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Liver and biliary

Glutathione deficiency in alcoholics: risk factor for paracetamol hepatotoxicity

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SUMMARY **Patients chronically abusing ethanol are more susceptible to the hepatotoxic effects of paracetamol. This could be due to an increased activation of the drug to a toxic metabolite or to a decreased capacity to detoxify the toxic metabolite by conjugation with glutathione (GSH). To test these hypotheses paracetamol 2 g was administered to five chronic alcoholics without clinical evidence of alcoholic liver disease and five control subjects. The urinary excretion of cysteine- plus N-acetyl-cysteine-paracetamol, the two major products of detoxification of the reactive metabolite of paracetamol, was not significantly higher in chronic alcoholics arguing against a substantially increased metabolic activation of paracetamol. Chronic alcoholics had significantly lower plasma concentrations of GSH than healthy volunteers, however (4·35 (1·89) μM *v* 8·48 (2·68) μM, $p<0·05$) before the administration of paracetamol, and plasma GSH reached lower concentrations in the alcoholics after paracetamol (2·40 (1·36) *v* 6·26 (2·96) μM). In a group of patients with alcoholic hepatitis intrahepatic GSH was significantly lower than in patients with chronic persistent hepatitis and patients with non-alcoholic cirrhosis, suggesting that low plasma GSH in alcoholics reflects low hepatic concentrations of GSH. The data indicate that low GSH may be a risk factor for paracetamol hepatotoxicity in alcoholics because a lower dose of paracetamol will be necessary to deplete GSH below the critical threshold concentration where hepatocellular necrosis starts to occur.**

Patients chronically abusing ethanol are more susceptible to the hepatotoxic effects of paracetamol such that relatively small doses of the analgesic may result in severe hepatic damage.[1 2] Animal studies have shown that the toxicity of paracetamol arises as a consequence of the metabolic activation of the drug by the cytochrome P-450 system to a reactive metabolite.[3] This metabolite is detoxified by intracellular glutathione (GSH), eventually resulting in cysteine- and N-acetylcysteine-paracetamol which are excreted in urine. Hepatocellular necrosis ensues when the capacity of the liver to resynthesize the consumed GSH and thus its capacity to detoxify the reactive metabolite is exceeded.[4] We have recently shown that even therapeutic doses of the analgesic markedly stimulate GSH turnover in man,[5] but no direct evidence that paracetamol ingestion depletes hepatic stores of GSH has been obtained in man. Because repeat liver biopsies are not feasible in man the effect of a drug on the hepatic GSH status is difficult to assess. Based on data obtained in experimental animals,[6 7] however, plasma GSH may reflect the intrahepatic concentration of GSH.

In view of the pathogenesis of paracetamol induced liver injury an increased formation of the toxic metabolite and/or a decreased detoxification could both account for the increased susceptibility of chronic alcoholics. Chronic ethanol consumption induces some cytochrome P-450- mediated processes in man,[8 9] and this could result in an increased formation of the toxic metabolite and increased toxicity. On the other hand, a defective regulation of hepatic GSH homeostasis might decrease the capacity of chronic alcoholics to detoxify the toxic metabolite of paracetamol.

The aim of this study was to test these hypotheses by measuring plasma GSH as an index of hepatic GSH and the formation of the toxic metabolite after a

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non-toxic dose of paracetamol in patients chronically abusing ethanol.

Methods

SUBJECTS

In the first part of the study plasma GSH was determined after an overnight fast in chronic alcoholics and control subjects. Male alcoholics were recruited from an alcohol treatment programme. They all had been drinking heavily (estimated average consumption of 180 g ethanol/day) up to two days before the study which was done on the second day of their hospitalisation. The patients were not receiving any medication except for chlorodiazepoxide in some cases (the last dose of 10 mg more than 10 hours before the study). This benzodiazepine is not conjugated with glutathione and is not known to induce drug metabolism within 48 hours of starting treatment. On physical examination none of the patients showed clinical evidence for alcoholic liver disease. All subjects had a normal serum albumin and serum bilirubin except for one whose bilirubin was 34 μmol/l. Serum glutamic oxaloacetic transaminase was less than twice the upper limit of normal in all subjects except for two with 2·5 and 3·5 times raised values. Normal, healthy men served as controls. They denied consumption of ethanol in excess of 10 g/day and were not taking any medication.

