

The hepatic glutathione content in liver diseases

H. E. POULSEN, L. RANEK & P. B. ANDREASEN

Medical Department A, Rigshospitalet, Copenhagen, Denmark

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We measured the total glutathione content in 38 liver biopsies from patients undergoing diagnostic liver biopsy to study whether liver diseases result in decreased glutathione content making the liver more sensitive to different toxic damages.

The glutathione concentrations ranged from 20.2 to 41.0 $\mu\text{mol/g}$ hepatic protein in six biopsies without light microscopic pathological changes (mean \pm SD = 26.9 ± 8.1). The mean concentrations \pm SD in patients with toxic hepatitis ($n = 3$), viral hepatitis ($n = 4$), chronic active hepatitis ($n = 4$), cirrhosis ($n = 14$) and steatosis ($n = 7$) were 62.5 ± 27.2 , 47.4 ± 25.9 , 38.3 ± 17.0 , 29.1 ± 15.7 and 21.0 ± 9.6 , respectively.

The hepatic glutathione content is not decreased in patients with moderate hepatic impairment.

Key-words: glutathione; liver diseases

Henrik Enghusen Poulsen, M.D., Medical Department A 2152, Rigshospitalet, 9 Blegdamsvej, DK-2100 Copenhagen Ø, Denmark

Glutathione (γ -L-glutamyl-L-cysteinylglycine) is a ubiquitous intracellular tripeptide. The liver has one of the highest concentrations of glutathione, 15-30 $\mu\text{mol/g}$ liver protein [3, 15]. Most of the glutathione is in the reduced form (GSH) and only a small amount of oxidized glutathione (GSSG) is found.

There is still much uncertainty concerning the physiological role of GSH. It is known to be the catalytic cofactor of hepatic enzymes producing vitamin K dependent coagulation

factors [7] and to act as a carrier in amino acid transportation over membranes [8, 9].

GSH protects cells from oxidative damage either as a free radical scavenger [14] or by elimination of peroxides by acting as a substrate for glutathione peroxidase [1, 4, 12].

In acute liver damage, due to massive overdose of paracetamol, GSH plays an important role as the toxic metabolite of paracetamol is detoxified by hepatic conjugation with GSH and subsequently excreted as paracetamol-mercapturate in the urine. If the liver tissue is depleted of GSH the metabolite may cause hepatic necrosis [11].

However, little is known of GSH function in man. So far only one paper has been published on the concentration in human liver tissue [2]. We have estimated the concentration of GSH in human liver tissue and investigated whether the glutathione concentration is changed in liver diseases.

MATERIALS AND METHODS

The material consisted of liver biopsies from 40 consecutive patients, 20 females and 20 males, who underwent Menghini biopsy for diagnostic or control purposes. The patients were fasted overnight and the biopsies were performed between 9 and 10 a.m.

Routine biochemical blood tests included prothrombin index, bilirubin, alanine amino transferase, alkaline phosphatases and albumin. In 17 patients the galactose elimination was determined [16]. One patient was excluded because the biopsy contained no liver tissue, and another because the patient received parenteral nutrition.

Patients were allocated to six diagnostic groups based on clinical history, biochemical data and liver histology.

Six patients with normal liver histology and only minor abnormalities of the biochemical tests were considered a control group.

The rest of the patients were grouped as follows: *Toxic hepatitis*: one halothane, one disulfiram, one paracetamol-induced hepatitis. *Viral hepatitis*: three type B, one type non-A non-B. *Chronic active hepatitis*: four. *Cirrhosis*: primary biliary (seven), alcoholic (two), cryptogenic (five). *Steatosis*: seven.

The patients with halothane, disulfiram and paracetamol-induced hepatitis had the biopsy performed 16, 7 and 7 days after admission to our department, respectively, which in each case was shortly after exposure. The patients with viral hepatitis had the biopsy performed shortly after admission, i.e. as soon as the coagulation status of the patient allowed. In patients with chronic liver disease, the duration of the disease is so uncertain that it cannot be used.

Glutathione analysis. The liver tissue obtained on biopsy was washed for a few seconds in isotonic saline to remove blood. A piece of the biopsy (range 1.7–12.3 mg) was cut off for

GSH estimation and the residue was fixed in formaldehyde for subsequent paraffin embedding, cutting and staining.

The GSH analysis was made according to Tietze [15] with the following modifications. The tissue was weighed and homogenized by ultrasound in 3 ml 10% trichloroacetic acid–9.92 mol/l hydrochloric acid and centrifuged for 10 min at 1000 g.

The supernatant was divided into two parts. One was washed five times in ice-cold diethyl ether and 50–150 μ l was used to determine the content of total glutathione. The other part of the supernatant was used to determine the protein content [6] using bovine albumin as standard.

Storage of biopsy or supernatant frozen at -22°C showed no change in GSH concentration after seven days but was reduced by 10% after 2 weeks.

The recovery of GSH (4.07–16.3 μ mol/ml homogenate added to rat liver homogenate) was $97 \pm 6.5\%$ (SD).

The correlation between biopsy weight and the GSH concentration in the 38 samples was $r^2 = 0.02$.

