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S-Adenosylmethionine but not glutathione protects against galactosamine-induced cytotoxicity in rat hepatocyte cultures

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Abstract

A gradual but extensive depletion of hepatic GSH has long been known to accompany development of galactosamine-induced hepatotoxicity in rats, and some protection from liver injury has been observed after administration of sulfhydryl-donating compounds. Although these observations support a key role for GSH in the underlying mechanism, the impact of GSH depletion and repletion on the hepatotoxic response to galactosamine is unclear. To investigate the role of GSH in galactosamine-induced liver injury, we examined the effect of modulating GSH content on galactosamine toxicity in rat primary hepatocyte cultures. Galactosamine (4 mM) cytotoxicity was assessed by release of lactate dehydrogenase into the culture medium, and hepatocellular GSH content was measured by HPLC with electrochemical detection. The data indicated that prior depletion of GSH with either diethyl maleate or buthionine sulfoximine significantly enhanced galactosamine toxicity; however, addition of GSH-ester or alternate sulfur nucleophiles at various times during the incubation did not abrogate toxicity. In contrast, co-addition of *S*-adenosylmethionine (SAMe) with galactosamine exerted a marked protective effect without significantly altering hepatocyte GSH content. These data suggest that GSH depletion is not directly involved in the sequelae for galactosamine-induced hepatotoxicity, and raise the possibility that SAMe may have hepatoprotective effects that are not dependent on its ability to enhance GSH synthesis.

Keywords: Hepatotoxicity; Hepatocytes; Hepatocyte cultures; Galactosamine; *S*-adenosylmenthionine; Glutathione; Glutathione ethyl-ester; Cysteamine; *N*-acetylcysteine

1. Introduction

Galactosamine has long been used as a model of chemical-induced liver injury because of its ability to induce a diffuse hepatic necrosis in rodents. Hepatotoxicity has been attributed to a rapid (30 min) and extensive loss of uridine nucleotides (<10% of normal levels), leading to inhibition of RNA and protein synthesis (Decker and Keppler, 1972, 1974; Keppler et al., 1969, 1970b). Hepatotoxicity can be prevented by co-treatment with uridine or uridine precursors if they are administered within 4 h of exposure to galactosamine (Decker and Keppler, 1974; Farber et al., 1973; Keppler et al., 1970a).

Accompanying galactosamine toxicity in vivo is a slow but extensive depletion (24 h, <20% of normal) in hepatic reduced glutathione (GSH) (MacDonald et al., 1984, 1985a; McMillan and Jollow, 1992). As noted above for uridine nucleotides, the loss of GSH may also contribute to galactosamine toxicity because

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administration of low molecular weight sulfhydryl compounds (which restore GSH content by stimulating GSH synthesis) up to 12 h after galactosamine has been shown to diminish the toxic response (MacDonald et al., 1984, 1985a,b).

The mechanism underlying the ability of sulfhydryl-donating compounds to protect against galactosamine-induced liver injury is unclear. Galactosamine does not conjugate with GSH (Maley et al., 1968), so it is unlikely that GSH depletion reflects metabolic utilization of GSH as an alternate nucleophile. Similarly, because the loss of GSH is slow and because available evidence suggests that lipid peroxidation is not causal in galactosamine hepatotoxicity (Watanabe et al., 1978; Yoshikawa et al., 1982; Yuasa et al., 1987), it is unlikely that the loss of GSH reflects its utilization as a co-substrate for GSH peroxidase.

Studies in our laboratory (McMillan and Jollow, 1992) have shown that a toxic concentration of galactosamine (4 mM) suppresses re-synthesis of GSH in rat primary hepatocyte cultures, suggesting that galactosamine has a direct or indirect inhibitory effect on GSH synthetase. In vivo studies by Seckin et al. (1995) suggested that galactosamine may affect liver GSH levels by enhancing γ-glutamyl transpeptidase activity. These studies raise the possibility of a third type of mechanism for GSH involvement in chemically induced hepatotoxicity. That is, a hepatotoxicant may interfere with the synthesis of GSH, thus, resulting in a gradual loss of GSH from the hepatocyte and thereby depriving the cell of part of its antioxidant defense system. The hepatocyte would then be more susceptible to oxidative stress resulting from other downstream cellular changes induced by the hepatotoxicant.

