Role of $S$-adenosylmethionine, folate, and betaine in the treatment of alcoholic liver disease: summary of a symposium$^{1-4}$

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ABSTRACT

This report is a summary of a symposium on the role of $S$-adenosylmethionine (SAM), betaine, and folate in the treatment of alcoholic liver disease (ALD), which was organized by the National Institute on Alcohol Abuse and Alcoholism in collaboration with the Office of Dietary Supplements and the National Center for Complementary and Alternative Medicine of the National Institutes of Health (Bethesda, MD) and held on 3 October 2005. SAM supplementation may attenuate ALD by decreasing oxidative stress through the up-regulation of glutathione synthesis, reducing inflammation via the down-regulation of tumor necrosis factor-$\alpha$ and the up-regulation of interleukin-10 synthesis, increasing the ratio of SAM to $S$-adenosylhomocysteine (SAH), and inhibiting the apoptosis of normal hepatocytes and stimulating the apoptosis of liver cancer cells. Folate deficiency may accelerate or promote ALD by increasing hepatic homocysteine and SAH concentrations; decreasing hepatic SAM and glutathione concentrations and the SAM-SAH ratio; increasing cytochrome P4502E1 activation and lipid peroxidation; up-regulating endoplasmic reticulum stress markers, including sterol regulatory element–binding protein-1, and proapoptotic gene caspase-12; and decreasing global DNA methylation. Betaine may attenuate ALD by increasing the synthesis of SAM and, eventually, glutathione, decreasing the hepatic concentrations of homocysteine and SAH, and increasing the SAM-SAH ratio, which can trigger a cascade of events that lead to the activation of phosphatidylethanolamine methyltransferase, increased phosphatidylcholine synthesis, and formation of VLDL for the export of triacylglycerol from the liver to the circulation. Additionally, decreased concentrations of homocysteine can down-regulate endoplasmic reticulum stress, which leads to the attenuation of apoptosis and fatty acid synthesis. Am J Clin Nutr 2007:86:14–24.

KEY WORDS

Alcohol, betaine, $S$-adenosylmethionine, folate, liver disease

INTRODUCTION

Alcoholic liver disease (ALD) is characterized by fatty liver, steatohepatitis, fibrosis, cirrhosis, and potentially hepatocellular carcinoma. Several mechanisms have been proposed for the pathogenesis of ALD, including acetaldehyde toxicity, oxidative stress, endotoxins, cytokines, chemokines, a compromised immune system, and nutritional deficiencies. Increasing evidence suggests that altered methionine folate metabolism can also contribute to the development of ALD (1, 2). Chronic ethanol exposure has been shown to decrease hepatic concentrations of $S$-adenosylmethionine (SAM) (3–5), increase plasma concentrations of homocysteine (6–8), increase hepatic concentrations of $S$-adenosylhomocysteine (SAH) (9–11), and decrease plasma concentrations of folate (12) in animal and human studies. These changes in methionine metabolism are associated with different degrees of liver injury. Conversely, exogenous administration of SAM has been shown to attenuate alcoholic liver injury in animal studies (3, 5, 13). In addition, betaine (trimethylglycine), a metabolite of choline, has been shown to attenuate alcoholic liver injury by increasing the concentrations of hepatic SAM and decreasing the concentrations of homocysteine and SAH in other animal studies (8, 11, 14, 15). Understanding the role of SAM, folate, and betaine in mitigating alcoholic liver injury may help to develop effective and safe therapies for ALD and non-ALD.

The National Institute on Alcohol Abuse and Alcoholism, in collaboration with the Office of Dietary Supplements and National Center for Complementary and Alternative Medicine of the National Institutes of Health, organized a symposium on the role of SAM, betaine, and folate in the treatment of ALD in Bethesda, MD, held on 3 October 2005. The following topics were discussed by 7 speakers: 1) methionine metabolism (Norlin Purohit, Duke University Medical Center, Durham, NC (MFA); the Department of Medicine, University of Louisville, Louisville, KY (SB and CJM); the Department of Animal Sciences, University of Wisconsin, Madison, WI (NJB); the VA Medical Center, Liver Study Unit R-151, Omaha, NE (SB and CJM); and the National Center for Complementary and Alternative Medicine (QYL) and the Office of Dietary Supplements (CS), National Institutes of Health, Bethesda, MD); 2) the effects of folate and betaine on ALD (Charles H Halsted, University of Southern California, Los Angeles, CA (CHM); the VA Medical Center, Liver Study Unit R-151, Omaha, NE (KKK); and the National Center for Complementary and Alternative Medicine (QYL) and the Office of Dietary Supplements (CS), National Institutes of Health, Bethesda, MD); and 3) the effects of SAM on ALD (Neel Kaplowitz, University of Virginia, Charlottesville, VA (NK and SCL); and the National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, MD (VP and SZ)). The opinions expressed herein are those of the authors and do not necessarily reflect the official position of NIAAA, ODS, NCCAM, or any other part of the National Institutes of Health.

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**Betaine**

Betaine is a methyl donor used in transmethylation reactions, whereby SAM is converted to SAH by transferring the methyl group to diverse biological acceptors (16). SAH is then converted to homocysteine and adenosine in a reaction catalyzed by homocysteine methyltransferase (BHMT). The conversion of homocysteine to methionine is an essential reaction to conserve methionine, detoxify homocysteine, and produce SAM.