Statistical analysis. All the groups were tested simultaneously with a Kruskal Wallis one-way analysis of variance, each group was then tested versus the control group with a Mann–Whitney *U*-test. For correlation the Spearman rank correlation coefficient was calculated. *P* values below 0.05 were considered statistically significant.

RESULTS

The six groups were comparable with regard to sex and age except for the group with acute viral hepatitis where the patients were younger. The mean GSH concentrations \pm SD in the patients with toxic hepatitis, viral hepatitis, chronic active hepatitis, cirrhosis and steatosis were 62.5 ± 27.2 , 47.4 ± 25.9 , 38.3 ± 17.0 , 29.1 ± 15.7 and 21.0 ± 9.6 , respectively, while that of the control group was 26.9 ± 8.1 .

The concentrations of GSH in the toxic hepatitis group were (μ mol/g): halothane hepatitis, 41.7; disulfiram hepatitis, 93.4 and paracetamol hepatitis, 52.7.

The hepatic total glutathione contents (μ mol per g protein) are shown in Fig. 1.

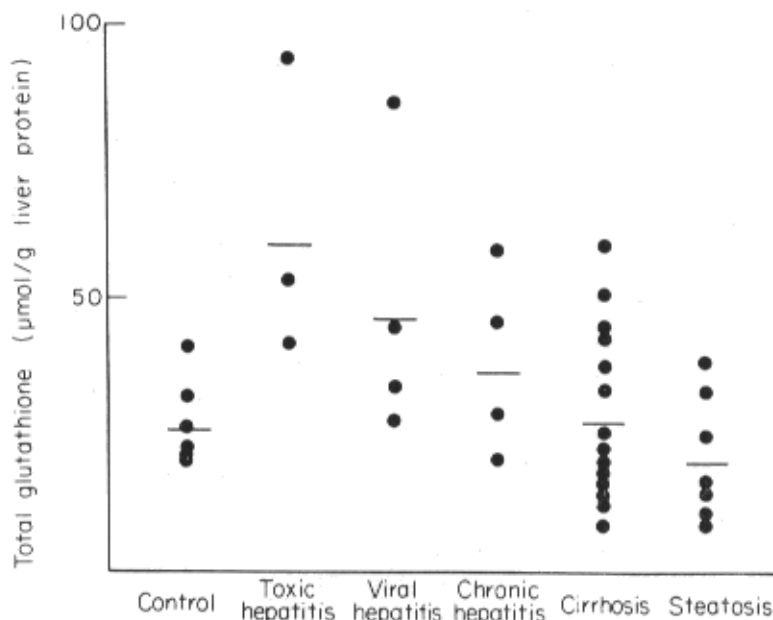


FIG. 1. Hepatic glutathione concentrations ($\mu\text{mol/g}$ liver protein) in patients with liver diseases and controls. The bars indicate the mean value of each group.

The mean levels of the groups were significantly different ($P < 0.05$) and the patients with toxic hepatitis and acute viral hepatitis had significantly higher concentrations than the control group, $P < 0.02$ and $P < 0.03$, respectively. There was no correlation between the prothrombin index or the serum alanine aminotransferase or the galactose elimination capacity and the hepatic concentration of glutathione.

DISCUSSION

This study shows that normal human liver contains glutathione in a concentration of 20–40 $\mu\text{mol/g}$ liver protein. None of the examined patients with liver disease had decreased GSH concentration in the liver and patients with acute toxic and viral hepatitis had elevated concentrations.

Glutathione is also found in erythrocytes but since the biopsies were washed to remove blood and since the concentration of GSH in blood is lower than in the liver [3] there is no reason to believe that the elevated values are due to blood contamination of glutathione.

The only other study on GSH concentration in human liver disease [2] demonstrated

concentrations 50% lower than ours in the one patient investigated as well as in the control group. However, both the method of estimation and the selection of the control group differ. By use of our method we have found glutathione levels in rat liver [10] similar to those reported by others. Human liver tissue from healthy individuals cannot be obtained for ethical reasons and consequently the controls represent a biased sample.

The two- to three-fold elevation found in acute liver disease, i.e. toxic hepatitis and viral hepatitis, was an unexpected finding especially in the patient with paracetamol-induced liver damage as the fall in hepatic glutathione is thought [11] to be an obligatory part of the hepatotoxic mechanism. However, GSH depletion after paracetamol overdose causes a GSH overshoot during the recovery phase [13] in rats and since the patient had the biopsy performed during convalescence the high value probably reflects a similar phenomenon. Whether glutathione is depleted initially in other toxic and viral liver damage in man remains to be investigated.

In the chronic liver diseases investigated in this study, i.e. chronic hepatitis, cirrhosis and steatosis, we have found GSH concentrations

identical to the control values. This is in accordance with the observation that GSH conjugation to paracetamol, in therapeutic doses, is unchanged in patients with cirrhosis [5].

The concentration of glutathione in liver was independent of liver function since it did not correlate to serum alanine aminotransferase or to the more quantitative measures of liver function: prothrombin index and galactose elimination capacity.

We conclude that the glutathione related detoxification mechanisms seem to be intact in patients with moderate liver disease. However, kinetic studies of the *de novo* synthesis of GSH have not yet been performed in patients with liver disease. If the capacity for synthesis is compromised, patients with liver disease may be more susceptible to toxic injury in spite of the normal or even elevated GSH concentrations demonstrated in this study.

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