S-adenosyl-L-methionine Treatment for Alcoholic Liver Disease: A Double-Blinded, Randomized, Placebo-Controlled Trial

Valentina Medici, Maria C. Virata, Janet M. Peerson, Sally P. Stabler, Samuel W. French, Jesse F. Gregory III, Anthony Albanese, Christopher L. Bowlus, Sridevi Devaraj, Edward A. Panacek, John R. Richards, and Charles H. Halsted

Background: S-adenosyl-L-methionine (SAM) is the methyl donor for all methylation reactions and regulates the synthesis of glutathione, the main cellular antioxidant. Previous experimental studies suggested that SAM may benefit patients with established alcoholic liver diseases (ALDs). The aim of this study was to determine the efficacy of SAM in treatment for ALD in a 24-week trial. The primary endpoints were changes in serum aminotransferase levels and liver histopathology scores, and the secondary endpoints were changes in serum levels of methionine metabolites.

Methods: We randomized 37 patients with ALD to receive 1.2 g of SAM by mouth or placebo daily. Subjects were required to remain abstinent from alcohol drinking. A baseline liver biopsy was performed in 24 subjects, and a posttreatment liver biopsy was performed in 14 subjects.

Results: Fasting serum SAM levels were increased over timed intervals in the SAM treatment group. The entire cohort showed an overall improvement of AST, ALT, and bilirubin levels after 24 weeks of treatment, but there were no differences between the treatment groups in any clinical or biochemical parameters nor any intra- or intergroup differences or changes in liver histopathology scores for steatosis, inflammation, fibrosis, and Mallory-Denk hyaline bodies.

Conclusions: Whereas abstinence improved liver function, 24 weeks of therapy with SAM was no more effective than placebo in the treatment for ALD.

Key Words: S-Adenosylmethionine, S-Adenosyl-L-Methionine, Alcoholic Liver Disease.

S-ADENOSYL-L-METHIONINE (SAM) IS the principal methyl donor for methyltransferase reactions that regulate gene expression and facilitates the generation of the antioxidant glutathione (GSH) from homocysteine. As depicted in Fig. 1, SAM is generated in the liver from methionine by methionine adenosyltransferase and is then converted by methyltransferases to S-adenosylhomocysteine (SAH), which is a potent inhibitor of the same reactions. SAM also activates the transsulfuration pathway that produces GSH and includes the vitamin B6-dependent enzymes cystathionine-β-synthase (CβS) and γ-cystathionase.

Prior studies established the association of abnormal hepatic methionine metabolism with the development of alcoholic liver disease (ALD) (Avila et al., 2000; Lee et al., 2004). Previously, our laboratory showed that ethanol-fed micropigs with the histopathology of ALD had reduced levels of liver SAM and elevated liver SAH (Halsted et al., 2002) with activation of genes relevant to steatohepatitis (Esfandiari et al., 2005), while all changes were prevented by the concurrent administration of SAM (Esfandiari et al., 2007; Villanueva et al., 2007). Our published analysis of baseline data from the present patients with ALD, from chronic alcoholics without liver disease, and from healthy subjects showed that the ratio of α-aminobutyrate, a byproduct of cystathionase, to cystathionine was a predictor of ALD among chronic alcoholics (Medici et al., 2010). Based upon these findings, we undertook the present double-blinded, placebo-controlled 24-week clinical trial to determine the efficacy of SAM in the treatment for ALD and to define its mechanistic effects on hepatic methionine metabolism.

METHODS AND MATERIALS

Study Design

Purified SAM (S-adenosyl-L-methionone-SD4) was provided as a gift from Abbott Laboratories (Abbott Park, IL) in the form of 400-mg tablets in blister packs. The ALD patient volunteers were

From the Department of Internal Medicine (VM, MCV, CLB, CHH), University of California Davis, Sacramento, California; Department of Nutrition (JMP), University of California Davis, Sacramento, California; University of Colorado School of Medicine (SPS), Aurora, Colorado; Department of Pathology (SWF), UCLA/Harbor Medical Center, Torrance, California; Food Science and Human Nutrition Department (JFG), Institute of Food and Agricultural Sciences, University of Florida, Gainesville, Florida; Gastroenterology Section (AA), Sacramento Veterans Affairs Medical Center, Mather, California; Department of Pathology (SD), University of California Davis, Sacramento, California; Department of Emergency Medicine (EAP, JRR), University of California Davis, Sacramento, California.