**FIGURE 1.** Hepatic methionine metabolism. The reaction in the first step of methionine metabolism is catalyzed by methionine adenosyltransferase (MAT), which generates S-adenosylmethionine (SAM). SAM is converted to S-adenosylhomocysteine (SAH) during transmethylation reactions. SAH hydrolysis then catalyzes the reversible hydrolysis of SAH to yield homocysteine and adenosine. In the liver, homocysteine can undergo 3 metabolic pathways, one of which is the transsulfuration pathway, which converts homocysteine to cysteine. Homocysteine condenses with serine to form cystathionine in a reaction catalyzed by cystathionine β-synthase, which requires vitamin B-6 as a cofactor. Cleavage of cystathionine, catalyzed by another vitamin B-6–dependent enzyme, γ-cystathionase, then releases free cysteine—the rate-limiting precursor of reduced glutathione (GSH) synthesis. GSH is synthesized in all mammalian cells via a 2-step process; the first step is rate-limiting and its reaction is catalyzed by glutamate-cysteine ligase (GCL), and the reaction in the second step is catalyzed by glutathione synthetase. The other 2 pathways that metabolize homocysteine resynthesize methionine from homocysteine. In one pathway, the reaction is catalyzed by methionine synthase (MS), which requires normal concentrations of folate and vitamin B-12; in the other pathway, the reaction is catalyzed by betaine-homocysteine methyltransferase (BHMT), which requires betaine—a metabolite of choline.

**ROLE OF S-ADENOSYL METHIONINE IN ALCOHOLIC LIVER DISEASE**

SAM, a metabolite of methionine, is an important molecule that is required for many vital functions and survival of cells in the body. It is the principal biological methyl donor required for methylation of DNA, RNA, biogenic amines, phospholipids, histones, and other proteins. It is the precursor for the synthesis of polyamines, which are required for cell proliferation and the maintenance of cell viability. In the liver, SAM is a precursor for glutathione—a major endogenous antioxidant that protects cells against injury by scavenging free radicals, which are involved in the pathogenesis of ALD. Thus, SAM deficiency can impair many vital functions of the liver, which render it susceptible to injury by toxic agents such as alcohol.

**Ethanol, hepatic SAM depletion, and consequences**

Animal and human studies suggest a link between ethanol consumption and hepatic SAM depletion. Chronic ethanol administration depleted the hepatic concentrations of SAM in rats (4, 21–23), in mice (5, 24), in baboons (3), and in micropigs (10, 25). Reduced hepatic SAM concentrations have also been reported in alcoholic hepatitis patients (26).

Hepatic SAM depletion by chronic ethanol administration is associated with liver injury of variable magnitude: fatty liver in rats (4, 21, 23); fatty liver, inflammation, and fibrosis in baboons (3); fatty liver and inflammation in micropigs (25); and hepatitis in humans (26). The effect of SAM depletion is well characterized in MAT1A knockout mice, which have markedly elevated serum methionine concentrations and reduced hepatic SAM and glutathione concentrations (27). At 3 mo, MAT1A knockout mice develop hepatic hyperplasia and are more prone to develop fatty liver due to a choline-deficient diet. At 8 mo of age, these

**METHIONINE METABOLISM CYCLE**

Methionine is an essential amino acid that is primarily metabolized in the liver (Figure 1). The first step in methionine metabolism is the formation of SAM in a reaction catalyzed by MAT (16, 17). Under normal conditions, most of the SAM generated is used in transmethylation reactions, whereby SAM is converted to SAH by transferring the methyl group to diverse biological acceptors (16). SAH is then converted to homocysteine and adenosine in a reversible reaction catalyzed by SAH hydrolase (16). In the liver, homocysteine is metabolized by transsulfuration and transmethylation pathways. In the transsulfuration pathway, homocysteine condenses with serine to form cystathionine in a reaction catalyzed by cystathionine β-synthase, which requires vitamin B-6 as a cofactor. The activity of cystathionine β-synthase is allosterically regulated by SAM (18, 19). Cystathionine is then cleaved by another vitamin B-6–dependent enzyme, γ-cystathionase, which results in the release of free cysteine, the rate-limiting precursor for reduced glutathione synthesis (16, 20). In the transmethylation pathway, homocysteine can be converted to methionine by 2 alternate reactions (16, 17). Normally, utilizing folate and through the action of methionine synthase (MS), a methyl group is transferred from N5-methyltetrahydrofolate (MTHF) to vitamin B-12 to form methylcobalamin, which in turn transfers the methyl group to homocysteine to produce methionine. Alternatively, when MS is compromised by exposure to ethanol (9, 10, 14), a methyl group is transferred from betaine to homocysteine to form methionine in a reaction catalyzed by betaine-homocysteine methyltransferase (BHMT). The conversion of homocysteine to methionine is an essential reaction to conserve methionine, detoxify homocysteine, and produce SAM.
Mechanisms of alcohol-induced hepatic SAM depletion

Several mechanisms have been proposed for SAM depletion: 1) inactivation of MAT, 2) excessive consumption of SAM by liver, and 3) inhibition of endogenous methionine synthesis due to impaired homocysteine methylation.

Inactivation of MAT impairs the metabolism of methionine to SAM, which leads to SAM depletion. For instance, MAT1A knockout mice have reduced hepatic concentrations of SAM and glutathione (27). Chronic alcohol exposure may decrease hepatic SAM concentrations by inactivating MAT. The activity of MAT was significantly reduced in liver biopsy samples from alcoholic and nonalcoholic cirrhotic patients (29, 30). This effect could be partly due to a decreased expression of MAT. Indeed, recent data indicate a 50% decrease in the expression of MAT1A (liver-specific MAT) in liver samples obtained from alcoholic hepatitis patients (26) and from ethanol-fed micropigs (31). The decrease in MAT activity may occur pre- and posttranslationally (17). MAT1A expression is diminished in end-stage cirrhotic patients independent of the etiology (32). MAT I/III (but not MAT II) can also be inactivated via covalent modification of a critical cysteine residue at position 121 (17). The inactivation can be reversed by glutathione and other thiol-reducing agents. Nitrosylation of Cys 121 of MAT I/III and its inactivation have been shown, both in vitro and in vivo, in animals treated with lipopolysaccharide (LPS) (33, 34). Ethanol metabolism is known to generate free radicals, both reactive oxygen species and reactive nitrogen species, which may inactivate hepatic MAT through the oxidation or nitrosylation of cysteine residue at position 121.

