Toxicology and Applied Pharmacology 247 (2010) 1–9

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/ytaap

Temporal study of acetaminophen (APAP) and S-adenosyl-L-methionine (SAMe) effects on subcellular hepatic SAMe levels and methionine adenosyltransferase (MAT) expression and activity

J. Michael Brown, John G. Ball, Amy Hogsett, Tierra Williams, Monica Valentovic *

Department of Pharmacology, Physiology, and Toxicology, 1 John Marshall Drive, Joan C. Edwards School of Medicine, Marshall University, Huntington, WV 25755, USA

ARTICLE INFO

Article history:
Received 3 January 2010
Revised 19 March 2010
Accepted 8 April 2010
Available online 4 May 2010

Keywords:
S-adenosyl-L-methionine
Acetaminophen
Hepatotoxicity
S-adenosylhomocysteine
Methionine adenosyltransferase

ABSTRACT

Acetaminophen (APAP) is the leading cause of drug induced liver failure in the United States. Previous studies in our laboratory have shown that S-adenosyl methionine (SAMe) is protective for APAP hepatic toxicity. SAMe is critical for glutathione synthesis and transmethylation of nucleic acids, proteins and phospholipids which would facilitate recovery from APAP toxicity. SAMe is synthesized in cells through the action of methionine adenosyltransferase (MAT). This study tested the hypothesis that total hepatic and subcellular SAMe levels are decreased by APAP toxicity. Studies further examined MAT expression and activity in response to APAP toxicity. Male C57BL/6 mice (16–22 g) were treated with vehicle (Veh; water 15 ml/kg ip injections), 250 mg/kg APAP (15 ml/kg, ip), SAMe (1.25 mmol/kg) or SAMe administered 1 h after APAP injection (SAMe and SAMe + APAP). Hepatic tissue was collected 2, 4, and 6 h after APAP administration. Levels of SAMe and its metabolite S-adenosylhomocysteine (SAH) were determined by HPLC analysis. MAT expression was examined by Western blot. MAT activity was determined by fluorescence assay. Total liver SAMe levels were depressed at 4 h by APAP overdose, but not at 2 or 6 h. APAP depressed mitochondrial SAMe levels at 4 and 6 h relative to the Veh group. In the nucleus, levels of SAMe were depressed below detectable limits 4 h following APAP administration. SAMe administration following APAP (SAMe + APAP) prevented APAP associated decline in mitochondrial and nuclear SAMe levels. In conclusion, the maintenance of SAMe may provide benefit in preventing damage associated with APAP toxicity.

Introduction

Acetaminophen (APAP) is the leading cause of drug induced liver disease in the United States resulting in over 56,000 emergency room visits and approximately 500 deaths each year (Nourjah et al., 2006). One of the problems associated with APAP toxicity is the wide availability of the drug. APAP is present in more than 180 over the counter (OTC) products, which increases the probability of accidental overdose. Acute overdose of APAP leads to severe hepatic centrilobular necrosis (Boyd and Bereczky, 1966; Golden et al., 1981). Rapid treatment with *N*-acetylcysteine (NAC) is currently the clinical treatment for APAP overdose.

The toxicity of APAP is mediated through its biotransformation into *N*-acetyl-p-benzoquinoneimine (NAPQI) by cytochrome P450 2E1, 3A4, and 1A2 (Corcoran et al., 1980; Dahlin et al., 1984; Patten et al., 1993). NAPQI is a strong electrophile that rapidly adducts sulfhydryl groups like those found on reduced glutathione (GSH) (Streeter et al., 1984). GSH depletion by NAPQI precedes APAP toxicity (Larrauri et al., 1987). In addition to adducting proteins, NAPQI also induces mitochondrial dysfunction leading to a severe energy debt and the formation of reactive oxygen species (ROS) that induce further damage in the hepatocytes (Andersson et al., 1990).

The current treatment for APAP overdose is *N*-acetylcysteine (NAC). NAC functions by replenishing cellular stores of cysteine which is involved in the rate-limiting step in the formation of GSH. NAC has been demonstrated to reduce protein adduction in response to APAP overdose (Corcoran et al., 1985). Also, NAC reduces mitochondrial dysfunction and reactive oxygen generation in hepatocytes following APAP overdose (Reid et al., 2005). In order to be effective as a treatment however, NAC must be administered within 8–10 h following APAP overdose, making the study of alternative therapies attractive (Smilkstein et al., 1988).