In the present studies we have determined the effect of prior depletion of GSH on galactosamine toxicity. Furthermore, we have determined whether repletion of GSH levels or the addition of alternate nucleophiles could abrogate galactosamine toxicity to the hepatocytes. We report that although GSH depletion was found to exacerbate hepatocyte toxicity, repletion of GSH or addition of cysteamine did not protect against galactosamine-induced hepatotoxicity. On the other hand, the methyl donor/GSH precursor, *S*-adenosylmethionine (SAMe), was found to exert a marked protective effect that appeared to be unrelated to its ability to replenish hepatocyte GSH. The data suggest that while loss of hepatocyte GSH content causes hepatocytes to become more susceptible to galactosamine-induced cell death, it does not have a specific role in the underlying mechanism of liver injury.

2. Materials and methods

2.1. Materials

Collagen, gentamicin sulfate, galactosamine, diethyl maleate (DEM), DL-buthionine-[*S*,*R*]-sulfoximine (BSO), 2-mercaptoethylamine (cysteamine), *N*-acetylcysteine (NAC), monoethyl-*O*-GSH (GSH-ester), and *S*-adenosyl-L-methionine (SAMe) were obtained from Sigma Chemical Company (St. Louis, MO). Collagenase Type 2 was obtained from Worthington Biochemicals (Freehold, NJ). Insulin, fetal bovine serum (FBS), penicillin/streptomycin, Dulbecco's phosphate buffered saline (DPBS), Hank's Balanced Salt Solutions, and William's medium E were purchased from Gibco-Invitrogen (Carlsbad, CA).

2.2. Hepatocyte isolation, culture, and treatment

Adult male Long Evans rats (100–122 g) were purchased from Harlan Lab Animals (Indianapolis, IN), housed in AAALAC-accredited animal facilities and maintained on a 12-h light/dark cycle with free access to food and water for at least 1 week prior to use.

Hepatocytes were isolated from the rats (150–250 g) by in situ collagenase perfusion as previously described (McMillan and Jollow, 1992) in accordance with an IACUC-approved protocol. Isolated hepatocytes (>85% viability) were suspended in William's medium E containing 10% FBS, 2 mM L-glutamine, 34 μg/ml insulin, and 0.1 mg/ml gentamicin sulfate and plated at confluence (1.25×10^6 cells/well) in collagen-coated six-well culture plates. Hepatocytes were allowed to attach for 2 h at which time old medium was removed and replaced with fresh medium.

Treatments were initiated at the time of cell attachment. DEM (1.0 mM) was added 30 min prior to addition of galactosamine (4.0 mM). BSO (5.0 mM) was added concurrently with galactosamine. GSH-ester (1.0 or 2.0 mM), cysteamine (4.0 mM), NAC (0.5 or 1.0 mM), or SAMe (1.0 mM) were added 15 min prior to galactosamine addition. DEM was added to the cultures in a small volume of ethanol, so that the concentration of ethanol did not exceed 0.1%. Galactosamine, BSO, cysteamine, NAC, GSH-ester, and SAMe were dissolved in serum-free William's medium E and added in a small volume to the culture medium.

2.3. Determination of hepatocyte GSH content

For GSH determination, hepatocytes were harvested by scraping them into 0.1N HCl. Acidified cell lysates were stored for no more than 24 h at −80 °C until analysis. Total cellular GSH content was determined in the acidified lysates using a specific HPLC assay (Jensen et al., 1986). Chromatography of the samples was performed on a Waters (Milford, MA) reversed-phase C_8 NovaPak column (3.9 × 150 mm) using an isocratic mobile phase consisting of helium-purged 15% methanol in water containing 5.0 mM heptanesulfonic