Ethanol may deplete hepatic SAM concentrations by increased SAM consumption. This was apparent in a rat study in which chronic alcohol administration decreased hepatic SAM and glutathione concentrations without affecting MAT activity (23). This suggests that the utilization of SAM is increased as a precursor for the synthesis of glutathione to counteract alcohol-induced oxidative stress.

Chronic ethanol administration has been shown to decrease the hepatic activity of MS and to reduce hepatic concentrations of folate and betaine (10, 12, 14, 31, 35). These factors are known to participate in the synthesis of endogenous methionine through methylation of homocysteine. Thus, alcohol may deplete hepatic SAM by inhibiting the synthesis of its endogenous precursor methionine.

SAM treatment in alcoholic liver injury

The fact that SAM is essential for multiple metabolic reactions and that chronic ethanol administration can deplete its hepatic concentration in association with liver injury prompted researchers to evaluate its role in the attenuation of ALD in animals and humans.

In intact rats, SAM administration attenuated alcohol-induced steatosis and restored hepatic glutathione concentrations (13), whereas it attenuated ethanol-induced depletion of mitochondrial glutathione and restored mitochondrial function in hepatocytes (36). In isolated perfused rat liver, SAM administration attenuated ethanol hepatotoxicity by 1) decreasing aspartate transaminase (AST) and lactate dehydrogenase release in the perfusate, 2) restoring mitochondrial and homogenate glutathione concentrations, and 3) restoring normal hepatic oxygen consumption (37). In mice, SAM treatment significantly attenuated acute alcohol-induced liver injury [steatosis, necrosis, and increased serum alanine transaminase (ALT) activity], which was associated with restoration of hepatic SAM and mitochondrial glutathione concentrations and attenuation of lipid peroxidation (5). In baboons, SAM attenuated alcohol-induced liver injury by repairing mitochondrial injury, which restored plasma glutathione concentrations and decreased plasma concentrations of AST (3).

The therapeutic potential of SAM was tested in a 24-mo randomized, placebo-controlled, double-blind, multicenter clinical trial in 123 patients with alcoholic cirrhosis. SAM treatment improved survival or delayed the need for liver transplantation in patients with alcoholic liver cirrhosis, especially in those with less advanced liver disease (38). In this trial, increased hepatic concentrations of glutathione may have contributed to the beneficial effect of SAM because, in another study, oral administration of 1.2 g SAM/d for 6 mo significantly increased hepatic glutathione concentrations in ALD patients (39). In a recent clinical meta-analysis review, Rambaldi and Gluud (40) could not find evidence to support or refute the use of SAM in the treatment of patients with ALD.

SAM treatment in non-alcoholic liver injury

SAM has also been shown to attenuate liver injury induced by other toxic agents such as CCl₄, acetonaminophen, and diethylnitrosamine. In rats, SAM treatment attenuated CCl₄-induced liver fibrosis by restoring hepatic MAT activity and glutathione concentrations and by reducing lipid peroxidation (41, 42). In cultured hepatocytes, SAM protected against CCl₄-induced hepatotoxicity by suppressing the leakage of glutamate-oxalate-transaminase and glutamate-pyruvate-transaminase (43). In acetonaminophen-treated mice, SAM significantly attenuated liver injury by preventing decreases in liver and blood SAM concentrations and by attenuating both cytosolic and mitochondrial glutathione depletion and mitochondrial dysfunction (44). In addition, SAM appears to have protective effects against hepatic carcinogenesis. SAM inhibited growth and induced phenotypic reversion and apoptosis of preneoplastic cells and decreased the development of hepatic diethylnitrosamine-induced neoplastic nodules in rats (45, 46). These changes were associated with restoration of the hepatic SAM pool and DNA methylation. Furthermore, exogenous SAM inhibits the growth of cultured hepatoma cells (47).

Mechanisms of SAM’s protective effects

SAM may provide protection against liver injuries through various mechanisms, and some of these mechanisms are discussed below.

Attenuation of oxidative stress by restoring glutathione concentrations

Oxidative stress plays a major role in the development of alcoholic liver injury. This can occur because of excess accumulation of free radicals, their delayed elimination, or both. Glutathione, an endogenous antioxidant, is capable of attenuating oxidative stress by scavenging free radicals. Therefore, one way to attenuate alcoholic liver injury is to increase hepatic concentrations of glutathione. In this regard, SAM administration restored mouse spontaneously develop NASH (27), and by 18 mo, the majority of the knockout mice develop hepatocellular carcinoma even when consuming a normal diet (28).
hepatic concentrations of glutathione depleted by alcohol in mice (5), rats (22, 36, 48), and patients affected with ALD and non-ALD (39). Importantly, SAM can restore the hepatic mitochondrial glutathione concentration (22, 36, 48), which is critical for the maintenance of mitochondrial function and rescue of mitochondria from free radical damage. The restoration of mitochondrial glutathione by SAM was associated with normalization of the fluidity of the mitochondrial inner membrane (48), which suggests a mechanism by which alcohol impairs and SAM repairs mitochondrial glutathione transport from the cytosol. In addition to protecting hepatocytes from oxidative stress, elevated glutathione concentrations also rescued hepatocytes from tumor necrosis factor-α (TNF-α) toxicity, such as necrosis (22).