Received for publication December 17, 2010; accepted March 7, 2011.

Reprint requests: Valentina Medici, Division of Gastroenterology & Hepatology, Department of Internal Medicine, University of California Davis, 4150 V Street, Suite 3500, Sacramento, CA 95817; Tel.: 916-734-3751; Fax: 916-734-7908; E-mail valentina.medici@ucdmc.ucdavis.edu

DOI: 10.1111/j.1530-0277.2011.01547.x

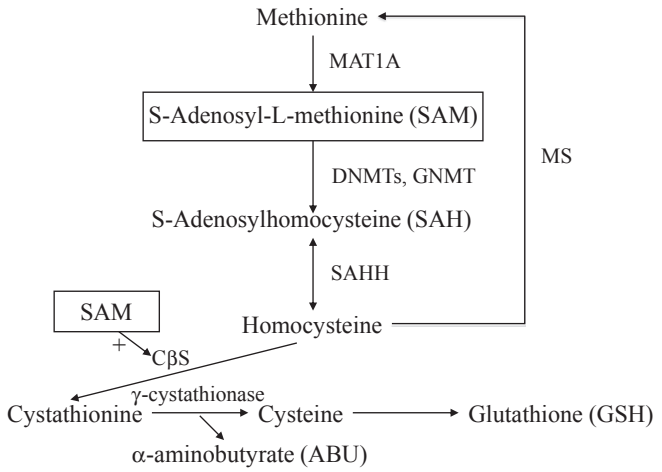

Fig. 1. Methionine metabolism. Methionine is converted by methionine adenosyltransferase (MAT1A) to S-adenosyl-L-methionine (SAM), which is subsequently metabolized to S-adenosylhomocysteine (SAH) by donating its methyl moiety to DNA methyltransferases (DNMTs) or to other methyltransferases that include glycine N-methyltransferase (GNMT). SAH hydrolase (SAHH) regulates the bi-directional reaction that leads to the synthesis of homocysteine from SAH or vice versa. Completing the cycle, homocysteine is converted to methionine by methionine synthase (MS), but is also reduced through the transsulfuration pathway that leads to the synthesis of glutathione (GSH). This pathway is regulated by the vitamin B6-dependent enzymes cystathionine β-synthase (CβS) and γ-cystathionase. Alpha-aminobutyric acid (ABU) is the collateral product of the reaction catalyzed by cystathionase.

randomly assigned by an independent UC Davis Medical Center Investigational Drug Service pharmacist in a 1:1 ratio to receive SAM or matching placebo, using a computer-generated allocation sequence. A sealed, opaque envelope was used to conceal the randomization scheme and was kept by the independent pharmacist. SAM or matching placebo capsules with identical appearance, smell, and taste were provided to each subject at 3 daily doses for a total of 1.2 g per day. The study subjects, care providers, study coordinator, and those assessing outcomes were all blinded to intervention. The patients were advised to continue their daily diets as well as their clinically indicated medications and daily multivitamins, and were required to discontinue all alcoholic beverages. Each subject was given a renewable 1-month supply of SAM or placebo capsules and was followed at 2-week intervals up to 8 weeks, then at 4-week intervals up to 24 weeks at the UC Davis Clinical and Translational Science Center (CTSC) Clinical Research Center. At each appointment, the remaining pills in each month's supply were counted to determine compliance with the treatment. On the initial and each follow-up visit, patients met with 1 of 2 physicians (VM or CHH) for histories and physical examinations, and blood was drawn. Patients who were noncompliant according to consumption of more than 2 alcoholic beverages in the interval between visits were dropped from the study. The protocol was approved annually by the UC Davis Institutional Review Board (# 200311168-7; most recent approval date: 09/24/2010), and all patients provided written informed consent. The study was conducted according to the guidelines of the Declaration of Helsinki under provisions of "Good Clinical Practices" as defined in the US Code of Federal Regulations on the Protection of Human Subjects for the United States.