In the second part of the study hepatic tissue was obtained from patients with alcoholic hepatitis undergoing percutaneous liver biopsy (Table 1) to document that low plasma GSH in alcoholics reflects low hepatic GSH. No medical indication for a liver biopsy was given in the subjects participating in the first and second part of the study. Plasma GSH was not measured in the biopsied patients because the equipment necessary for the rapid derivatisation of plasma sulphydryls was not available on the wards. Patients with chronic persistent hepatitis and non-alcoholic cirrhosis (cirrhosis as a result of chronic active liver disease, cryptogenic cirrhosis) who had a percutaneous biopsy for diagnostic purposes, and patients without liver disease undergoing open biopsy at the time of cholecystectomy provided control values for intrahepatic GSH.

Table 1 *Concentration of glutathione in liver of patients with alcoholic hepatitis*

Patient	*GSH/ g liver μmol*	*GSH/ mg prot nmol*	*GOT U/l*	*GPT U/l*	*Albumin g/l*	*Bilirubin μM*	*Alk phos U/l*
			<40	<40		<21	<115
1	0·58	13·3	235	25	38	325	122
2*	0·81	11·0	130	11	26	53	214
3	0·86	10·4	222	71	27	63	355
4	1·15	8·0	99	15	34	49	475
5*	1·34	24·7	127	16	26	20	322
6*	1·59	12·7	58	19	25	53	268
7*	1·78	21·8	85	69	26	23	168
8*	2·28	17·8	50	32	26	20	120

*Histology showed cirrhosis in addition to alcoholic hepatitis.

In the third part of the study the effect of paracetamol on plasma GSH was determined in five alcoholics and five healthy volunteers. A heparin lock was placed in a vein of the forearm for repeat blood sampling. The subjects who had been fasting for 10 hours then received 2 g paracetamol dissolved in lemonade between 8 and 9 am, and blood was obtained at hourly intervals for four hours for the determination of plasma GSH. Pilot studies had shown that plasma GSH varies by less than 10% over four hours. In order to estimate the metabolic activition of paracetamol to the toxic metabolite, urine was collected for six hours.

The study was approved by the Institutional Review Board and all subjects gave informed consent to participate.

ANALYTICAL METHODS

Blood was immediately centrifuged at 3000 g for two minutes. None of the samples was visibly haemolysed. Within three minutes of collection 200 μl plasma were added to 20 μl 5 mM monobromobimane in acetonitrile and penicillamine was added as an internal standard. After three minutes proteins were precipitated with 40 μl 20% perchloric acid. After centrifugation the protein free supernatant was analysed by reverse phase high performance liquid chromatography and fluorometric detection[10] using 0·005 M octanesulphonic acid/1% acetic acid/5% acetonitrile as the mobile phase. Standard curves were obtained each day by spiking plasma samples with known amounts of cysteine and GSH.

Urinary metabolites of paracetamol were measured by high performance liquid chromatography as described by Corcoran *et al.*[11] Glutathione in liver biopsies was measured by the method of Tietze.[12]

Because the hypothesis that the values of plasma GSH were normally distributed could not be rejected at the 95% confidence level, the statistical analysis of the group means of the time course study of plasma GSH was done by paired *t*-test, and statistical differences between alcoholics and controls were assessed by 2-sided *t*-test.

Results

As shown in Figure 1 the plasma concentration of

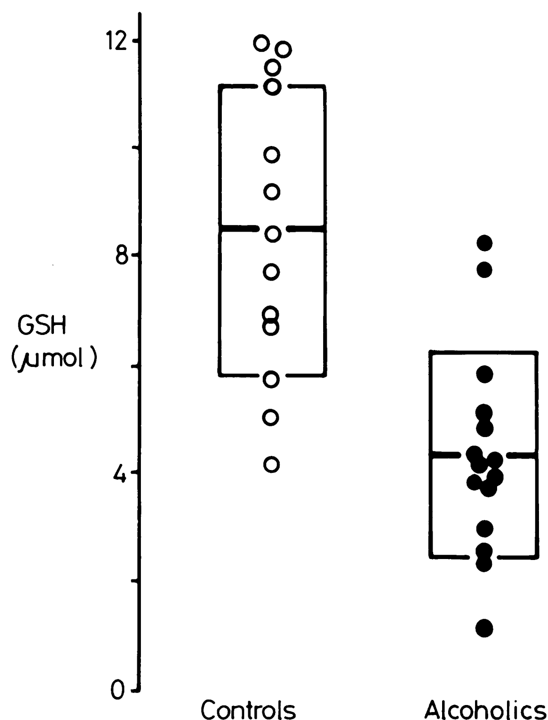

Fig. 1 *Concentration of glutathione in plasma in chronic alcoholics and healthy volunteers. Mean (SD) indicated by bars.*

GSH was significantly ($p<0.05$) lower in alcoholics (4·35 (1·89) µM, mean (SD)) than in healthy volunteers (8·48 (2·68) µM). The values in the two patients with biochemical evidence for alcoholic liver disease were comparable with the other patients. In contrast to GSH, the plasma concentration of free cysteine was similar in the alcoholics (9·71 (2·42) µM) and the controls (9·46 (2·29) µM).