**Attenuation of inflammation**

The inflammatory response is an important feature of ALD. Cytokines play an important role in both the initiation and the attenuation of alcoholic liver injury. In this regard, TNF-α is a proinflammatory cytokine, whereas interleukin-10 (IL-10) is an antiinflammatory cytokine. Evidence that SAM may attenuate alcoholic liver injury by decreasing TNF-α concentrations is based on the following information: 1) SAM attenuated LPS-induced increases in serum TNF-α concentrations in rats maintained on a methionine-restricted and choline-deficient diet (49), 2) SAM inhibited TNF-α production in an LPS-stimulated RAW 264.7 murine monocyte cell line (50), and 3) 5′-methylthioadenosine (MTA), a metabolite of SAM, suppressed LPS-stimulated TNF-α concentrations in mice and in RAW 264.7 cells (51). SAM may also attenuate alcoholic liver injury by increasing IL-10 concentrations on the basis of the following information: 1) SAM treatment enhanced LPS-induced IL-10 protein production and gene expression in a RAW 264.7 murine macrophage cell line (50, 52), and 2) MTA increased LPS-stimulated IL-10 synthesis in mice and in RAW 264.7 cells (51). These results suggest that SAM may attenuate alcoholic liver injury by decreasing TNF-α concentrations and increasing IL-10 concentrations. The inhibitory effect of SAM on TNF-α expression most probably occurs at the transcriptional level. Work done in murine macrophages has shown that SAM and its metabolite MTA can significantly down-regulate LPS-inducible nuclear transcription factor κB transcriptional activity and consequent TNF-α expression (53). On the other hand, a SAM-mediated increase in intracellular adenosine concentrations may contribute to the increase in LPS-inducible IL-10 expression. Data obtained from a murine macrophage cell line suggest that increased adenosine concentrations affected by SAM modulate IL-10 expression via binding to the adenosine A2 receptor (50).

**Prevention of apoptosis of hepatocytes**

Apoptosis is a form of cell death characterized by organized nuclear and ultimately cellular fragmentation. Increasing evidence suggests that apoptosis of hepatocytes plays an important role in the initiation of alcoholic liver injury (54). SAM and its metabolite MTA protected rat hepatocytes from okadaic acid-induced apoptosis in a dose-dependent manner. This effect was mediated through attenuation of mitochondrial cytochrome-c release, caspase-3 activation, and poly(ADP-ribose) polymerase cleavage (55). SAM treatment also inhibited bile acid–induced apoptosis of cultured rat hepatocytes (56, 57). Thus, SAM may attenuate ALD by preventing apoptosis of hepatocytes.

The mechanism of this effect is targeted at the mitochondria because SAM prevented okadaic acid–induced cytochrome-c release. However, the molecular mechanism of this effect remains unclear.

**Induction of apoptosis of liver tumor cells**

Chronic alcohol consumption is a risk factor for hepatocellular carcinoma in humans. In a rat study, chronic ethanol administration significantly decreased hepatic concentrations of methionine, SAM, and DNA methylation by ≈40% (58). In addition, c-myc was hypomethylated and its mRNA concentration increased, and genome-wide DNA strand breakage increased. Studies in the ethanol-fed micropig model showed an association of a decrease in the ratio of SAM to SAH (SAM:SAH) with hepatocellular apoptosis (10) and with increased DNA oxidation and strand breaks (25). These changes may predispose the liver to malignancy, which suggests a role of ethanol in the development of hepatocellular carcinoma. Apoptosis is a mechanism by which tumor cells can be eliminated. In this regard, SAM has been shown to induce apoptosis in the liver cancer cell lines HepG2 and HuH-7 (55). SAM induced apoptosis of preneoplastic cells in rats induced by diethylnitrosamine (45, 46). Furthermore, SAM has been shown to selectively induce an apoptotic factor, Bcl-xS, in a time- and dose-dependent manner in HepG2 cells, but not in normal hepatocytes, by increasing alternative splicing of Bcl-x (59), which suggests a mechanism whereby SAM can induce the apoptosis of neoplastic cells.

**SAM summary**

SAM supplementation may attenuate ALD through various mechanisms (Figure 2). By being a precursor of glutathione as well as by activating cystathionine β-synthase, SAM up-regulates the trans-sulfuration pathway, which leads to the increased synthesis of glutathione, which in turn attenuates oxidative stress.

The down-regulation of oxidative stress is expected to attenuate inflammation, fibrosis, and eventually ALD. SAM may attenuate inflammation and, thus, ALD by down-regulating TNF-α and up-regulating IL-10 synthesis. SAM also may attenuate liver injury by inhibiting the apoptosis of normal hepatocytes as well as by stimulating the apoptosis of liver cancer cells.

**ROLE OF FOLATE IN ALCOHOLIC LIVER DISEASE**

Folate is a water-soluble vitamin that plays an integral role in methionine metabolism and DNA synthesis. Folate in its 5-methyltetrahydrofolate (5-MTHF) form can transfer a methyl group to homocysteine via an MS-catalyzed reaction to form endogenous methionine, which is a precursor of SAM. Thus, folate helps maintain normal concentrations of homocysteine, methionine, and SAM. Folate deficiency can impair methionine metabolism, which leads to hyperhomocysteinemia as well as depletion of methionine and SAM, which are important features of ALD (60).