Patients

Inclusion criteria were a positive history for chronic alcohol abuse according to World Health Organization definition (O'Connor and Schottenfeld, 1998), and the AUDIT screening test was used to quantify alcohol abuse (Bush et al., 1998). Patients with clinical evidence of liver disease were graded for severity by Child-Pugh and MELD scores (Pugh et al., 1973; Wiesner et al., 2003). Patients were excluded who had a Child-Pugh score >10 because a prior European trial showed that those with higher scores were less likely to respond to SAM treatment (Mato et al., 1999). We also excluded patients with positive laboratory tests for chronic hepatitis B or C, primary biliary cirrhosis, autoimmune hepatitis, Wilson disease, hemochromatosis, or hepatocellular carcinoma with alpha-fetoprotein >10 times the upper limit of normal. Patients with cancer, congestive heart failure, renal insufficiency with serum creatinine >1.2 mg/ml, use of antifolate drugs or corticosteroids, or infectious illness were also excluded.

Biochemical Parameters

Fasting blood samples were obtained at each visit for measurements of complete blood counts, serum AST, ALT, alkaline phosphatase, total bilirubin, albumin, INR, folate, vitamin B6 and B12, SAM, SAH, and homocysteine, and a modified measurement of serum carbohydrate-deficient transferrin (%CDT) (Myrick et al., 2001) to screen for recent alcohol use.

Liver Biopsies

The safety criteria for liver biopsies included an INR value of <1.5, platelet count $>50{,}000/\text{mm}^3$, and absence of palpable ascites and encephalopathy (Rockey et al., 2009). Percutaneous biopsies were obtained from the right lobe of the liver by ultrasonic guidance using a 16G Jamshidi needle, and biopsies were considered valid if they were greater than 15 mm in length and 1.4 mm in width. The blinded liver biopsy grading by SWF used a Nikon E400 microscope equipped with a digital camera and Nikon Metomorph computer software (Molecular Devices Corporation, Downingtown, PA). Using published criteria (French et al., 1993), quantitative scores included 4 grades for steatosis (0, $<10\%$; 1, up to 30%; 2, 30 to 60%; and 3, $>60\%$ of hepatocytes), 5 grades for fibrosis by Sirius red stain (0, none; 1, zone 3 perisinusoidal fibrosis; 2, perisinusoidal and periportal fibrosis; 3, bridging fibrosis, 4, overt cirrhosis), and 3 grades of inflammation (0, none; 1, 1 to 2 foci; 2, 3 to 4 foci; and 3, more than 4 foci). Fibrosis was also quantified as the percentage of stained pixels per square field. Mallory-Denk hyaline bodies, an index of hepatocyte necrosis, were counted in 10 fields and scored as average number per field.

Methionine Metabolites and Vitamins Levels

After sample preparation, frozen serum specimens were sent to SPS at Metabolite Laboratories, University of Colorado Health Sciences for measurements of SAM, SAH, and homocysteine by stable isotope dilution gas or liquid chromatography/mass spectrophotometry (Medici et al., 2010). Vitamin B6 was measured by JFG as pyridoxal-5′-phosphate as the semicarbazone-derivative by reverse-phase HPLC with fluorescence detection (Ubbink et al., 1985).

Primary and Secondary Endpoints

The primary outcomes were median changes in serum aminotransferases from baseline to week 24 and in histopathology scores between baseline and final biopsies by 1 or more grades of steatosis and inflammation with no increase in fibrosis. Secondary outcomes were median changes in serum SAM, SAH, and homocysteine from baseline to week 24.

