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The effect of cysteine and N-acetyl cysteine on rat liver glutathione (GSH)

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Cysteine and its derivatives are extensively used as mucolytics or as protective agents against acetaminophen (paracetamol) toxicity [1].

However, several reports in the literature indicate that moderate doses of cysteine can be toxic when added to tissue cultures [2], when injected to rats [3–5] or when given orally with the diet [6].

We reported [7] that intraperitoneal injections of large doses of N-acetyl cysteine (NAC) decreased GSH content of rat liver and that incubation of isolated hepatocytes in saline solution of Krebs and Henseleit [8] with cysteine (above 0.5 mM) decreased their GSH content [9]. However, in a recent study, Beatty and Reed [10] reported that incubation of hepatocytes with cysteine did not induce GSH depletion. This, together with the fact that NAC is used as a treatment of paracetamol overdose, prompted us to undertake a systemactic study of the effects of cysteine and NAC administered orally or intraperitoneally to control rats and to rats treated with high doses of paracetamol.

Experimental

Animals. Wistar rats (150–200 g body wt.) were fed *ad libitum* on a standard diet for rats and mice (Prada, Vara de Quart, Valencia, Spain). They always had free access to food and water. Although some previous studies on acetaminophen toxicity in rats have been carried out in fasted rats [11], we used well fed animals because fasting significantly decreases hepatic GSH content in rats [12].

Acetaminophen was injected intraperitoneally in a small volume of a 10% dimethyl sulfoxide solution in a physiological saline. Amino acids were dissolved in a small volume of physiological saline and administered orally or intraperitoneally. It was tested that injection of similar amounts of both solvents did not affect hepatic GSH content.

The dose of paracetamol injected was 0.5 g/kg body wt. (3.3 mmol/Kg). A similar dose had been previously used by other authors [11].

Chemicals. Paracetamol was a gift of the department of Pharmacy of the Faculty of Medicine (Valencia, Spain). Dimethyl sulfoxide was purchased from Sigma Chemical Company, St. Louis, MO., U.S.A. L-cysteine was from Merck (Darmstadt, Germany) and N-acetyl cysteine from Zambon Laboratories (Barcelona, Spain). All other chemicals were of the highest purity available.

Table 1. Effect of cysteine and NAC on rat liver GSH*

Dose administered (g/Kg body wt.)	Cysteine		NAC	
	Oral	i.p.	Oral	i.p.
0.125	4.32 ± 0.25 (5)	4.20 ± 0.41 (4)	5.69 ± 0.12 (3)	5.21 ± 0.18 (3)
0.25	4.17 ± 0.42 (4)	3.10 ± 0.33 (3)	5.60 ± 0.31 (3)	4.75 ± 0.36 (3)
0.50	3.83 ± 0.36 (5)	2.22 ± 0.53 (4)	5.04 ± 0.32 (3)	3.64 ± 0.27 (3)
1.00	0.43 ± 0.02 (3)	N.D.	4.91 ± 0.43 (3)	2.34 ± 0.37 (3)

* The amino acids were given orally or i.p. two hours before killing the animals. Control GSH values were 5.40 μmoles/g [4]. Data were given as means ± S.D. with the number of observations in parentheses. N.D: Non-Detectable.

Table 2. Effect of cysteine and NAC on paracetamol induced GSH depletion*

Dose administered (g/Kg body wt.)	Cysteine		NAC	
	Oral	i.p.	Oral	i.p.
0.125	2.31 ± 0.56 (5)	0.76 ± 0.26 (3)	2.10 ± 0.85 (5)	2.13 ± 0.54 (5)
0.25	2.84 ± 0.34 (4)	1.05 ± 0.30 (3)	3.59 ± 0.47 (3)	2.90 ± 0.05 (3)
0.50	2.01 ± 0.12 (4)	0.22 ± 0.04 (3)	2.52 ± 0.24 (4)	2.37 ± 0.38 (5)
1.00	N.D.	N.D.	1.92 ± 0.31 (4)	1.83 ± 0.46 (5)

* Rats were treated with 0.5 g/Kg body wt. of acetaminophen and amino acids, at various doses, two hours before killing the animals. Initial GSH in acetaminophen injected rats was 1.86 ± 0.23 μmoles/g [6]. Data are means ± S.D. with the number of observations in parentheses. N.D.: Non-Detectable.

Assays. Reduced glutathione (GSH) was determined by the glyoxalase method of Racker under the conditions described in [9]. The rate of cysteine oxidation was measured using a Clark type electrode as described in [13].

Results and discussion

Effect of cysteine and NAC on rat liver GSH. Table 1 shows that intraperitoneal injection of cysteine and NAC decreased the GSH content of rat liver. However, injection of cysteine always caused greater GSH depletions than injection of similar amounts of NAC.

The effect of oral administration of cysteine or NAC was always less marked than when the amino acids were given intraperitoneally. Indeed, even relatively large doses of cysteine (0.5 g/kg), given orally, caused only a moderate depletion of liver GSH (about 30% of the controls). Even large doses of NAC, given orally, failed to affect liver GSH.