**Folate concentrations in alcoholics**

Studies performed in the United States before folic acid fortification and more recently in several European countries have reported decreased serum or red blood cell folate concentrations in the majority of chronic alcoholic patients who consume >80 g ethanol/d (6, 12, 61, 62). More than 40 y ago, a US study
reported a greater incidence of very low serum folate concentrations (<3.0 ng/mL) in patients with ALD than in alcoholics without liver disease (61), whereas another survey found low serum folate concentrations in 78% of 140 ALD patients admitted to a large US city hospital (63).

Mechanisms of folate depletion in alcoholics

The possible causes of folate deficiency in chronic alcoholism include: 1) diet lacking in folate-rich foods (61), 2) intestinal malabsorption (64) that may be due to decreased transcription of the reduced folate carrier required for folate transport across intestinal membranes (65), 3) decreased liver uptake (66, 67), and 4) increased urinary excretion (68–70). Decreased liver folate storage may be a critical cause of folate deficiency in ALD patients.

Folate deficiency and homocysteine concentrations in alcoholics

Increasing evidence suggests that homocysteine is involved in the pathogenesis of alcoholic liver injury (71). Because folate is required for the metabolism of homocysteine, its deficiency can lead to the elevation of serum homocysteine concentrations. Indeed, folate deficiency was shown to be associated with elevated serum homocysteine concentrations in 50–60% of chronic alcoholics in Spanish (12) and Portuguese (6) studies. The Spanish study of 103 heavy drinkers included 19 ALD patients whose mean serum homocysteine concentration was not different from that of the alcoholics without liver disease. In these subjects, elevated serum homocysteine correlated inversely with the reduced folate-sufficient diet group together with an 8-fold increase in serum homocysteine concentrations and was highest in a subgroup of patients who carried the methylenetetrahydrofolate reductase (MTHFR) 677T→T genotype (12). MTHFR converts 5,10-methylene tetrahydrofolate to methylenetetrahydrofolate, the substrate for MS. Because 5,10-methylene tetrahydrofolate is also a substrate for thymidine synthase, which converts uracil to thymidine, its diversion to the MTHFR reaction results in less availability of uracil for the thymidine synthase reaction, with subsequent decrease in thymidine synthesis and hence nucleotide imbalance that may contribute to an increased risk of carcinogenesis (10). Serum homocysteine concentrations were elevated in >80% of a series of 42 chronic alcoholics admitted to a Swedish detoxification center, and these concentrations declined rapidly with abstinence and a nutritious diet (72).

Role of dietary folate deficiency in the development of alcoholic liver injury

The micropig has been used as a model to understand the role of folate deficiency in the development of ALD (25). In this study, 4 groups of 6 animals each were administered the following diets for 14 wk: folate-sufficient diet (control), folate-deficient diet, ethanol-containing (40% of total kcal) folate-sufficient diet, and ethanol-containing folate-deficient diet. The effects of diets were determined by evaluating histopathologic changes, alterations in methionine metabolism, and markers of oxidative stress. After 3 mo of feeding, serum homocysteine concentrations were increased maximally in the combined folate-deficient ethanol diet group together with an 8-fold increase in serum AST and the histopathology of steatohepatitis (25). In contrast, 12 mo of ethanol feeding was required for induction of similar histopathology in micropigs fed ethanol with a folate-sufficient diet (74). These findings were associated with elevated hepatic SAH concentrations, reduced SAM:SAH and
SAM and glutathione concentrations, decreased global DNA methylation (25), increased DNA strand breaks, and increased concentrations of hepatic lipid peroxide, malondialdehyde, and the DNA oxidation product 8-oxo-2’-deoxyguanosine. It is important to note that folate deficiency alone did not affect liver histology, whereas ethanol alone induced only steatosis in some animals.

Using liver specimens from the same groups of micropigs, studies of transmethylation regulatory enzymes found that folate deficiency or ethanol exposure, singly or in combination, reduced concentrations of MTHFR, MS, MAT, and SAH hydrolase (31). A subsequent study of molecular mechanisms using liver samples from the same animals found that hepatocellular apoptosis was maximal in pigs fed the combined folate-deficient and ethanol diet. This finding was associated with increased transcription and protein concentrations of CYP2E1 and activation of ER stress markers, including sterol regulatory element–binding protein (SREBP), lipid synthesis enzymes, and activated caspase-12 (75). The findings of correlations of concentrations of CYP2E1, apoptosis, and ER stress signals with elevated concentrations of homocysteine and SAH and decreases in SAM:SAH were consistent with the notion that the pathogenesis of ALD is mediated through effects of ethanol feeding, magnified by folate deficiency, on methionine metabolism.

It is important to note that, with folic acid fortification of the American diet, chronic ethanol exposure may not deplete hepatic folate to an extent sufficient to impair homocysteine metabolism, although ethanol may still impair homocysteine metabolism by decreasing the activity of MS. This notion is corroborated by a study in which 4 wk of ethanol feeding of rats with 20 times the basal folate requirement did not reduce plasma or hepatic folate concentrations, although plasma homocysteine concentrations were significantly elevated (7).

In summary, in the absence of dietary fortification with folic acid, folate deficiency and the accompanying hyperhomocysteinemia are common findings in chronic alcoholics who consume >80 g ethanol/d. ALD patients are probably at greater risk of folate deficiency because of the decreased liver storage of folate. Studies using the micropig model have shown that the onset of ALD is accelerated in the presence of folate deficiency, which also magnifies the effect of chronic ethanol exposure on altered methionine metabolism. A significant role of altered methionine metabolism in the pathogenesis of ALD is supported by recent studies that have linked the induction of elevated SAH and homocysteine concentrations to increased activation of CYP2E1 and ER stress pathways of apoptosis and steatosis in folate-deficient ethanol-fed micropigs. Folate deficiency alone does not lead to liver injury, but it can accentuate or promote the development of ALD. Whether exogenous folate administration would attenuate ALD needs further investigation.