Statistical Methods

Data were analyzed to determine relationships between metabolic and disease responses in the SAM and placebo groups. Results were

analyzed for the intention to treat (ITT) and per protocol (PP) treated sets. ITT analyses were performed using all subjects (n = 37) with baseline data who took at least 1 dose of randomized medication and had at least 1 postbaseline blood test, while the PP cohort was comprised of all subjects (n = 26) who completed the treatment trial. The variables that were analyzed according to changes at each time point in both the ITT and PP cohorts included serum levels of methionine metabolites, liver biochemical function tests, and clinical severity of liver disease according to Child and MELD scores. Quantitative histological scores were compared between the 2 groups in 14 liver biopsies obtained before and after treatment in the same patient. Variables were assessed for conformance to the normal distribution and were transformed as necessary. The baseline values of the SAM and placebo treatment groups were compared with 2-sample t-tests and variables which, by chance, were significantly different between groups at baseline, were controlled for in subsequent analyses. The effect of SAM treatment was examined by analysis of covariance, with treatment group as the main effect. Least-squares means were estimated for each group to adjust for covariates, and the resulting values were back-transformed (if needed) to obtain adjusted medians. In the case of SAM, a logarithm transformation was performed, so the value is also the adjusted geometric mean. The differences in serum SAM levels between the 2 groups over multiple time points from baseline to 24 weeks were assessed by mixed models analysis of covariance, controlling for the baseline value of SAM. All analyses were conducted with SAS v. 8.2 or higher (SAS Institute, Cary, NC).

Sample Size and Power

We estimated a sample size of 20 subjects per treatment group for our single center trial, anticipating the low yield of eligible patients owing to the extensive screening procedures and budgetary constraints (Fig. 2). It was calculated that the sample size of 20 subjects per treatment group would detect correlations between variables of 0.39 or higher, and differences between groups of 0.9 within-subject standard deviations or higher at 80% power and a 5% level of significance. We anticipated that, for most variables, the intrasubject correlation would be high enough to enable us to detect changes in serum aminotransferases and methionine metabolites and histological scores from liver biopsies.

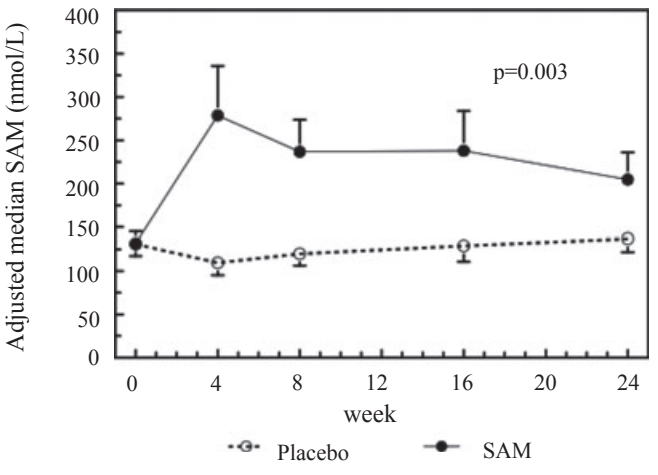

Fig. 2. Adjusted serum SAM levels. SAM treatment group presented overall higher serum SAM levels over time than placebo group during the 24 weeks of the trial. All serum SAM levels were obtained after 12-hour fasting.

RESULTS

From July 1, 2005 through June 30, 2009, we screened 294 patients for eligibility, and after informed consent, 37 (12.5%) were randomized to participate in the clinical trial, including 18 to receive SAM and 19 to receive the placebo in the ITT cohort. Among those excluded, 128 did not meet the inclusion criteria, 105 met the inclusion criteria but could not be enrolled because of homelessness with no telephone contact, while 24 subjects declined to participate for concerns about ability to adhere to the requirement for abstinence, the trial schedule, or the potential risk of side effects. Subsequently, there were 5 dropouts in the SAM treatment group (27.7%) and 6 dropouts in the placebo group (31.5%, p = n.s.), all of whom had resumed active drinking. Consequently, the PP cohort consisted of 13 subjects in each group who completed the full 24-week trial. In the ITT cohort, 13 patients declined or were ineligible for liver biopsy, leaving 24 patients in whom the baseline liver biopsy was performed including 12 subjects in the placebo group and 12 subjects in the SAM group, whereas in the PP cohort the posttreatment liver biopsy was performed in 8 subjects in the placebo group and in 6 in the SAM group (p = n.s.). According to pill counts at each visit, a median of 86% (range 64 to 93) of pills were taken in the SAM group versus 81% (60 to 90) in the placebo group (p = n.s.). Among the patients who completed the study, 7 subjects in the placebo group and 1 subject in the SAM group reported a maximum of 2 episodes of drinking during which they had no more than 2 drinks in each occasion, and were permitted to stay in the study without further drinking. The measured outcomes were not affected by these episodes of drinking or by pill compliance. There were no differences in serum %CDT at timed intervals among the groups.

