Biochemical Pharmacology, Vol. 46, No. 7, pp. 1295–1297, 1993.
Printed in Great Britain.

0006-2952/93 $6.00 + 0.00

SHORT COMMUNICATIONS

Synthesis rates of glutathione and activated sulphate (PAPS) and response to cysteine and acetaminophen administration in glutathione-depleted rat hepatocytes

(*Received* 24 *March* 1993; *accepted* 7 *July* 1993)

Abstract—The effects of cysteine and acetaminophen (AA) on the synthesis rates of glutathione (GSH), adenosine 3'-phosphate 5'-phosphosulphate (PAPS, activated sulphate) and the AA metabolites, AA–GSH and AA–sulphate were studied in rat hepatocytes depleted of GSH by diethyl maleate (DEM). The synthesis rates were determined simultaneously by a previously described radioactive tracer method. Preincubation of the hepatocytes with 0.7 mM DEM for 30 min depleted GSH by 59% ($P < 0.05$) and PAPS by 28% ($P < 0.05$). Incubation with a toxic AA concentration resulted in GSH synthesis at a rate of 95 nmol/(10^6 cells · min) which increased to 281 nmol/(10^6 cells · min) ($P = 0.05$) after addition of cysteine. However, increased GSH synthesis was not followed by increased AA–GSH synthesis [4.7 vs 4.8 nmol/(10^6 cells · hr)]. Also, PAPS synthesis increased after cysteine administration [10.2 to 19.1 nmol/(10^6 cells · min)] ($P < 0.05$) without any change in AA–sulphate synthesis 18.5 vs 18.3 nmol/(10^6 cells · hr)]. Thus, in contrast to hepatocytes with normal GSH concentration, cysteine stimulated both GSH and PAPS synthesis rates in GSH-depleted rat hepatocytes incubated with a toxic AA concentration without stimulation of AA–GSH or AA–sulphate synthesis rates, indicating that the hepatoprotective effect of cysteine on AA toxicity is primarily due to stimulation of a GSH-mediated reduction of the reactive AA metabolite *N*-acetyl-*p*-benzoquinoneimine back to AA.

Conjugation with glutathione (GSH*) and inorganic sulphate (sulphation) represent major detoxification reactions and both share cysteine as a common precursor. The γ-glutamyl cycle utilizes cysteine in the formation of GSH [1]. Inorganic sulphate deriving from cysteine is activated before sulphation by formation of the cosubstrate adenosine 3'-phosphate 5'-phosphosulphate (PAPS, activated sulphate) [2]. The hepatotoxic analgesic acetaminophen (AA) is predominantly detoxified by sulphation, glucuronidation and P450 oxidation/GSH conjugation [3]. A radioactive tracer method measured simultaneously GSH and PAPS synthesis rates together with synthesis rates of two AA metabolites, AA–GSH and AA–sulphate, in isolated rat hepatocytes with normal GSH content [4]. The aim of the present study was to evaluate further the regulation of these two detoxification pathways, i.e. to investigate the effects of cysteine under conditions of GSH depletion and hepatotoxic AA concentration. The hepatocytes were GSH depleted by diethyl maleate (DEM) [5] prior to incubation with AA.

Materials and Methods

Hepatocyte isolation, analytical analyses and calculations of synthesis rates were performed as described in detail previously [4]. The hepatocytes were GSH depleted by preincubation of the cell suspension with 0.7 mM DEM for 30 min, preceding incubation with 5.0 mM AA and 6.8 mM cysteine, or 5.0 mM AA alone. All values are expressed as medians with interquartile distances. Differences were tested by a Wilcoxon test for paired observations. The level of significance was 0.05.

Results

DEM treatment resulted in a 59% reduction of the hepatocellular GSH concentration from 26.0 (20.6–30.0) to 10.5 (5.8–10.9) nmol/10^6 cells ($P < 0.05$). In addition to GSH depletion DEM also depleted 28% of PAPS from 2.3 (2.2–3.1) to 1.7 (1.3–1.8) nmol/10^6 cells ($P < 0.05$). Addition of cysteine to GSH-depleted hepatocytes exposed to AA resulted in increased GSH synthesis (corresponding to an increased turnover rate) and increased GSH concentration (Table 1). However, cysteine administration was not followed by increased AA–GSH synthesis. Also PAPS synthesis (corresponding to the turnover rate) as well as the PAPS concentration increased in GSH-depleted hepatocytes exposed to AA and cysteine, whereas AA–sulphate synthesis was unaffected by cysteine administration (Fig. 1).