Currently, *S*-adenosyl-L-methionine (SAMe) is available over the counter and has gained acceptance as beneficial for depression and alcoholic liver disease (Purohit et al., 2007; Williams et al., 2005). SAMe is a ubiquitous cofactor in a variety of biological reactions. SAMe is found in most tissues and is produced at a rate of 6–8 g per day in the liver. The production of SAMe is catalyzed by methionine adenosyltransferase (MAT) (Lu, 2000). *MAT1A* is expressed constitutively in the adult liver and encodes the α1 subunit which composes

* Corresponding author. Fax: +1 304 696 7391.
E-mail address: Valentov@marshall.edu (M. Valentovic).

0041-008X/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.taap.2010.04.018

MAT I (tetramer) and MAT III (dimer). The gene coding for MAT II is *MAT2*, which is widely distributed throughout the body with the exception of the adult liver (Kotb et al., 1997). However, MAT II is expressed in the adult liver during liver regeneration and hepatic cancer (Martinez-Chantar et al., 2003; Paneda et al., 2002). Furthermore, *MAT1A* expression is increased during liver regeneration following partial hepatectomy (Chen et al., 2004).

The protective action of SAMe upon the liver is hypothesized to be mediated via the transmethylation and transsulfuration pathways. SAMe is the principal biological methyl donor in cells. Following methyl group donation, SAMe becomes *S*-adenosylhomocysteine (SAH) that can enter the transsulfuration pathway leading to replenishment of cellular GSH (Finkelstein, 1990). Alterations in either SAMe or its ratio with SAH have been associated with toxicant exposure. For example, SAMe levels were decreased in humans with alcoholic liver disease (Purohit et al., 2007). Furthermore, any decline in SAMe or decrease in the ratio of SAMe:SAH has been demonstrated to inhibit cellular transmethylation reactions (Purohit et al., 2007) as SAH is a competitive inhibitor of transmethylation reactions (Kharbanda 2007). Previous research by our lab and others has demonstrated that SAMe protects against APAP induced hepatotoxicity when administered just prior to APAP overdose (Bray et al., 1992; Terneus et al., 2007). Recent studies in our laboratory showed that SAMe was protective for APAP hepatotoxicity when SAMe was administered 1 h after APAP overdose (Terneus et al., 2008). SAMe administration after APAP overdose is a more clinically relevant experimental model since antidotes are not normally administered to humans until after a toxic exposure.

In our laboratory, SAMe and NAC displayed a comparable level of protection for APAP toxicity in mice, when comparisons were made on the basis of a mmol/kg dosage (Terneus et al., 2008). However, the mechanism for SAMe protection of APAP toxicity remains to be elucidated. Diminished hepatic SAMe levels have been linked to liver damage mediated by toxicants including ethyl alcohol and acetaminophen. Alcohol exposure was associated with diminished hepatic SAMe levels in baboons and mice (Lieber et al., 1990 and Song et al., 2007). SAMe hepatic levels were lower in rats fed 600 mg/kg in food for 4 weeks (Verala-Moreiras et al., 1993). Hepatic SAMe levels were diminished 24 h after a very high acute dose of 750 mg/kg APAP in fed BALb/c mice (Oz et al., 2004) which is higher than most human overdose ingestions. Very little research has been done to examine first, the effects of lower APAP doses that are similar to human exposure and second the temporal changes in intracellular SAMe levels following APAP overdose. A decrease in intracellular SAMe levels following APAP overdose would have deleterious effects on DNA methylation, phospholipid formation and GSH synthesis. Additionally, given that expression of MAT appears to be required for liver regeneration following damage, the exploration of APAP overdose effects on MAT levels warrants further study. Therefore, the purpose of the current study was to investigate the effect of APAP overdose on hepatic, 15,000 ×*g* supernatant, nuclear and mitochondrial SAMe levels, as well as alterations of hepatic MATI/III and MATII. By examining these components of SAMe metabolism, the present study hopes to shed light on the mechanism of SAMe protection against APAP toxicity.

Methods and materials

Materials. SAMe toluenesulfonate salt was used in all experiments (Sigma Chemical Co., St. Louis, MO). The ALT reagent kit (TR-71021) was purchased from Thermo Electron Corporation (Louisville, CO). All solvents were of HPLC grade and other reagents were of comparable quality and purchased from Sigma Chemical Co. (St. Louis, MO) or Fisher Scientific (Pittsburgh, PA).

