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Alcohol-induced S-adenosylhomocysteine accumulation in the liver sensitizes to TNF hepatotoxicity: Possible involvement of mitochondrial S-adenosylmethionine transport

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ABSTRACT

Hepatocytes are resistant to tumor necrosis factor-α- (TNF) induced killing/apoptosis under normal circumstances, but primary hepatocytes from rats chronically fed alcohol have increased TNF cytotoxicity. Therefore, there must be mechanism(s) by which alcohol exposure "sensitizes" to TNF hepatotoxicity. Abnormal metabolism of methionine and S-adenosylmethionine (SAM) are well-documented acquired metabolic abnormalities in ALD. S-adenosylhomocysteine (SAH) is the product of SAM in hepatic transmethylation reactions, and SAH hydrolase (SAHH) is the only enzyme to metabolize SAH to homocysteine and adenosine. Our previous studies demonstrated that chronic intracellular accumulation of SAH sensitized hepatocytes to TNF cytotoxicity in vitro. In the current study, we extended our previous observations by further characterizing the effects of chronic alcohol intake on mitochondrial SAM levels in liver and examining its possible involvement in SAH sensitization to TNF hepatotoxicity. Chronic alcohol consumption in mice not only increased cytosolic SAH levels, but also decreased mitochondrial SAM concentration, leading to decreased mitochondrial SAM to SAH ratio. Moreover, accumulation of hepatic SAH induced by administration of 3-deaza-adenosine (DZA-a potent inhibitor of SAHH) enhanced lipopolysaccharide (LPS)/TNF hepatotoxicity in mice in vivo. Inhibition of SAHH by DZA resulted not only in accumulation of cytoplasmic SAH, but also in depletion of the mitochondrial SAM pool. Further studies using mitochondrial SAM transporter inhibitors showed that inhibition of SAM transport into mitochondria sensitized HepG2 cells to TNF cytotoxicity. In conclusion, our results demonstrate that depletion of the mitochondrial SAM pool by SAH, which is elevated during chronic alcohol consumption, plays a critical role in SAH induced sensitization to TNF hepatotoxicity.

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Abbreviations: SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; MAT, methionine adenosyltranserase; DZA, 3′-deazaadenosine; PLP, pyridoxal 5-phosphate; SAHH, s-adenosylhomocysteine hydrolyse; TUNEL, Terminal Deoxynucleotidyl Transferase Biotin-dUTP Nick End Labeling; GSH, glutathione; ALD, alcoholic liver disease; TNF, tumor necrosis factor α; HPLC, high performance liquid chromatography; LPS, lipopolysaccharide; ELISA, enzyme-linked immunosorbent assay

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1. Introduction

Alcoholic liver disease (ALD) continues to be an important health problem in the United States. Compelling evidence generated over the past 1–2 decades demonstrates that tumor necrosis factor-α (TNF) is a critical effector molecule in ALD [1], and abnormal hepatic methionine metabolism is a major feature of ALD [2,3].

Although the fact that TNF plays an etiologic role in ALD is now widely accepted, the exact molecular mechanism(s) involved in its hepatotoxicity are not well defined. TNF can induce both proliferative and cytotoxic responses in hepatocytes [4], and under normal conditions hepatocytes are resistant to TNF induced killing. However, primary hepatocytes isolated from rats chronically fed alcohol have increased TNF cytotoxicity [5], therefore a ''sensitization'' process must be induced by alcohol through which hepatocytes become vulnerable to TNF-induced cytotoxicity. Several factors have been demonstrated to sensitize hepatocytes to TNF cytotoxicity. Some of these factors include agents inhibiting protein synthesis or inducing transcriptional arrest [6]; agents causing total GSH depletion or selective mitochondrial GSH depletion [7,8]; and agents inhibiting proteosome function [9].