Patient Characteristics at Baseline

All baseline demographic characteristics, biochemical measurements of liver function, MELD, and Child scores were similar between the placebo and SAM groups except for higher mean levels of AST and ALT in the placebo group (Table 1). Also, liver biopsies from the placebo group presented higher baseline steatosis and inflammation scores compared with the SAM group. A fibrosis score of 3 or more, indicative of bridging or cirrhosis, was present in 7 of 12 (58.3%) in both the SAM and the placebo group. The serum levels of methionine metabolites and serum folate and vitamins B12 and B6 were also similar in the 2 groups (Table 2).

Treatment Effects on Primary Outcomes and Clinical Parameters

Table 3 describes the median changes and ranges in clinical and biochemical parameters according to treatment group and PP and ITT analyses. Whereas the combined groups showed reductions in AST (65 U/l, 27–228 to 42, 22–113;

Table 1. Baseline Clinical, Biochemical, and Histological Characteristics of Subjects[a]

Parameter	SAM (n = 18)	Placebo (n = 19)	p-Value
Age	46.5 (34, 66)	44 (34, 65)	0.63
Gender F/M	6/12	6/13	0.91
Duration of abstinence (days)	60 (0.5, 447)	6 (1, 210)	0.08
Duration of drinking (years)	25 (2, 45)	21 (4, 37)	0.06
AUDIT	16.5 (8, 24)	14.5 (8, 21)	0.11
AST U/l [15 to 37]	54 (42, 81)	110 (27, 228)	**0.002**
ALT U/l [5 to 37]	31 (17, 54)	55 (14, 201)	**0.009**
Total Bilirubin mg/dl [0.3 to 1.3]	1.5 (1, 7.3)	1.6 (0.6, 3)	0.72
Alkaline Phosphatase U/l [35 to 115]	124 (67, 425)	167 (60, 239)	0.62
Albumin g/dL [3.2 to 4.6]	3.4 (2.4, 4.1)	3.3 (2.5, 4)	0.32
Creatinine mg/dl [0.44 to 1.4]	0.8 (0.5, 1.4)	0.8 (0.6, 1.1)	0.44
INR [0.75 to 1.19]	1.25 (1.03, 1.56)	1.14 (0.96, 1.48)	0.49
%CDT	3.6 (2.53, 6.62)	4.11 (1.88, 5.23)	0.22
MELD score[b]	10 (7, 17)	10 (6, 17)	0.87
Child score[b]	7 (5, 9)	7 (5, 9)	0.98
	Baseline biopsy n = 12		
Steatosis	1 (0, 4)	4 (0, 4)	**0.03**
Fibrosis	4 (1, 4)	3 (0, 4)	0.5
Percent fibrosis per square field	4.12 (0.32, 15.3)	2.98 (0.77, 17.2)	0.7
Inflammation	0 (0, 3)	2 (0, 3)	**0.01**
Mallory-Denk bodies	0 (0, 4)	1 (0, 4)	0.58

[a]Data in this and all other tables are presented as median and range or percentage.

[b]Meld and Child scores were calculated only for patients with clinical and/or histological evidence of cirrhosis (n = 12 patients in the SAM group and n = 11 patients in the placebo group).

$p < 0.05$ are indicated in bold.

