

No Net Splanchnic Release of Glutathione in Man during *N*-Acetylcysteine Infusion

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Glutathione and amino acid concentrations were measured in arterial and hepatic vein plasma in four healthy volunteers and two patients with cirrhosis. There was no significant splanchnic efflux of glutathione (95% confidence limits, -0.501 to 0.405 $\mu\text{mol}/\text{min}$). After infusion of *N*-acetylcysteine (NAC) in a high dose (150 mg/kg body weight primer plus 15 mg/(h \times kg BW), corresponding to treatment of acetaminophen overdose, there was no change in the splanchnic glutathione efflux (95% confidence limits, -0.531 to 0.375 $\mu\text{mol}/\text{min}$). NAC increased hepatic plasma flow rate from 0.90 ± 0.53 l min^{-1} to 0.97 ± 0.11 (mean \pm SEM; $p < 0.05$). The effects of NAC treatment on plasma amino acids corresponded to an increased load on hepatic metabolic *N* conversion and transamination among non-essential amino acids. Splanchnic uptake of serine, alanine, cystine, isoleucine, and phenylalanine increased after NAC compatible with stimulated hepatic glutathione synthesis. In contrast to the rat, plasma glutathione in man probably originates mainly from extrahepatic tissues.

Key words: *N*-acetylcysteine; amino acid analysis; glutathione; glutathione synthesis; indocyanine green; liver; liver vein catheterization

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Glutathione (γ -glutamyl-cysteinyl-glycine, GSH) plays an important role in the detoxification of toxic metabolites of drugs and oxygen intermediates (1). It is found mainly in reduced form; less than 10% is found as the oxidized disulphide. GSH metabolism and its regulation have been studied primarily in experimental animals and cellular systems, particularly isolated hepatocytes. It is believed that GSH present in blood is derived from hepatic synthesis, since isolated hepatocytes release GSH. This is also taken to be the case in man, on the basis of indirect measurements of the synthesis of GSH and of concentrations in accessible organs and body fluids (2–6). However, few direct measurements of organ gradients have been conducted in man.

It has been demonstrated in rats that GSH synthesis increases after administration of the precursor *N*-acetylcysteine (NAC) (7), which protects against paracetamol hepatitis both in rats (7) and in man (8). From these data together with some indirect measurements in man (9) it has been inferred (3) that NAC also stimulates the hepatic synthesis and release of GSH in man. If so, blood GSH concentration and splanchnic GSH release should increase after NAC administration.

We have preliminarily reported, however, that in man there does not appear to be a splanchnic efflux of glutathione (10), in contrast to both the findings in rats (4, 11, 12) and the indirect synthesis studies in man (3, 9). The lack of hepatic efflux has recently also been described by others

(13). These reports raise basic questions pertaining to the origin of GSH in the blood and the validity of the indirect GSH synthesis measurements.

The purpose of this study was to measure directly net splanchnic GSH balance in normal man and the effects hereupon of NAC.

MATERIALS AND METHODS

Subjects

The subjects were four volunteers (female/male = 1:3; age, 21–28 years) and two patients (male; age, 47 and 56 years) with stable alcoholic cirrhosis of the liver. The volunteers were recruited among medical students and medical staff. They had no history of liver disease, had not been admitted to hospital, and received no medication. The two patients had a liver vein catheterization performed for haemodiagnostic studies.

All subjects participated after informed written consent. The protocol was approved by the local ethical committee.

Catheterization and sampling

After a 12-h fast, an intravenous infusion of indocyanine green (ICG) dissolved in isotonic saline was commenced at a constant rate of 0.30 $\mu\text{mol}/\text{min}$ and maintained throughout the study. A steady arterial plasma concentration of 0.35 $\mu\text{mol}/\text{l}$ was attained after 90 min. An indwelling catheter (diameter, 1.1 mm) was placed in an antecubital or femoral

artery, and a hepatic vein catheterization was performed percutaneously via an antecubital vein (with No. 8 Cournand catheters in the patients in whom the wedge hepatic vein pressure was measured) or via a femoral vein (with No. 6 Oedmann catheters in the volunteers, to avoid passage of the right atrium). The position of the catheters was repeatedly controlled by fluoroscopy. After the procedures, the subjects were observed overnight in the ward. There were no complications or adverse effects.

There were two experimental periods that each lasted 15–18 min and were separated by an intermission of 40 min. During both experimental periods, a total of four equidistant sets of blood samples were taken simultaneously from the arterial and the hepatic vein catheters as described below. Immediately after the last sample of the first period, an intravenous NAC infusion was started via a separate venous line as a 150 mg/kg primer given over 15 min, followed by a constant infusion of 15 mg/(h × kg body weight).