S-adenosylhomocysteine (SAH) is the product of methionine in the hepatic transmethylation pathway whereby methyl groups from S-adenosylmethionine (SAM) are transferred to a vast number of molecules including DNA, RNA, biogenic amines, phospholipids, histones, and other proteins via specific methyltransferases. SAH is a potent competitive inhibitor of most methyltransferases. Either a decrease in SAM levels or an increase in SAH levels, or both, results in a decrease in the SAM:SAH ratio which is associated with inhibition of transmethylation reactions [10,11]. Abnormal hepatic methionine metabolism is an acquired metabolic abnormality in ALD. Previous studies, including ours, reported that chronic alcohol in experimental animals not only caused SAM deficiency, but also elevation of SAH [3,12,13]. These observations are consistent with the study by Lieber et al. showing that alcohol consumption decreased hepatic phosphatidylethanolamine N-methyltransferase activity in a baboon model of alcohol-induced hepatic fibrosis [14]. Furthermore, a recent study by Lu's group documented decreased mRNA for multiple methyltransferases in liver biopsies from patients with alcoholic hepatitis [15]. More importantly, our recent studies have shown for the first time that elevation of intracellular SAH levels can sensitize to TNF hepatotoxicity [12]; however, the mechanism for this is still not fully understood.

SAM is the source for methyl groups in almost all biological methylation reactions [16]. Methylation reactions occur in both cytosol and mitochondria. Normal intra-mitochondrial SAM concentrations play a pivotal role in mitochondrial functions because methylation reactions are required for the methylation of RNA and proteins, and they function as intermediates in the biosynthesis of lipoic acid, ubiquinone and biotin [17–20]. Since mitochondria have a relatively large pool of SAM [21], and the enzyme required for SAM synthesis (methionine adenosyltransferase, MAT) is present only in cytosol and not in the mitochondria [22], a specific SAM transporter is needed to maintain normal mitochondrial SAM levels. A human mitochondrial SAM transporter has been recently identified [23]. Moreover, it has been reported that increased cytosolic SAH caused a decrease in SAM concentration in the mitochondria in rat hepatocytes [24]. Furthermore, studies by Colell et al. [25] demonstrated that exogenous SAM supplementation prevented the depletion of mitochondrial GSH pool induced by alcohol consumption and protected hepatocytes from alcohol-consuming rats from TNF-α induced hepatotoxicity. A very recent study by Bailey et al. [26] showed that SAM treatment prevented mitochondrial dysfunction induced by chronic alcohol consumption in rats. It is our hypothesis that SAH accumulation caused by chronic alcohol consumption inhibits mitochondrial SAM transport in hepatocytes, and the subsequent depletion of the mitochondrial SAM pool contributes to the well-documented sensitization to TNF hepatotoxicity observed in ALD.

In this study, we first examined the effects of chronic alcohol consumption on hepatic SAM and SAH levels in both cytosol and mitochondria, and documented that mitochondrial SAM was indeed significantly decreased in a mouse model of ALD. We next evaluated the effect of endogenously elevated SAH levels by 3-deaza-adenosine (DZA), a potent inhibitor of SAH hydrolase (SAHH), on mitochondrial SAM levels, as well as on the sensitization to TNF-induced hepatotoxicity both in vivo and in vitro. Based on these studies, we then performed in vitro studies to evaluate the effects of inhibition of SAM transport into mitochondria (using inhibitors of the mitochondrial SAM transporter) on sensitization to TNF induced hepatotoxicity.

2. Materials and methods

2.1. Materials

Lipopolysaccharide (LPS) (*Escherichia coli* O111:B4) was purchased from Difco Laboratories (Detroit, MI). Before use, LPS was dissolved in sterile, pyrogen-free water, sonicated, and diluted with sterilized saline. Penicillin, streptomycin, Dulbecco's modified Eagle's medium (DMEM), trypsin, and fetal bovine serum were purchased from Invitrogen (Grand Island, NY); cell culture plates were from Corning (Corning, NY). Both human and rat recombinant TNF-α were from R&D Systems (Minneapolis, MN). DNA fragmentation ELISA kit was from Roche (Indianapolis, IN). All other reagents were of the highest purity available and, unless indicated otherwise, were obtained from Sigma (St. Louis, MO).

2.2. Primary rat hepatocyte isolation and culture

A two-step collagenase perfusion technique was used for primary hepatocyte isolation. Briefly, 6–8 weeks old Sprague–Dawley male rats were anesthetized and the portal vein was cannulated thereafter and perfused with Ca^{2+}-free Hanks bicarbonate perfusion buffer. The perfusion was then switched to a re-circulating system with the perfusion medium (100 ml) as above but also containing $CaCl_2$ (4 mM) and 0.05% collagenase (type IV) and continued for another 4–6 min. The digested liver was then chopped, filtered, and centrifuged at $50 \times g$ and 4 °C. The sediment, containing hepatocytes was washed 2–3 times with Ca^{2+}-free Hanks, resuspended, counted, and evaluated for viability by Trypan