Discussion

The present data show that GSH-depleted hepatocytes exposed to a toxic AA concentration were able to stimulate both GSH and PAPS synthesis rates, if they were incubated with cysteine.

The GSH depletion observed in the present study is in accordance with other studies using DEM as the GSH depleting agent. DEM is spontaneously conjugated or conjugated by catalysis of cytosolic GSH transferase to GSH. However, the effect of DEM is not restricted to depletion of GSH. In the present study DEM also depletes the hepatocellular PAPS pool after a 30-min preincubation period. This effect may be of a transient nature. The PAPS concentration in livers from rats treated with DEM at a much higher concentration (6 mmol/kg) given intraperitoneally was not different from control values, if the PAPS concentration was measured 3 hr after the GSH depleting agent was administered [6]. DEM has been shown to affect hepatic microsomal monooxygenase activities [7] which renders it probable that a similar inhibitive effect of DEM on PAPS-generating enzymes is responsible for the PAPS depletion seen in the present study. Increased lipid peroxidation, which is also seen after DEM treatment [8], may also affect viability of the hepatocytes resulting in

* Abbreviations: AA, acetaminophen; GSH, glutathione; PAPS, activated sulphate, adenosine 3'-phosphate 5'-phosphosulphate; DEM, diethyl maleate.

Table 1. Median (interquartile distances) turnover rates, concentrations and synthesis rates of PAPS, GSH, AA-sulphate and AA-GSH in isolated hepatocytes preincubated with 0.7 mM DEM and subsequently incubated with 5 mM AA and 6.8 mM cysteine, or 5 mM AA alone

	Turnover rate (min^{-1})		Concentration (nmol/10^6 cells)		Synthesis [nmol/(10^6 cells · min)]		Synthesis [nmol/(10^6 cells · hr)]	
	PAPS		PAPS		PAPS		AA-sulphate	
Controls	8.0	(5.5–10.2)	1.5	(1.3–1.8)	10.2	(10.0–19.2)	18.5	(12.3–27.1)
Cysteine	11.4*	(9.2–20.0)	1.7*	(1.4–2.1)	19.1*	(11.9–34.0)	18.3	(13.3–27.6)
	GSH		GSH		GSH		AA-GSH	
Controls	14.0	(9.0–26.6)	8.8	(6.5–10.8)	95	(70–394)	4.7	(4.3–7.9)
Cysteine	21.8*	(9.9–40.3)	11.7*	(9.1–12.9)	281*	(64–405)	4.8	(4.7–8.1)

* Significantly different from control experiments ($P < 0.05$).

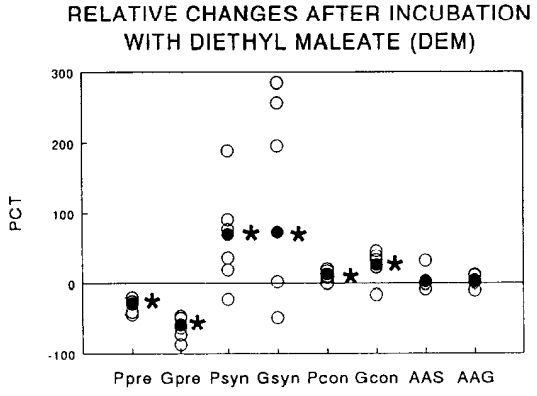

Fig. 1. Relative changes in concentrations of PAPS (Ppre) and GSH (Gpre) in seven single paired experiments with isolated hepatocytes after preincubation with 0.7 mM DEM, and relative changes in synthesis rates of PAPS (Psyn) and GSH (Gsyn), in concentrations of PAPS (Pcon) and GSH (Gcon) and in synthesis rates of the AA metabolites, AA-sulphate (AAS) and AA-GSH (AAG) after subsequent incubation with 5 mM AA and 6.8 mM cysteine, or 5 mM AA alone. Filled circles represent median values. Asterisks indicate significant changes ($P < 0.05$).

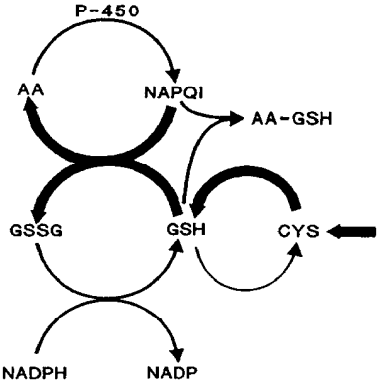

Fig. 2. A scheme proposed for the effect of cysteine (CYS) supplementation on GSH biosynthesis in GSH-depleted rat hepatocytes exposed to a toxic AA concentration. Thick arrows indicate which metabolic pathways are stimulated after cysteine administration. GSSG, oxidated GSH; NAPQI, *N*-acetyl-*p*-benzoquinoneimine.

decreased concentration of hepatocytes in the cell suspension, thus diminishing the PAPS concentration.