The sampling procedure in the two patients did not adhere strictly to the above regimen; from one of them only three sets of samples were taken during the first experimental period and five samples during the second period with NAC infusion; from the other, five sets of samples were obtained at intervals of 15 min, and no NAC was given.

Before the sampling, 15 ml was drawn (and later reinjected) at a rate of 1 ml/sec simultaneously from both the arterial and hepatic vein catheter. At the 15th sec 3 ml of blood was sampled into pre-chilled glass tubes with buffered L-serine/Na-borate (final concentration, 20 mmol/l; pH 7.4). The tubes were immediately transferred to a chilled fast-accelerating centrifuge and spun at maximum speed for 1 min. Plasma was precipitated with trichloroacetic acid (TCA)/HCl for analysis of GSH. The flushing, sampling, centrifugation, and precipitation were completed within 120 sec. Additional sets of blood samples were taken for amino acid analysis and measurement of ICG.

Analyses

The fast processing of the samples ensures negligible loss of glutathione, whereas a 20- to 30-min delay leads to substantial loss of plasma GSH (data not given). The advantage of the fast procedure is that it was not necessary to stabilize GSH in plasma by sulphosalicylic acid, which inhibits the recycling reductase assay (14). The measurement of GSH concentration was performed on the same day, since there was significant loss of GSH in plasma stored at -20°C (data not given), in contrast to the stable GSH contents of frozen acidified liver tissue (15). The total plasma GSH concentration—that is, oxidized plus reduced—was measured with double-beam photometry in the enzyme-coupled assay described by Tietze (16), with modifications reported elsewhere (15). ICG does not interfere with the assay.

Plasma ICG concentration was estimated colorimetrically at 800 and 900 nm (17–19). Samples were kept in the dark until analysis, performed on the same day.

Plasma amino acid concentrations (after storage at -20°C) were measured by high-performance liquid chromatography after separation in a lithium ion-exchange column and fluorescence detection after post-column orthothal-aldehyde (OPA) derivatization. Storage of samples results in exchange between glutamine and glutamate, and the sum of the two measurements is given together.

Calculations

Splanchnic plasma flow (SPF) was calculated in accordance with Fick's principle (19):

$$\text{SPF} = (\text{Inf.} - \text{BW} \times 0.05 \times d\text{ICG}_a/dt) / (\text{ICG}_a - \text{ICG}_{\text{hv}})$$

where Inf. = ICG infusion rate, $\text{BW} \times 0.05$ = the volume of distribution of ICG (that is, the plasma volume), $d\text{ICG}_a/dt$ the rate of change in arterial plasma concentration between two adjacent samples, and $(\text{ICG}_a - \text{ICG}_{\text{hv}})$ = the mean plasma concentration difference between arterial and hepatic venous samples.

The net splanchnic GSH efflux was estimated for each of the eight plasma sample pairs (four before and four during NAC infusion) as

$$\text{GSH efflux} = (\text{GSH}_{\text{hv}} - \text{GSH}_a) \times \text{SPF}$$

Statistical analyses

A two-way analysis of variance was performed, including the variables 'subject' and 'NAC treatment'. A similar analysis was performed including SPF instead of subject. Changes in splanchnic amino acid uptake by NAC treatment was examined by paired *t* tests after one-way analysis of variance. *P* values less than 5% were considered statistically significant.

RESULTS

Glutathione

Splanchnic plasma flow rate was $0.90 \text{ l/min} \pm 0.53$ (SD) and increased slightly, to 0.97 ± 0.11 (SD) during NAC treatment ($p < 0.05$, analysis of variance). In the volunteers the basal splanchnic GSH effluxes equalled zero, with 95% confidence limits of -0.501 to $0.405 \mu\text{mol/min}$. The average was $-0.0480 \mu\text{mol/min} \pm 0.5297$ (16 observations: mean and SD of 4 estimations on 4 subjects) (Fig. 1). During the NAC treatment the efflux did not change significantly from zero, with 95% confidence limits of -0.531 to $0.375 \mu\text{mol/min}$ (mean, -0.0780 ; SD, 0.9744). The range of observations was -2.17 to $1.61 \mu\text{mol/min}$. The grand mean of the basal arterial total GSH plasma concentrations was $4.54 \pm 0.90 \mu\text{mol/l}$ (mean \pm SEM). Individual measurements ranged from 1.16 to 7.9 and did not change significantly during the NAC treatment ($p > 0.05$).