Table 2. Baseline Methionine Metabolites and Vitamins of Subjects

Parameter	SAM (n = 18)	Placebo (n = 19)	p-Value
HCY umol/l [4.1 to 10]	10 (5.4, 17.3)	10.2 (7.4, 16)	0.21
SAH nmol/l [14 to 64]	34 (16, 87)	39 (16, 142)	0.82
SAM nmol/l [72 to 160]	132 (86, 328)	113 (63, 412)	0.52
Folate ng/ml [3.5 to 16.1]	14.6 (0.4, 20)	13.6 (3.3, 20)	0.08
Vitamin B12 pg/ml [200 to 600]	683 (421, 805)	681 (353, 1708)	0.78
Vitamin B6 nmol/l [15 to 120]	47.2 (8.6, 140)	36.3 (9.5, 187)	0.06

$p < 0.0001$), ALT (35 U/l, 14–211 to 24, 16–109; $p = 0.004$), and bilirubin levels (1.5 mg/dl, 0.6–7.3 to 1.1, 0.4–6.7, $p = 0.004$), there were no differences in changes between the 2 treatment groups. Also, there was no treatment effect in either group on any of the histopathology scores (Table 4).

Treatment Effects on Methionine Metabolites

According to PP and ITT analyses, there were no changes between baseline and 24 weeks in serum SAM, SAH, or homocysteine in either treatment group. However, the adjusted fasting serum SAM levels were overall higher in the SAM treatment group when the PP data were analyzed by treatment interaction with time ($p = 0.003$) (Fig. 2).

Adverse Events

There were no severe adverse events in either treatment group. In the SAM group, 4 subjects complained of transient diarrhea, 3 complained of abdominal pain and bloating, and 2 complained of headaches. One subject in the SAM group reported transient hair loss, xerostomia, and night sweats. In the placebo group, 4 subjects complained of diarrhea, 2 of nausea, 2 of abdominal pain, and 2 of bloating.

DISCUSSION

The present study of the efficacy of 24 weeks of oral SAM in the treatment for ALD found no differences between the SAM and placebo groups in baseline and posttreatment parameters of serum liver biochemistries, SAM, SAH, or homocysteine levels, or liver histopathology scores (Tables 3 to 5). However, analyses at all intermediate time points found a significant increase in fasting serum SAM levels in response to SAM treatment, consistent with its adequate intestinal absorption and systemic response (Fig. 2). In prior studies, the addition of SAM in the same doses used here attenuated or prevented reductions in liver SAM and GSH in ethanol-fed baboons (Lieber et al., 1990), and in ethanol-induced liver injury in micropigs by prevention of its histopathology and the over-expressions of genes relevant to oxidative liver injury (Villanueva et al., 2007) and hepatic lipogenesis (Esfandiari et al., 2007). Two previous European clinical studies suggested that a longer treatment period with oral SAM at the identical doses used in our study may be effective in ALD. In a 6-month Italian study of 17 randomized patients with ALD, initially low hepatic GSH levels were normalized by treatment with oral SAM at 1.2 g/d (Vendemiale et al., 1989). In a later 2-year multicenter European treatment trial of 123 patients with ALD, the mortality or liver transplant incidence was reduced from 30% in the placebo group to 16% in the SAM group, but the differences were significant only after exclusion of Child class C patients from the analysis (Mato et al., 1999). However, a recent meta-analysis of results from 434 patients in 9 studies found insufficient evidence to support or refute the clinical use of SAM in the treatment for ALD (Rambaldi and Gluud, 2006). Furthermore, none of these prior studies evaluated the effect of SAM treatment on liver histopathology, and the largest clinical trial did not exclude patients with coexistent chronic viral hepatitis (Mato et al., 1999). The present data indicate that 24-week treatment with SAM at the same dose that was used in prior studies (Mato et al., 1999; Vendemiale et al., 1989) is not effective in patients with moderately severe ALD according to MELD scores and Child criteria.

Table 3. Changes in Clinical and Biochemical Parameters[a]