To test the hypothesis that low plasma GSH might reflect low intrahepatic GSH as it does in experimental animals,[6] hepatic GSH was measured in a group of alcoholic patients in whom a percutaneous liver biopsy was indicated for medical reasons. The biopsied patients all had histological evidence of alcoholic hepatitis with and without cirrhosis, and as a group had more severe liver disease (Table 1) than the alcoholic subjects in whom plasma GSH was measured. The hepatic concentration of GSH in these alcoholic patients was significantly lower than in patients without liver disease and patients with a mild inflammatory process or non-alcoholic cirrhosis (Table 2). In the four patients with GOT activities of less than 2·5 times the upper limit of normal the hepatic concentration of GSH per mg protein was not higher than in the other patients. Both, the plasma and the hepatic concentrations of GSH, were thus

Table 2 *Glutathione in human liver*

	µmol/g wet weight	*nmol/mg protein*
Alcoholic hepatitis (8)	1·30 (0·57)*	15·0 (5·9)
Normal liver (3) (surgical biopsies)	4·62 (0·79)	39·9 (14·7)
Chronic persistent hepatitis (5)	3·79 (1·73)†	32·2 (15·2)‡
Non-alcoholic cirrhosis (7)	2·39 (1·64)	32·6 (16·7)‡

*mean (SD), the number of patients are indicated in brackets; †$p<0.01$ *v* alcoholic hepatitis; ‡$p<0.05$ *v* alcoholic hepatitis; values mean (SD).

decreased by approximately 50% in alcoholics.

The time course of the plasma concentration of GSH after the administration of paracetamol to five alcoholics and five control subjects is shown in Figure 2. After the administration of the drug the plasma concentration of GSH decreased from 8·37 (2·65) µM (mean (SD)) to 6·26 (2·96) µM at three hours ($p<0.02$ by paired *t*-test) in normal volunteers. In the alcoholics the values at two hours (3·10 (1·83) µM, $p<0.02$) and three hours (2·40 (1·36) µM, $p<0.05$) were significantly lower than the baseline GSH concentration of 4·66 (1·87) µM and significantly ($p<0.05$) lower than the corresponding values in the

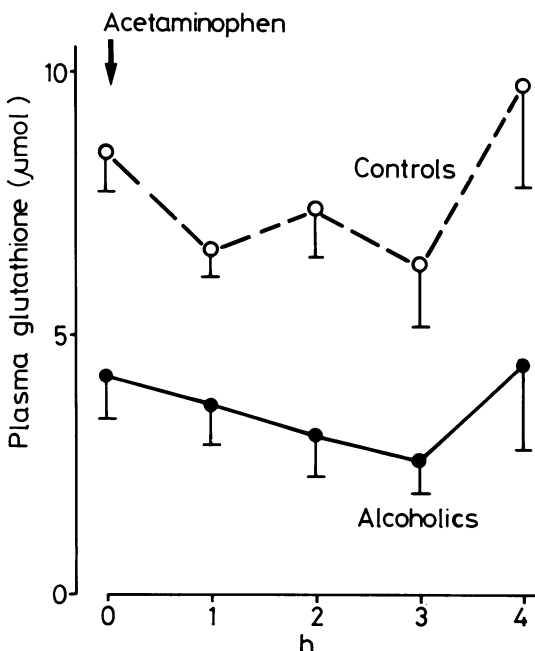

Fig. 2 *Concentration of glutathione in plasma in five healthy volunteers and five chronic alcoholics before and after ingestion of 2 g of paracetamol (mean (SE)). The values at 0, 1, 2, and 3 h are significantly lower in the alcoholics ($p<0.05$).*

Table 3 *Metabolites of paracetamol excreted in urine (µmol in six hours)*

	Sulphate	Glucuro-nide	Acetamino-phen	Mercaptu-ric acid	Cysteine	Urine vol ml
Controls						
1	1599	1829	184	121	96	365
2	2445	5702	229	147	143	385
3	1581	2448	180	160	111	105
4	1656	2734	155	115	89	470
5	1229	1177	83	102	75	75
	1702	2778	166	129	103	
	(448)	(1741)	(54)	(24)	(26)	
Alcoholics						
1	670	970	37	53	30	180
2	2363	2713	132	173	163	625
3	2068	3630	195	198	172	550
4	4959	6216	384	290	264	435
5	1021	3082	72	265	216	155
	2216	3322	164	196	169	
	(1687)	(1899)	(137)	(93)	(87)	

Values mean (SD).

controls. The plasma concentration of cysteine decreased to 5·0 (2·6) µM in the normal volunteers ($p<0·05$) and to 8·3 (2·3) µM in the alcoholics (not significant).