In the two patients with liver disease the basal arterial plasma GSH concentrations were 2.68 ± 0.98 and $3.88 \pm 0.71 \mu\text{mol/l}$ (mean \pm SD). The basal efflux of GSH in one

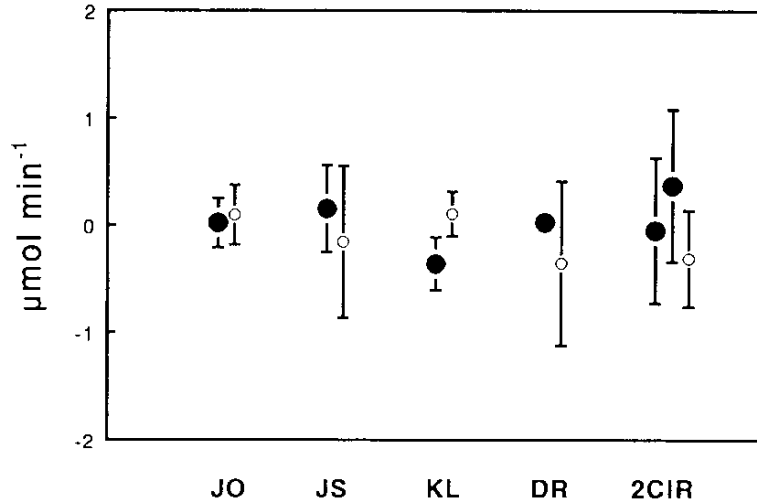
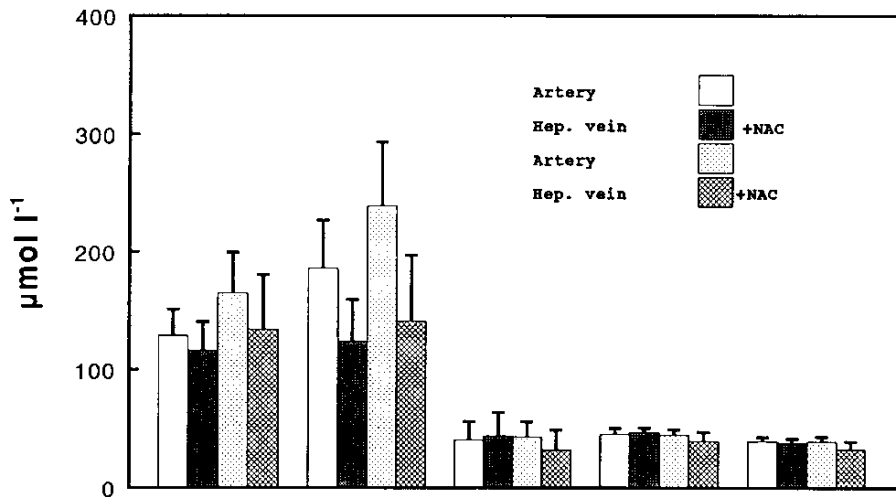


Fig. 1. Hepatic glutathione efflux in four healthy volunteers (JO, JS, KL, DR) and two patients with hepatic cirrhosis (2CIR), estimated from hepatic plasma vein-artery concentration difference times hepatic plasma flow. Symbols indicate mean; bars, SEM before (closed symbols) and after *N*-acetyl cysteine infusion (open symbols), corresponding to the treatment for paracetamol intoxication (150 mg/kg over 15 min, followed by 15 mg/kg/h for 25 min). The efflux of glutathione from the liver is not different from zero ($p > 0.05$).



	SER	ALA	CY2	ILE	PHE
Grad -NAC	-13	-62	3	2	-1
Grad +NAC	-31	-98	-11	-5	-6

Fig. 2. Amino acids with increased ($p < 0.05$) hepatic uptake after *N*-acetylcysteine (NAC) infusion (see legend to Fig. 1). Serine (SER) and alanine (ALA) were taken up by the liver in the basal state ($p < 0.05$) and after NAC infusion. Cystine (CY2), isoleucine (ILE), and phenylalanine (PHE) uptake by the liver was significant after NAC infusion. Boxes indicate mean, bars SEM. The table below the figure gives the mean hepatic gradient in $\mu\text{mol/l}$ of four volunteers before (Grad -NAC) and after (Grad +NAC) NAC infusion.

patient was $0.37 \pm 0.71 \mu\text{mol/min}$, and in the other patient -0.05 ± 1.68 . During NAC treatment the GSH efflux in the latter patient was $-0.31 \pm 0.45 \mu\text{mol/min}$ ($p > 0.05$). These values are within the 95% confidence limits of the GSH efflux of the volunteers and are not significantly

different from 0. The splanchnic GSH effluxes are shown in Fig. 1.