ROLE OF BETAINES IN ALCOHOLIC LIVER DISEASE

Betaine (trimethylglycine) is an important human nutrient obtained from a variety of foods, and it is also available as a dietary supplement. As a methyl donor, betaine provides a methyl group to homocysteine to form methionine in a reaction that is catalyzed in the liver by BHMT. This helps to maintain an adequate supply of liver methionine for the synthesis of SAM and the regulation of the homocysteine concentration. Betaine is synthesized in the liver from choline in a reaction that is catalyzed by choline oxidase. To understand the role of betaine in the treatment of ALD, researchers have used various animal models of alcoholic liver injury, which are described below.

Rat model of alcoholic fatty liver injury and betaine

In this model, animals are fed a Lieber-DeCarli ethanol-containing diet (36% of total energy) for up to 4 wk. This treatment results in the development of fatty liver, which is associated with significant alterations in methionine metabolism. The liver injury does not progress to necroinflammation or fibrosis despite continuous ethanol feeding. In this model, betaine treatment attenuates fatty liver and restores (normalizes) methionine metabolism. These protective effects of betaine are presented below in detail.

Betaine lowers homocysteine concentrations

Alcoholics have elevated concentrations of plasma homocysteine (6, 72), and chronic ethanol administration elevates total plasma homocysteine concentrations in rats (7, 77) and in micropigs (10, 25). Because betaine is known to methylate homocysteine to form methionine, the effect of betaine on the ethanol-induced release of homocysteine was determined in vitro (15). In this study, hepatocytes isolated from rats fed the Lieber-DeCarli control or ethanol diet for 4 wk were incubated in vitro for 4 h, and the concentration of homocysteine was measured in media as a reflection of cellular concentrations. The hepatocytes from ethanol-fed rats were found to release twice as much homocysteine into the media as controls. Furthermore, the addition of methionine to the incubation mixtures of control and ethanol-fed hepatocytes resulted in a marked increase in homocysteine generation in both cell types (15). Betaine supplementation in the incubation medium prevented the increases in homocysteine by methionine-treated control cells as well as by the cells from ethanol-treated rats. The inhibiting effect of betaine on the release of homocysteine from hepatocytes was recently confirmed by the same group of investigators (78); however, in this study, SAM failed to inhibit the release of homocysteine. Because homocysteine concentrations are associated with the development of fatty liver, betaine may attenuate alcoholic fatty liver, at least in part by preventing the intracellular accumulation of homocysteine through its methylation to form methionine. In another study, betaine supplementation increased hepatic BHMT activity in the control animals, which was further increased in the ethanol-fed rats (14). Thus, dietary betaine appears to promote
the metabolism of homocysteine by providing a methyl group as well as by increasing the activity of BHMT, which catalyzes the transfer of a methyl group from betaine to homocysteine.

**Betaine attenuates SAH concentrations**

SAH is formed when SAM transfers its methyl group to various compounds, catalyzed by many different methyltransferases. SAH is further metabolized to homocysteine and adenine through a reaction catalyzed by SAH hydrolase. This reaction is reversible, and the generation of SAH from homocysteine is thermodynamically favored over the synthesis of homocysteine. The reaction proceeds toward homocysteine synthesis only when the products (homocysteine and adenine) are removed by further metabolism. However, if the products are allowed to accumulate, the hepatic concentrations of SAH can be elevated, which could inhibit the activities of many SAM-dependent methyltransferases.

Chronic ethanol feeding has been shown to increase hepatic concentrations of SAH in rats (9), mice (24), and micro pigs (10, 25). Furthermore, hepatocytes obtained from ethanol-fed rats showed a significant 2-fold increase in SAH concentrations, which were further elevated when the hepatocytes were incubated with methionine (11). When betaine was added to the incubation medium, the concentrations of SAH were significantly reduced. In another study, betaine was shown to attenuate adenosine-induced increases in SAH concentrations in isolated hepatocytes from rat liver (79). Thus, betaine may prevent alcoholic fatty liver, at least in part, by attenuating SAH production.

Researchers further investigated whether betaine could attenuate SAH-induced hepatocyte apoptosis. Various concentrations of adenosine were used to increase intracellular concentrations of SAH in cultured rat hepatocytes. Adenosine-induced increases in SAH concentrations were associated with increases in caspase-3 activity and DNA fragmentation, both of which are markers of apoptosis (79). The addition of betaine to the incubation medium significantly attenuated adenosine-induced caspase-3 activity and DNA fragmentation by attenuating the adenosine-induced increases in SAH concentrations. These results were further corroborated by using tubercidin, a potent inhibitor of SAH hydrolase, which has also been used to increase intracellular concentrations of SAH (80). Betaine could also prevent tubercidin-induced hepatocyte apoptosis. The results of the studies obtained by using adenosine and tubercidin in vitro indicate that betaine may prevent alcoholic liver injury by attenuating the SAH-induced apoptosis of hepatocytes. Inhibition of the critical methyltransferase (such as isoprenyl cysteine methyltransferase) by elevated intracellular SAH concentrations may be responsible for the adenosine- or tubercidin-induced apoptosis in hepatocytes (79, 80). Recent studies have shown that the carboxyl methylation reaction of small GTPases is a crucial activation step that facilitates these proteins to participate in anti-apoptotic signaling pathways (81). Although increased homocysteine concentrations can induce ER stress and cause caspase activation, homocysteine concentrations are not increased by adenosine exposure (79).