Addition of cysteine to the GSH-depleted hepatocytes increased GSH synthesis three times without affecting AA-GSH synthesis, which remained about 5 nmol/(10^6 cells · hr), indicating that the hepatoprotective effect in AA toxicity, provided by the antidote *N*-acetylcysteine, is caused by other mechanisms than by increasing the formation of the GSH conjugate. The observed stimulation of GSH synthesis may be used in a GSH-mediated reduction of *N*-acetyl-*p*-benzoquinoneimine (NAPQI) back to AA, resulting in oxidation of GSH. This is substantiated by the finding that administration of a toxic AA concentration inhibits thiol-transferase activity for 1 hr in mice [9]. The same study shows that the cytosolic GSH reductase activity was not affected by AA, resulting in a preserved GSSG (oxidated GSH) reducible capacity consistent with increased GSH synthesis. A scheme proposed for the effect of cysteine supplementation on GSH biosynthesis in GSH-depleted cells exposed to 5 mM AA is shown in Fig. 2.

There seems to be a balanced correlation between cysteine availability, hepatocellular GSH concentration and concentration of the substrate (AA) for GSH-*S*-transferase activity on GSH synthesis. As shown previously, GSH synthesis is inhibited by cysteine if administered to rat hepatocytes incubated with a low, non-toxic AA concentration [4]. Furthermore, GSH synthesis is unaltered if cysteine is given together with a high, toxic AA dose under conditions in which GSH has not yet been depleted. In the present study in which GSH was depleted to one third of pretreatment values, cysteine was able to stimulate GSH synthesis without affecting AA-GSH synthesis. These data correspond to those from Lauterburg and Mitchell [10] who demonstrated a doubling of GSH synthesis in rats treated with DEM and a non-toxic AA concentration using a similar methodological approach. Furthermore, Lauterburg *et al.* [11] was able to demonstrate increased GSH synthesis if rats, treated with a toxic AA concentration, were given *N*-acetylcysteine at the time when GSH was almost depleted to two thirds of pretreatment values.

The increase in GSH turnover after DEM treatment may use available cysteine or methionine which also are

precursors for inorganic sulphate formation and PAPS generation. The diversion of sulphur contained in cysteine away from oxidation to inorganic sulphate may trigger stimulation of PAPS synthesis in order to maintain the PAPS pool at a certain level. Under conditions without hepatocellular GSH depletion, opposite effects of cysteine on PAPS synthesis are seen [4], i.e. PAPS synthesis is inhibited after cysteine administration. This effect was explained by increased formation of PAPS intermediates which are known to inhibit the PAPS-generating enzyme sulphate adenylyltransferase (ATP-sulfyrulase) [2], thus resulting in decreased PAPS synthesis. This effect is in GSH-depleted hepatocytes overcome by a diversion of cysteine (containing sulphur) to GSH biosynthesis, indicating that the size of the GSH pool is a strong regulator of cysteine metabolism in isolated hepatocytes. When the cysteine pool is further depleted the increased PAPS synthesis is no longer able to preserve the PAPS pool without affecting AA–sulphate synthesis which eventually will decrease. This is in accordance with the data by Galinsky [12] who showed decreased AA–sulphate formation and AA total clearance in rats pretreated for 30 min with 3.9 mmol/kg DEM given intraperitoneally preceding administration of a toxic AA concentration. When the GSH pool returns to pretreatment size, GSH synthesis will probably decrease, and sulphur from cysteine will again be diverted into inorganic sulphate and PAPS formation.

An alternative explanation for increased PAPS synthesis after DEM pretreatment is that the PAPS depletion itself (26%), via a feed-back regulation of PAPS on a PAPS generating enzyme, enhances PAPS synthesis.

Acknowledgements—Supported by grant from (a) the Danish Hospital Foundation for Medical Research, Region of Copenhagen, The Faroe Islands and Greenland, (b) Christian X's Foundation, (c) Danish Foundation for the Advancement of Medical Science and (d) Arvid Nilsson's Foundation. The technical assistance of Miss M. Poulsen is gratefully acknowledged.

Department of Medicine A-2152 KIM DALHOFF*
Rigshospitalet, and HENRIK E. POULSEN†
†*Department of Pharmacology*
University of Copenhagen
Copenhagen, Denmark

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* Corresponding author: Dr Kim Dalhoff, Department of Medicine A-2152, Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen, Denmark. FAX (45) 31 35 29 13.