in the subgroups for glutathione concentration.(Tab.II).

TABLE II

Total Hepatic GSH in Subgroups of Alcoholic Patients divided on the basis of:
1) Degree of Histologic Alterations;
2) Presence/Absence of Biochemical Tests Abnormalities.

			GSH (μmol/g liver)	
LIVER HISTOLOGY	No change or steatosis only	(12)	2.4 ± 0.2	ns
	Advanced lesions (hepatitis-fibrosis-cirrhosis)	(23)	2.3 ± 0.1	
LIVER FUNCTION	Normal	(11)	2.8 ± 0.2	ns
	Abnormal	(24)	2.4 ± 0.1	

Values are expressed as mean ± SEM. The number of patients in each group is reported in parentheses.

The liver GSH was already below the normal range even in patients with no abnormalities in the biochemical tests or with no major histological alterations, which confirms that GSH is a poor index for discrimination of mild versus advanced alcohol induced liver injury whereas it may represent an early marker of liver impairment (7). Since in the non-alcohol group no patients exhibited biochemical tests within normal limits, the GSH values were also grouped on the basis of the degree of histological abnormalities and the

results are reported in Fig. 1.

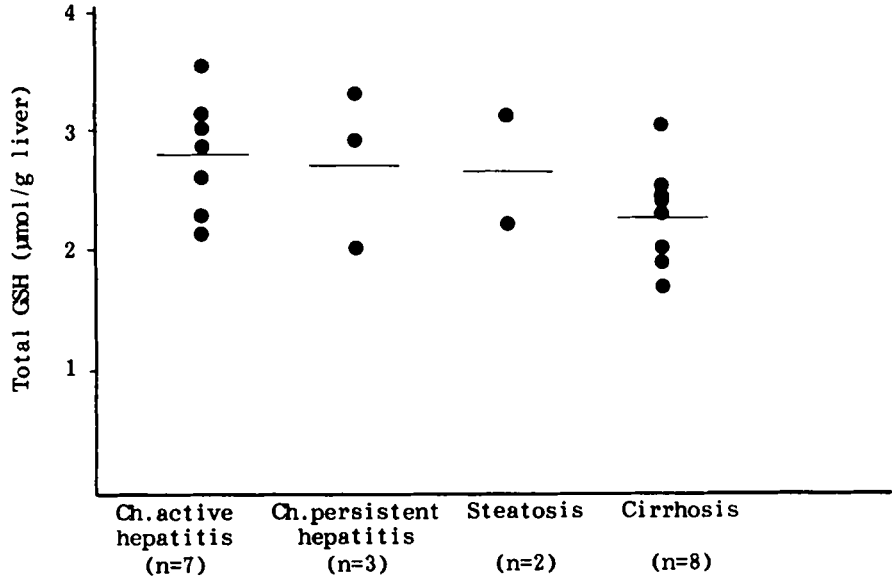

Fig.1
Total hepatic GSH concentrations in patients with non alcoholic liver disease. The bars represent the mean value of each subgroup.

No major differences were found in GSH levels among these groups, confirming the lack of relationship between GSH concentration and the progression of liver disease, also in patients with non alcoholic liver diseases. Small sample sizes of these subgroups, however, require consideration.

Discussion

While there is abundant literature on liver glutathione in animal species, relatively little work has been carried out on hepatic glutathione in humans. Furthermore, in the available data some discrepancies among these studies are reported. In control subjects Bonkowsky et al. (13) and Selden et al. (14) reported hepatic GSH values of 13.30 and 2.75 nmol/mg liver protein, respectively. The marked difference between these two studies may pertain to the criteria for selection of control patients. Other investigators (6) reported decreased hepatic GSH levels in alcoholics compared to non alcoholics with liver disease, although no comparison was made to a control group, whereas Poulsen et al (9) found unaltered or elevated GSH concentrations in patients with liver diseases of mixed aetiology. In that study, however, no real control group and no clear distinctions between alcoholic and non alcoholic patients were reported. In our present study we observed decreased

hepatic GSH levels in both alcoholic and non alcoholic patients with liver disease in respect to a selected group of control subjects with no alterations of liver function tests and histology. Even if GSSG concentrations were comparable in the three groups studied, alcoholic and non alcoholic patients showed higher levels of GSSG (as percentage of total) compared to controls. This may be of interest since it has been shown that excess of oxidized glutathione (as would occur with reduction of peroxides or with a change in the redox potential) leads to the interaction of GSSG with protein-SH groups, forming mixed disulfides (15), which in turn may alter a variety of cell function, including enzyme function, protein synthesis, cell integrity, microtubular function, transport processes and release mechanisms (2,16). In a previous report (7) we found a marked decrease of hepatic glutathione levels in alcoholics even in absence of other clear signs of alcoholic liver damage; it was inferred that hepatic GSH is a poor index for discrimination between the mildly versus more seriously affected subjects. In the present study the finding of low hepatic GSH levels in non alcoholic patients with severe as well as mild histological abnormalities, suggest a lack of relationship between GSH concentration and the progression of liver injury also in patients with non alcoholic liver disease.