In order to study the capacity of cysteine or NAC to promote GSH replenishment after paracetamol induced GSH depletion [14], we studied the effect of cysteine or NAC on liver GSH of rats treated with paracetamol. Table 2 shows that oral administration of 0.5 g/kg body wt. of NAC maintained the level of liver GSH of rats treated with paracetamol at values of about 80% of those found in controls. However, larger doses of this agent did not prevent paracetamol induced GSH depletion. NAC injected intraperitoneally was less effective in preventing paracetamol-induced GSH depletion. Administration of free cysteine, orally or intraperitoneally, failed to prevent the fall of GSH content due to paracetamol.

The relevant fact concerning cysteine or NAC toxicity is that if large doses of these amino acids enter the blood plasma, they may be oxidized very rapidly, particularly cysteine (see below). Autooxidation of cysteine generates hydroxyl and thiyl radicals [13] which cause GSH depletion and other signs of cell damage. However, if administered orally, the gastric pH maintains the amino acids reduced and they are slowly released into the small intestine and absorbed into the plasma. This, together with the fact that oxidation of NAC is relatively slow, explains the lack of GSH depletion after oral administration of NAC. In this context, it is significant that tissue levels of cysteine are always kept very low, around 0.1 mM [15]. Such low levels are capable of supporting protein synthesis [16] and glutathione synthesis [17]. However, the levels of glutathione are, at least, an order of magnitude higher than those of cysteine and GSH has been proposed to be a physiological reservoir of cysteine [18].

The rate of autooxidation of cysteine and NAC. We have recently reported that free thiyl and hydroxyl radicals are formed during autooxidation of cysteine [13]. These highly reactive radicals are quenched by hepatocytes resulting in the observed GSH depletion and cell damage [9, 19, 20]. It was, therefore, very important to determine the rate of autooxidation of cysteine and NAC in blood plasma and in water, with physiological concentrations of Cu(II) added. The rates of oxidation of 4 mM cysteine or NAC dissolved in water containing 8 μM Cu(II) were 1020 and 42 nmoles of thiol oxidized per minute, respectively, at 37°. The rates of oxidation of cysteine or NAC in human blood plasma were 1000 and 125 nmoles of thiol oxidized per minute, respectively. The fact that the rate of oxidation of cysteine was always higher than that of NAC, provides an explanation for the greater GSH depletion observed when cysteine was used than with NAC.

In contrast with the effects reported here *in vivo* and with those previously found in isolated hepatocytes [9, 20], Beatty and Reed [10] did not observe an effect of cysteine on GSH in isolated hepatocytes. This discrepancy can be explained by important differences in the incubation conditions. Those authors used complex semisynthetic media containing foetal calf serum, which contains high concentrations of lactate (17 mM). We have shown that lactate protects against cysteine toxicity in isolated hepatocytes [20]. These incubation conditions result in a very low rate

of cysteine oxidation. Thus, Beatty and Reed reported a rate of 30 nmoles of cysteine oxidized per minute. However we show here that in water with physiological amounts of Cu(II) added or in blood plasma, cysteine is oxidized at a rate that is, at least, an order of magnitude higher than that reported by Beatty and Reed [10]. This difference in oxidation rate is important because the very toxic thiyl and hydroxyl radicals are generated only when cysteine is oxidized [13].

The question of finding an optimal treatment against paracetamol intoxication remains open. An interesting new approach is the use of liposomally entrapped glutathione [21, 22]. We found [7] that methionine protects against paracetamol induced GSH depletion. N-acetyl cysteine, given orally may also protect against GSH depletion induced by paracetamol. The fact that cysteine induces a depletion of GSH may be of both theoretical and practical importance.

The relevant facts reported in this paper are. (1) Cysteine administered to rats, i.p. or orally, results in a depletion of hepatic GSH. (2) N-acetyl cysteine, given i.p., also caused a depletion of hepatic GSH. However, when given orally, NAC did not affect liver GSH. (3) Neither cysteine, administered orally or i.p. nor NAC given i.p. prevented the paracetamol induced depletion. However, NAC administered orally prevented paracetamol-induced GSH depletion. (4) The rate of oxidation of cysteine in water or in blood plasma was always higher than that of NAC. Since the rapid thiol oxidation, which generates free radicals, is essential to explain cysteine cytotoxicity, the lower rate of autooxidation of NAC when compared with cysteine explains the different effects of both amino acids on liver GSH and other cytotoxic effects of cysteine.

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Inhibition of oxygen radical metabolism in phorbol ester-activated polymorphonuclear leukocytes by an antitumor promoting copper complex with superoxide dismutase-mimetic activity

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A relationship between chronic inflammation and the induction of malignancy has been proffered following observations that metabolically activated polymorphonuclear leukocytes (PMNs) produce genetic lesions in bacterial and mammalian cells [1–3], presumably as a consequence of their generation of reactive oxygen species. Although oxygen radical production by leukocytes is readily elicited by tumor promoting phorbol esters [4, 5], the role of inflammation in the promotion stage of carcinogenesis is unclear. Most all epidermal promoters are irritants; however, not all inflammatory agents are promoters [6]. That oxygen radicals may mediate a component of the promotion process appears likely though. Free radical generating compounds, such as benzoyl peroxide, are tumor promoters [7]. A previous study has shown that there appears to be a correlation among phorbol esters as to their relative activities as tumor promoters in mouse epidermis and their abilities to stimulate oxygen radical metabolism in PMNs [5]. Moreover, phorbol esters provoke a rapid and sustained decrease in epidermal superoxide dismutase (SOD) and