Animals. Male C57BL/6 mice were obtained from Hilltop Lab Animals Inc. (Scottsdale, PA). Animals included in the study were between 4 and 8 weeks of age and weighed 16–24 g. Mice were maintained in a facility in compliance with the American Association for Accreditation of Laboratory Animal Care. Mice were maintained at controlled temperature (21–23 °C), humidity (40–55%), and 12 h light cycles (lights on 6:00 AM to 6:00 PM). An acclimation period of 7 days was observed prior to the beginning of any experiment. The animals received Purina rodent chow and water *ad libitum*. The mice were fasted for 16 h prior to any experiment, but maintained free access to water.

SAMe and NAC treatment following APAP overdose. Mice were randomly allocated into the following groups: vehicle (Veh; 15 ml/kg water by intraperitoneal (ip) injection), SAMe (1.25 mmol/kg 5 ml/kg ip injection), APAP (250 mg/kg 15 ml/kg ip injection), and SAMe administered 1 h after APAP (SAMe + APAP; doses same as previously listed). Mice treated with NAC were randomly divided into the following groups: Veh, NAC (1.25 mmol/kg with 5 ml/kg ip injection), APAP, and NAC + APAP. Mice were fasted for 16 h prior to the administration of APAP. SAMe and NAC were administered 1 h after APAP. Mice were anesthetized with carbon dioxide 2, 4, and 6 h after APAP administration with SAMe treatment, and 4 h following APAP treatment when NAC was used as a comparison for SAMe. Blood was collected by cardiac puncture in heparin-rinsed 1 ml syringes for determination of serum ALT activity, which serves as an indicator of liver injury. Livers were then isolated and placed in ice cold Krebs buffer (126 mM NaCl, 5 mM KCl, 3 mM $MgSO_4$, 3 mM Na_2HPO_4, and 1 mM $CaCl_2$; pH 7.4), blotted, and weighed.

Mitochondrial isolation. Mitochondria were isolated using a modification of a previously published protocol (Gogvadze et al., 2004). Briefly, the liver was isolated, blotted, weighed and placed in Mitochondrial Isolation Buffer A (225 mM sucrose, 3 mM KH_2PO_4, 5 mM $MgCl_2$, 20 mM KCl, 20 mM triethanolamine, and 2 mM EGTA; pH 7.4). The liver was minced and homogenized in a Dounce homogenizer on ice. Following homogenization, the liver was centrifuged at 600 ×*g* for 10 min. The resultant pellet was discarded and the supernatant was centrifuged at 15,000 ×*g* for 5 min. After the final centrifugation, the supernatant was retained for analysis of SAMe levels. The pellet containing the mitochondria was resuspended in Mitochondrial Isolation Buffer B (same as Buffer A except lacking EGTA) for a final concentration of 1 mg tissue weight/1 ml Buffer B. Samples were stored at −80 °C until analysis.

Nucleus isolation. The protocol for isolating the nuclei of liver cells was adapted from Graham (2001). Briefly, the liver was homogenized in ice cold nuclear isolation medium (0.25 M sucrose, 25 mM KCl, 5 mM $MgCl_2$, and 10 mM Tris–Cl; pH 7.4) using a Dounce homogenizer and adjusted to a final 3 ml volume. The homogenate was centrifuged at 800 ×*g*, the supernatant was discarded, new buffer was added and centrifuged at 800 ×*g* and the supernatant was discarded. The pellet was resuspended in 1 ml nuclear isolation medium followed by 2 ml of sucrose density barrier (1.15 M sucrose, 10 mM KCl, 2.5 mM $MgCl_2$, and 5 mM Tris–Cl; pH 7.4) and vortexed. Six mL of sucrose density barrier was then layered under the homogenate and centrifuged for 1 h at 100,000 ×*g*. The pellet was resuspended in 1 ml nuclear isolation medium and stored at −80 °C until use.

HPLC analysis of hepatic SAMe levels. A 200 mg aliquot of liver was homogenized on ice in 0.4 mM $HClO_4$ and adjusted to a final 1 ml volume. Mitochondrial and 15,000 ×*g* supernatant suspensions were added to an equal volume of 0.4 mM $HClO_4$ to precipitate protein. Nuclear samples were concentrated by lyophilizing 1 ml of sample (total liver weight 600–900 mg) and reconstituting the sample in 125 µl 0.4 mM $HClO_4$. The samples were centrifuged at 10,000 ×*g* for 10 min at 4 °C and filtered through 0.45 µM MIllex®-HV filters (Millipore; Billericia, MD). A 20 µl sample of the filtrate was analyzed