Our data also show no correlation between hepatic GSH levels and blood parameters both in alcoholic and non alcoholic patients. The decrease in GSH, during alcohol consumption, could be due to either non-specific systemic toxicity or other modulators of GSH turnover, such as other hormones (17). Another possible mechanism is based on the antioxidant properties of GSH: acute and chronic ethanol intoxications result in increased free radicals and lipid peroxide formation (18,19) which favor GSH consumption and oxidation to GSSG by glutathione peroxidase (20). In addition, other non alcohol related mechanisms could lead to GSH depletion including an impairment of transulfuration pathway during cirrhosis. It has been well established, indeed, that methionine can provide a significant source of sulfur incorporated into GSH (21) through S-adenosyl-methionine (SAM) and cysteine formation via the cystathionine pathway. Horowitz et al (22) observed an impaired clearance of a methionine load, in cirrhotic patients compared to controls. In that study, the absence of accumulation of intermediates of the pathway in plasma and urines, suggested that the rate-limiting block is early in these patients, most likely in the formation or use of SAM. This hypothesis has been recently confirmed by other investigators (23) who reported a significant decrease of SAM-synthetase activity both in alcoholic and post-hepatitic cirrhotic patients compared to controls. It is therefore reasonable to speculate that the reported reduction of hepatic GSH levels in patients with chronic liver disease could be the result of several ethanol and non ethanol mediated mechanisms, such as consumption in detoxification reactions and impairment of transulfuration pathway.

Since glutathione is a vital substance in detoxification and cell physiology, its depletion may represent an important contributing factor of liver injury and may enhance the risk of toxicity in these patients.

AKNOWLEDGEMENT

The authors wish to thank Mrs. A. D'Amico for the helpful collaboration in revising the manuscript.

References

1) MEISTER A. The Liver:Biology and Pathobiology. I.ARIAS, H. POPPER, D. SCHACHTER, D.A. SHAFRITZ, eds, p. 297-308, Raven Press, New York, (1982).
2) N.S. KOSOWER and E.M. KOSOWER. Int. Rev. Cytol. 54 109-160 (1978).
3) N. KAPLOWITZ. Am. J. Physiol. 239 439-444. (1980).
4) D.J. REED, and P.W. BEATTY. Rev. Biochem. Toxicol. 2 213-242 (1980).
5) P. HOLDENS and B. JERNSTROM. Functions of Glutathione. A. LARSSON, S. ORRENIUS, A. HOLMGREN, B. MANNERVIK, eds, p. 99-108, Raven Press, New York, (1983).
6) S. SHAW, K.P. RUBIN and C.S. LIEBER. Dig. Dis. Sci. 28 585-589 (1983).
7) S.A. JEWELL, D. DI MONTE, A. GENTILE, A. GUGLIELMI, E. ALTOMARE and O. ALBANO. J. Hepatol. 3 1-6 (1986).
8) E.C.P SHI, R. FISHER, M. Mc EVOY, R. VANTOL, M. ROSE and J.M. HAM. Clin. Sci. 66 279-283 (1984).
9) H.E. POULSEN, L. RANEK and P.B. ANDREASEN. Scand. J. Clin. Lab. Invest. 41 573-576 (1981).
10) Y.A. EDLUND and L.S. ZETTERGREN. Acta Chir. Scand. 113 201-210 (1957).
11) D.J. REED, J.R. BABSON, P.W. BEATTY, A.E. BRODIE, W.W. ELLIS, and W. POTTER. Anal. Biochem. 106 55-62 (1980).
12) O.H. LOWRY, N.J. ROSEBROUGH, A.L. FARR, and R.J. RANDALL. J. Biol. Chem. 193 265-275 (1951).
13) H.L. BONKOWSKY, G.H. MUDGE and R.J. Mc MURTRY. Lancet i 1016-1018 (1978).
14) C. SELDEN, C.A. SEYMOUR and T.J. PETERS. Clin. Sci. 58 211-219 (1980).
15) J. ISAACS and F. BINKLEY. Biochim. Biophys. Acta 497 192-204 (1977).
16) J. OLIVER, D.F. ALBERTINI and R.D.BERLIN. J. Cell. Biol. 75 921-932 (1976).
17) J.C. FERNANDEZ-CHECA, M. OOKHTENS and N. KAPLOWITZ. J. Clin. Invest. 80 57-62 (1987).
18) R.C. REITZ. Biochim. Biophys. Acta 380 145-154 (1975).
19) L.A. VIDELA, V. FERNANDEZ, G. UGARTE and A. VALENZUELA. Febs Lett. 11 6-10 (1980).
20) L. FLOHE', W.A. GUNZLER, R. LADENSTEIN. Glutathione: Metabolism and Function. I.M. Arias, W.B. Jakoby, eds., p. 115-135, Raven Press, New York, (1976).
21) D.J. REED and S. ORRENIUS. Biochem. Biophys. Res. Commun. 77 1257-1264 (1977).
22) J.H. HOROWITZ, E.B. RYPINS, J.M. HENDERSON, S.B. HEYMSFIELD, S.D. MOFFITT, R.P. BAIN, R.K. CHAWLA, J.C. BLEIER and D. RUDMAN. Gastroenterology 81 668-675 (1981).
23) A.M. DUCE, P. ORTIZ, C. CABRERO and J.M. MATO. Hepatology 8 65-68 (1988)