Hepatoprotection by L-Cysteine-Glutathione Mixed Disulfide, A Sulfhydryl-Modified Prodrug of Glutathione

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ABSTRACT: L-Cysteine-glutathione disulfide, a ubiquitous substance present in mammalian cells, was shown to be highly effective in protecting mice against acetaminophen-induced hepatotoxicity. Since the corresponding D-cysteine-glutathione disulfide was totally ineffective in this regard, an enzymatic mechanism that provides glutathione directly to cells is postulated. © 2003 Wiley Periodicals, Inc. J Biochem Mol Toxicol 17:95–97, 2003; Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/jbt.10069

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Drug-induced hepatotoxicity is a major cause of new drug withdrawal from the market. It also limits further development of promising therapeutic agents even prior to clinical trials. Over-the-counter drugs are not exempt from hepatotoxic liability; for example, acetaminophen (ACP), a widely used (and misused) analgesic/antipyretic agent, when taken acutely in large doses, or chronically in greater than recommended dosages, can lead to liver and kidney damage. While individual pharmacogenetic profiles of hepatic cytochrome P-450 isozyme patterns, when correlated with chemical structures of the drugs and their possible metabolic activation pathways, hold promise as means to preclude susceptible subjects from drug exposure, the concept of therapeutic intervention or prevention methods have not yet attracted much attention, despite the fact that the standard clinical option for protecting the liver from ACP overdoses is to administer intravenous N-acetyl-L-cysteine (NAC) within 8 h of the overdose [1]. NAC, following deacetylation in the liver [2], provides L-cysteine, the sulfhydryl amino acid required for the rate-limiting first step in the biosynthesis of glutathione (GSH) [3]. GSH is the body’s natural defense against endogenously generated reactive oxidant species as well as reactive species such as N-acetyl-p-benzo-quinoneimine (NAPQI) produced in the metabolism of ACP [4].

Experimentally, the administration of high doses of ACP to mice produces fulminant hepatic necrosis, manifested by quantum elevations in serum transaminase levels and histological evidence of centrilobular necrosis leading eventually to death. Postadministration of NAC, a prodrug of L-cysteine, or other cysteine prodrugs that have been sulfhydryl-modified, effectively protects mice against this ACP-induced hepatotoxicity [5–7].

Using a 14C-glycine/HPLC assay method to determine the extent of incorporation of the cysteinyl moiety of the cysteine prodrugs into GSH in rat lens [8], we discovered a radioactive peak near GSH, which appeared to be produced metabolically. This substance was tentatively identified as the mixed disulfide of L-cysteine with GSH, viz., L-cysteine-GSH disulfide (L-CySSG). L-CySSG is produced endogenously via a thiol-disulfide exchange reaction between GSH and L-cystine [9], and possibly, the reaction of L-cysteine with GSSG (the oxidized form of GSH). L-CySSG, postulated to be a storage form of L-cysteine [10], has been detected in small quantities (relative to GSH) in liver and kidney samples from rats, but is present in comparable amounts as GSH, cysteine, and cystine in rat and human plasma [11,12].

Except for the monesters (on the glycyl moiety) and the diethyl ester of GSH, prodrugs of GSH [13] have not been systematically investigated as protective agents against xenobiotic-induced hepatotoxicity. Since L-CySSG can be considered to be a sulfhydryl-modified (vs. carboxyl-modified) prodrug of GSH, we
examined its protective effects against ACP-induced hepatotoxicity using our recently developed mouse model [14,15].

In this communication, we report that L-CySSG is a highly effective liver protective agent in this model, even surpassing the activities of glutathione monoethyl ester (GSH-OEt) [16] and the cysteine prodrug, CySSME [5-(2-hydroxymethylmercapto)-L-cysteine] [14] (Figure 1). As can be seen, the plasma alanine aminotransferase (ALT) levels of ACP-treated mice, when L-CySSG was implemented as the hepatoprotective agent, were not different from that of vehicle control animals at the 99% confidence level. In contrast, D-CySSG, prepared from D-cysteine in the same manner as L-CySSG (unpublished), was totally ineffective. The relative hepatoprotective properties of L-CySSG, CySSME, and GSH-OEt can be compared readily by studying the data of Figure 1.

The remarkable efficacy of L-CySSG in protecting mice against ACP toxicity, contrasted to the lack of protection by D-CySSG, suggests that GSH is being released from L-CySSG. This could be the consequence of an enzymatic reduction of the disulfide bond (for which no evidence is presently available), or from the GSH-dependent thiol-disulfide exchange reaction with L-CySSG catalyzed by liver glutathione reductase (GR) [17], viz.,

\[ \text{L-CySSG} + \text{GSH} \xrightarrow{\text{GR}} \text{GSSG} + \text{CySH} \]

In either case, the result would be the overall reduction of the disulfide bond leading to the net intracellular release of GSH as well as L-cysteine. Thus, L-CySSG provides not only GSH itself, but also the key amino acid for de novo GSH biosynthesis.

A structural feature of L-CySSG, one that has profound practical ramifications, is that both reactive sulfhydryl groups of the GSH and cysteine moieties are masked in a disulfide linkage. Unlike GSH or its carboxy esters with free βSH groups that are subject to oxidation, L-CySSG is stable [9,17], and can, theoretically, be used as a prophylactic agent to abort hepatotoxicity by coformulation with potentially hepatotoxic drugs. Also, because L-CySSG is a ubiquitous endogenous product of cells and, now, a demonstrated prodrug of GSH, it may be beneficial as a dietary supplement to maintain GSH homeostasis and cellular antioxidant levels. Indeed, L-CySSG may have therapeutic application in preventing oxidative stress caused by GSH depletion known to manifest in alcoholic liver disease [18], AIDS [19,20], cataracts [21,22], cystic fibrosis [23], ischemic reperfusion injury [24], and acute respiratory distress syndrome, [25,26], among others [27,28].

REFERENCES

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