**Betaine elevates hepatic SAM concentrations**

Because betaine can methylate homocysteine to form methionine, which is a precursor of SAM, it is logical to assume that dietary betaine supplementation would affect hepatic concentrations of SAM, which may in turn attenuate liver injury. In a rat model of alcoholic fatty liver, betaine administration for 4 wk increased the hepatic concentrations of SAM 2-fold in control animals and 4-fold in the ethanol-fed rats (4, 14). The higher concentrations of SAM were associated with attenuated fatty liver in ethanol-fed rats. Another study showed that betaine administration, even for a short period (2 wk) generated increased hepatic concentrations of SAM in both control and ethanol-fed rats and significantly lower ethanol-induced accumulation of hepatic triacylglycerol (21). The stimulating effect of betaine on SAM production was further confirmed in an in vitro study in which betaine significantly increased the concentrations of SAM in isolated hepatocytes from both control and ethanol-treated rats (11). These studies suggest that betaine may attenuate alcoholic fatty liver by increasing SAM production in the liver.

**Betaine elevates SAM:SAH**

Whereas SAM is the major source of methyl groups required for the methylation of many compounds in the liver, SAH is a competitive inhibitor for many SAM-dependent methyltransferases because it acts on the same site as SAM on these enzymes. The potential pathogenicity of SAH lies in its high affinity binding to the catalytic region of most SAM-dependent methyltransferases, which enables it to act as a potent product inhibitor. The $K_i$ value for SAH is often less than the $K_m$ value for SAM for many of the methyltransferases (82). Therefore, the ratio of SAM to SAH in cells appears to be the prime regulator of the activities of most methyltransferases, and any significant decrease in the ratio will negatively affect methylation reactions.

Chronic ethanol exposure is associated with increased hepatic concentrations of SAH and decreased hepatic concentrations of SAM. This effect of ethanol is expected to decrease the SAM:SAH, which can inhibit the activities of many SAM-dependent methyltransferases and may contribute to alcoholic fatty liver. Researchers have investigated the role of betaine in the correction of the SAM:SAH altered by ethanol. In one study, the intracellular SAM:SAH in isolated hepatocytes from ethanol-fed rats was significantly lower than that from control rats. The addition of betaine to the incubation medium significantly increased the SAM:SAH in hepatocytes from both the control and ethanol-fed rats (11). These results were confirmed in a recent study in which researchers compared the potencies of betaine and SAM supplementation in increasing the intracellular SAM:SAH in the hepatocytes from ethanol-fed and pair-fed control rats (78). Supplementation of betaine or SAM in the incubation media increased this ratio in hepatocytes from both the control and ethanol-fed rats and attenuated the ethanol-induced increase in hepatocellular triglyceridol concentrations by $\approx 20\%$. Although the effects of both compounds in enhancing the SAM:SAH were similar, the mechanisms of their effects appear to be different. Betaine is likely to increase the SAM:SAH by lowering intracellular SAH concentrations as a result of lowering homocysteine concentrations and by increasing SAM concentrations via increases in methionine concentrations through the activity of BHMT. On the other hand, SAM supplementation is likely to increase the SAM:SAH by increasing intracellular SAM concentrations. The difference in the mechanisms is due to the fact that betaine, but not SAM, can effectively prevent the accumulation of homocysteine via its methylation and subsequent formation of methionine and then SAM. These results suggest that betaine may attenuate alcoholic liver injury by correcting the SAM:SAH altered by ethanol exposure.
Phosphatidylethanolamine methyltransferase (PEMT) catalyzes the methylation of phosphatidylethanolamine (PE) to form phosphatidylcholine (PC), where SAM acts as a methyl donor. Although PC can also be synthesized via the Kennedy pathway in the liver, which accounts for ≈60–70% of PC synthesized in the liver (83), it was recently shown that the PC synthesized by the PEMT pathway is an important and essential constituent for the synthesis and secretion of VLDL, which is required for the export of liver triacylglycerol (84). It was recently shown that PEMT knockout mice spontaneously develop steatosis, despite the ingestion of the recommended dietary intake of choline (85).

To determine the effect of an altered SAM:SAH on PEMT activity, hepatic microsomal fractions were incubated with a constant amount of SAM and different amounts of SAH so that the SAM:SAH was equal to either 5.0 or 2.5 to correspond to the ratios seen in the livers of control-fed or ethanol-fed rats, respectively. The PEMT activity at an SAM:SAH of 2.5 was only 50% of that observed at an SAM:SAH of 5.0 (11). These researchers also showed that ethanol significantly inhibits the conversion of PE to PC in isolated hepatocytes (86), which suggests that this effect of ethanol is mediated through the inhibition of PEMT activity. Taken together, these results support the notion that the decrease in PEMT activity due to the decreased intracellular SAM:SAH seen in hepatocytes of ethanol-fed rats can inhibit PC synthesis. A reduced amount of PC can lead to defective synthesis and secretion of VLDL, which may be partly responsible for the hepatic steatosis seen in these rats. Because betaine has been shown to elevate the SAM:SAH, this may be a mechanism by which betaine restores PEMT activity, elevates PC concentrations, normalizes VLDL secretion, and attenuates alcoholic fatty liver.