Amino acids

In the basal state aspartic acid, threonine, serine,

Table I. Plasma glutathione concentrations reported in the literature

Plasma (μM)	Hepatic vein (μM)	Year and Ref. no.
Man		
0.34	—	1980 (25)
0.91	—	1985 (26)
1.11	0.79	1990 (13)
1.1–1.7	—	1971 (22)
2–4.5	—	1984 (27)
4.5	—	1978 (28)
1.2–7.9	2.4–6.8	Present study
8.4	—	1988 (2)
Rat		
5	—	1965 (16)
13.6	25.4	1990 (13)
14.5	26.0	1980 (11)
10.5–18.2	28.4	1984 (12)
26.8	—	1984 (21)
22–27	—	1980 (30)

glutamate + glutamine, glycine, and alanine were taken up by the splanchnic bed, and during the NAC treatment the arterial concentrations of aspartic acid, threonine, serine, glutamate + glutamine, glycine, and alanine increased significantly but less than 10% ($p < 0.05$). NAC infusion resulted in increased splanchnic uptake of serine, alanine, cystine, isoleucine, and phenylalanine ($p < 0.05$). The arterial and hepatic vein amino acid concentrations before and after NAC are depicted in Fig. 2, in which the splanchnic gradients are given below the figure.

DISCUSSION

In the present study in man there was no significant net efflux of GSH from the splanchnic bed, either in the basal state or during infusion of NAC in the dose used for treatment of paracetamol intoxication. Nor did the blood GSH concentration increase during NAC treatment, but there were small increases in arterial concentrations of the glycconeogenic and ureagenic amino acids plus threonine and splanchnic uptake of serine, alanine, cystine, ileucine, and phenylalanine.

Recently, our preliminary data presentation (10) was confirmed independently by hepatic gradient observations in man and the rat (13). To our knowledge, these studies are the only data available on GSH concentration in the hepatic veins in man.

Recently, a 14% improvement by acetylcysteine in cardiac output in fulminant hepatic failure was reported; however, after recovery this effect disappeared (20). We found a slight increase of about 7% in hepatic blood flow rate by acetylcysteine in healthy volunteers. This could indicate that the effect on the splanchnic bed is present in healthy volunteers, although the overall effect on the circulation is minimal.

Reported peripheral venous plasma GSH concentrations

in man vary by a factor of 25 (Table I). This may reflect methodologic difficulties; the sampling procedure seems to be of paramount importance. Our technique is optimized so as to prevent haemolysis and to complete precipitation of plasma proteins and acidification within 2 min. Moreover, the deproteinization with 5-sulphosalicylic acid used by other investigators inhibits the recycling assay specific for GSH (14). Reports on very low plasma GSH (25, 26) probably partly indicate loss of GSH due to variation in the time from blood sampling to acidification and analysis and, partly, use of 5-sulphosalicylic acid.

Organic anions such as ICG may inhibit transport of GSH from the liver to the hepatic sinusoid (23). This, however, requires much higher and toxic concentrations of the dye than the low concentrations used for estimation of hepatic flow. In agreement with this there was a high efflux of ICG in rats whether ICG was given or not (11, 12). We therefore conclude that the present lack of splanchnic GSH release in man is probably not due to ICG.

Burgunder & Lauterburg (3), from the decay of exogenously administered GSH, estimated the input of GSH to the circulation to be about $28 \mu\text{mol min}^{-1}$ in healthy volunteers and inferred that this corresponds to hepatic production. In rats hepatic GSH synthesis can be estimated by combined use of ^{35}S -cysteine and paracetamol (4, 12). Unfortunately, such data are of no use in man, since the fractional turnover of the tracer reflects turnover of the cysteine pool available for GSH synthesis rather than turnover of hepatic GSH (12). However, the 95% confidence limits and the observed range of splanchnic effluxes measured by the direct method in the present study constitute less than 4% of the total GSH release into the systemic circulation, as estimated by the exogenous GSH decay method (3). The hepatic GSH concentration in man corresponds to that in rats (15). Considered together, these data suggest lower hepatic synthesis of GSH in man compared with rats.

The NAC treatment had the effects on plasma amino acids expected in a situation with increased load on hepatic metabolic *N* conversion and transamination among non-essential amino acids (24). The almost identical increase in splanchnic serine and cystine uptake may, moreover, be interpreted in the light of the need for GSH synthesis for these two amino acids. If they are incorporated mole-to-mole in GSH, the basal and NAC-stimulated serine uptake gradients are of the same magnitude as the decay-based data in man and correspond to the hepatic GSH synthesis values measured in rats. On this assumption, the increase in serine and cystine uptake may reflect more than a doubling of intrahepatocytic GSH synthesis by NAC despite the lack of net release.

In conclusion, in man there is no net GSH release into the blood stream from the splanchnic bed, even during ample supply of the precursors taken to stimulate synthesis of the peptide. GSH in blood therefore does not originate from the liver. Based on circumstantial data it remains possible,

however, that intrahepatocytic GSH synthesis is increased by the precursor, which may be one explanation for the protective effect of the precursor towards GSH depletion and cell damage by alkylating metabolites of, for example paracetamol.

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