The urinary excretion of metabolites of paracetamol during the critical first six hours is shown in Table 3. There was no statistically significant difference in the recovery and the relative proportions and absolute amounts of metabolites between alcoholics and controls.

Discussion

Our data indicate that chronic excessive ethanol consumption is associated with decreased circulating concentrations of GSH. Either an increase in the catabolism of plasma GSH or a decreased release of GSH by the liver, possibly because of malnutrition or a direct effect of ethanol, could account for this observation. Degradation of GSH by gamma glutamyl transferase, an enzyme the activity of which is commonly found to be raised in serum of alcoholic patients, is the major route of catabolism of GSH. An increase in the activity of gamma glutamyltransferase, however, does not measurably increase the clearance of plasma GSH.[13] Low plasma GSH in the presence of decreased concentrations of GSH in the liver of alcoholics even in the presence of good nutrition[14] suggest a decreased efflux of GSH from the liver. In rats the liver is the major source of plasma GSH[15] and the plasma concentration of the tripeptide reflects its intrahepatic concentration.[6] A similar relationship can be inferred from the observation that hepatic and plasma GSH are decreased by approximately 50% in chronic alcoholics. A comparable decrease in hepatic GSH has been reported in patients with alcoholic fatty liver,[14] whose clinical status might correspond better to the subjects in whom we measured plasma GSH.

The interpretation that plasma GSH reflects intrahepatic GSH also in man is further supported by the decrease in plasma GSH following paracetamol. The dose of paracetamol of which approximately 8% or 1060 µmol will be activated to the toxic metabolite requires the same amount of GSH for its detoxification. Assuming a liver weight of 1500 g this consumption of GSH will decrease the concentration of intrahepatic GSH from approximately 4 µmol/g to 3·3 µmol/g. The approximately 25% decrease in plasma GSH seen in our subjects (Fig. 2) is consistent with this intracellular depletion of GSH. The compensatory stimulation of hepatic GSH synthesis[5] increases the consumption of cysteine and could thus explain the decrease in plasma cysteine. The lesser fall in plasma cysteine seen in alcoholics could possibly be due to a decreased capacity to stimulate GSH synthesis in response to a stress on the hepatic GSH pool.

The mechanism by which chronic alcoholism decreases hepatic glutathione in man is not clear at present. In rats, ethanol acutely depletes GSH, most likely by inhibiting its synthesis.[16] After chronic administration of ethanol to experimental animals, the hepatic concentration either decreases, increases, or remains unchanged.[17-19] The administration of a nutritionally adequate diet, however, together with ethanol in these animal studies may not be a good model for our patient population who required treatment in a facility. In this population, malnutrition[20] as well as a specific effect of ethanol on GSH homeostasis could be responsible for the decrease in GSH. Whatever the mechanism, a decreased availability of GSH in the liver and the circulation might render alcoholics more susceptible to the toxic effects of certain xenobiotics and reactive oxygen species.

Based on experiments with rodents[21,22] enzyme induction by chronic ethanol consumption has generally been thought to be the major factor explaining the increased hepatotoxicity of paracetamol in chronic alcoholics. Enzyme induction would contribute to the toxicity of paracetamol during the first few hours after ingestion of the drug when the rate of formation of the toxic metabolite is highest.[23] In agreement with data reported by other investigators,[24] however, the alcoholics participating in our study did not excrete significantly more cysteine- and N-acetyl-cysteine-paracetamol, the major metabolites of the toxic intermediate, during that critical period of time.

In conclusion, the concentrations of GSH in liver and plasma are significantly lower in alcoholics, and plasma GSH reaches lower levels after the ingestion of a therapeutic dose of paracetamol. In experimental animals covalent binding and hepatocellular necrosis is seen *in vivo* when the hepatic concentration of GSH falls below 0·5 to 1·0 μmol/g.[4] Provided that plasma GSH reflects intrahepatic GSH also in man as our data suggest, a lower dose of paracetamol will be necessary to deplete GSH in alcoholics below the critical threshold concentration where hepatocellular necrosis starts to occur.

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