Mouse model of alcoholic liver injury (fatty liver and necroinflammation) and betaine

The intragastric ethanol-fed mouse model of alcoholic liver injury was used to further understand the role of betaine in the treatment of ALD (8, 71, 87). In this model, 4 wk of ethanol feeding lead to histopathologic features of early ALD, namely prominent steatosis, accompanied by necroinflammatory foci and scattered apoptosis of hepatocytes. These histopathologic changes are associated with an elevated ER stress response, as indicated by the up-regulation of the proapoptotic gene GADD153 and of the lipogenic transcription factor SREBP-1. The mechanisms of ER stress in this model are not clear. In addition, this model showed increased plasma concentrations of ALT and increased hepatic mRNA concentrations of TNF-α, CD14, and CYP2E1. Furthermore, this model showed many features of altered methionine metabolism, including several-fold increases in plasma homocysteine concentrations, decreased hepatic SAM concentrations, and decreased hepatic mRNA concentrations of MS and BHMT. In this model, betaine feeding attenuated the pathologic features of alcoholic liver injury (fatty liver, necroinflammation, and apoptosis) and decreased plasma ALT concentrations.

“These features were associated with attenuated plasma homocysteine concentrations, increased hepatic SAH concentrations, several-fold increased hepatic SAM concentrations, and several-fold increased SAM:SAH ratios (8, 71, 87). In addition, betaine attenuated the alcohol-induced ER stress response as shown by the down-regulation of proapoptotic gene GADD153 and lipogenic transcription factor SREBP-1. Betaine feeding did not abolish the induction of CYP2E1 by ethanol, nor did it attenuate ethanol-induced increases in hepatic TNF-α or CD14 mRNA. These results suggest that betaine attenuates alcoholic liver injury by decreasing homocysteine concentrations, increasing SAM concentrations, and increasing the SAM:SAH in the liver. This beneficial effect of betaine does not appear to be mediated through the gene expression of CYP2E1, TNF-α, or CD14.

Rat model of alcoholic liver fibrosis and betaine

To understand the role of betaine in the treatment of liver fibrosis, researchers have used a rat model of ethanol plus CCl4-induced liver fibrosis (88). In this model, administration of ethanol in drinking water and a low dose of CCl4 results in liver fibrosis, which is associated with hepatic lipid peroxidation and increases in plasma concentrations of AST and ALT. Betaine treatment prevented liver fibrosis, attenuated lipid peroxidation, and decreased plasma transaminase activities.

Nonalcoholic steatohepatitis and betaine

The role of betaine in the treatment of NASH has been evaluated in 3 human studies. In a prospective, randomized, double-blind therapeutic trial (n = 191 patients), oral administration of betaine glucuronate for 8 wk reduced hepatic steatosis by 25%, reduced hepatomegaly by 8%, and significantly attenuated the hepatic concentrations of AST, ALT, and γ-glutamyl transferase in NASH patients (89). In addition, betaine treatment significantly improved discomfort in the abdominal upper right quadrant.

In a pilot study of 10 subjects with NASH, 7 of 10 patients completed 1 y of treatment with betaine. A significant improvement in serum concentrations of AST (P = 0.02) and ALT (P = 0.007) occurred during treatment. Aminotransferases normalized in 3 of 7 patients decreased by >50% in 3 of 7 patients and remained unchanged in one patient when compared with baseline values. A marked improvement in serum concentrations of aminotransferases (ALT, −39%; AST, −38%) also occurred during treatment in those patients who did not complete 1 y of treatment. Similarly, a marked improvement in the degree of steatosis, necroinflammatory grade, and stage of fibrosis was noted at 1 y of treatment with betaine (90). In an ongoing study of patients affected with NASH, betaine treatment attenuated serum ALT concentrations and improved the grades of steatosis, inflammation, and fibrosis (91).

Betaine summary

Dietary betaine can be absorbed from the intestine and transported to the liver, which leads to increased hepatic concentrations of betaine (Figure 3). In the liver, betaine can transfer its one methyl group to homocysteine to form methionine. This can result in decreased concentrations of homocysteine and increased concentrations of methione in the liver. Consequently, the former results in decreased hepatic concentrations of SAH, whereas the latter can increase hepatic SAM concentrations, which leads to an increased SAM:SAH. An elevated SAM:SAH can trigger a cascade of events leading to PEMT activation, PC synthesis, formation of proper VLDL, export of triacylglycerol, and attenuation of fatty liver. Decreased hepatic concentrations
of homocysteine can attenuate ER stress, which may result in the down-regulation of proapoptotic genes and consequently the attenuation of apoptosis, inflammation, and fibrosis. Down-regulation of another ER stress gene, SREBP-1, can reduce hepatic fatty acid synthesis, which may result in a reduction in fatty liver. Increased hepatic concentrations of SAM can activate cystathionine β-synthase, which leads to the up-regulation of the trans-sulfuration pathway, the increased synthesis of glutathione (GSH), and the attenuation of oxidative stress. Thus, betaine can ameliorate ALD by attenuating fatty liver, inflammation, and fibrosis.

OVERALL SUMMARY

It is evident that altered methionine metabolism can contribute to the development of ALD. Furthermore, information available from in vitro and animal studies clearly suggests that SAM and betaine have the potential to treat ALD, partly via the restoration of transmethylation and transsulfuration pathways of methionine metabolism. However, clinical trials conducted thus far on the effectiveness of SAM for the treatment of ALD are not conclusive. These studies neither support nor refute the use of SAM for the treatment of patients with ALD. In addition, clinical trials on the effectiveness of betaine for the treatment of ALD have not been carried out. Therefore, long-term randomized clinical trials are required to evaluate the safety and efficacy of SAM and of betaine for the treatment of ALD. It is important that factors such as dose, duration of treatment, and bioavailability of compounds should be taken into consideration in the design of these studies. Before clinical trials of folate are considered, additional animal studies are required to determine the potential of folate for the treatment of ALD.

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