	SAM changes (PP) (n = 13)	Placebo changes (PP) (n = 13)	p-Value	SAM changes (ITT) (n = 18)	Placebo changes (ITT) (n = 19)	p-Value
AST U/l [15 to 37]	−13.5 (−35, 156)	−56 (−140, 5)	0.36	−11 (−52, 40)	−53.5 (−200, 77)	0.74
ALT U/l [5 to 37]	−6.5 (−17, 126)	−21 (−107, 19)	0.85	−6 (−31, 9)	−31.5 (−114, 36)	0.68
Total Bilirubin mg/dl [0.3 to 1.3]	−0.35 (−2.4, 0.4)	−0.5 (−4.7, 0.5)	0.72	−0.2 (−2.4, 0.9)	−0.35 (−4.7, 0.5)	0.55
Alkaline Phosphatase U/l [35 to 115]	−2 (−140, 82)	−22 (−150, 58)	0.17	−1 (−140, 82)	−11.5 (−150, 58)	0.21
Albumin g/dl [3.2 to 4.6]	−0.05 (−0.3, 1.1)	0.6 (−0.5, 1.2)	0.59	−0.1 (−0.5, 1.1)	0.6 (−0.5, 1.6)	0.16
INR [0.75 to 1.19]	−0.04 (−0.2, 0.13)	−0.08 (−0.25, 0.13)	0.54	−0.04 (−0.2, 0.13)	−0.06 (−0.25, 0.13)	0.62
%CDT	0.01 (−1.96, 2.52)	0.07 (−2.47, 1.41)	0.82	0.34 (−1.96, 6.67)	0.02 (−2.4, 1.4)	0.96
MELD score[b]	−1 (−4, 1)	−1 (−8, 1)	0.92	−1 (−3, 1)	−1 (−6, 1)	0.88
Child score[b]	0 (−2, 2)	−0.5 (−5, 1)	0.96	−1 (−3, 1)	−0.5 (−5, 2)	0.92
BMI (kg/m^2)	0.8 (−4.8, 3.7)	0.3 (−6.8, 4.7)	0.14	1 (−4.8, 3.7)	0.3 (−6.8, 4.7)	0.06

[a]PP, Per Protocol cohort, ITT, Intention to Treat cohort.
[b]Meld and Child scores were calculated only for patients with clinical and histological evidence of cirrhosis (n = 12 patients in the SAM group and n = 11 patients in the Child group).

Table 4. Histology Scores with Changes in Each Group

	SAM pre (n = 6)	SAM post (n = 6)	SAM median changes	Placebo pre (n = 8)	Placebo post (n = 8)	Placebo median changes	p-Value
Steatosis	1 (0, 3)	1 (0, 2)	0 (−2, 0)	4 (0, 4)	1 (0, 4)	−1.25 (−4, 1)	0.28
Fibrosis	4 (1, 4)	3.5 (2, 4)	0 (−1, 2)	3 (0, 4)	3 (0, 4)	0 (−2, 1)	0.36
Percent fibrosis per square field	5.4 (1.5, 13.2)	10.7 (1.5, 21.7)	2.22 (−1.5, 9)	4.6 (1.18, 17.2)	5.3 (1.6, 16.7)	−0.16 (−1.3, 5.3)	0.29
Inflammation	0.5 (0, 3)	1 (0, 3)	1 (−1, 1)	1.5 (1, 3)	1 (0, 2)	−0.5 (−2, 1)	0.34
Mallory-Denk bodies	1 (0, 4)	1 (0, 1)	−0.5 (−3, 1)	2 (0, 4)	1 (0, 3)	−0.5 (−2, 0)	0.46

Table 5. Changes in Methionine Metabolites[a]

	SAM changes (PP) (n = 13)	Placebo changes (PP) (n = 13)	p-Value	SAM changes (ITT) (n = 18)	Placebo changes (ITT) (n = 19)	p-Value
HCY umol/l[b] [4.1 to 10]	−1.1 (−5.1, 4)	−0.8 (−6.6, 3.4)	0.45	−1.3 (−34.8, 4)	−0.75 (−34.8, 4)	0.24
SAH nmol/l [14 to 64]	−1 (−52, 32)	−2 (−93, 24)	0.61	−1 (52, 32)	−6.5 (−93, 24)	0.29
SAM nmol/l [72 to 160]	54 (−19, 393)	7 (−261, 97)	0.08	54 (−88, 393)	−7.5 (−261, 97)	0.054

[a]PP, Per Protocol cohort, ITT, Intention To Treat cohort.
[b]Ranges for matched healthy subjects as previously published (Medici et al., 2010). HCY, homocysteine; SAH, S-adenosylhomocysteine; SAM, S-adenosyl-L-methionine.

The major strengths of our study are that it is the only one to date to demonstrate a systemic response to oral SAM (Fig. 2), to evaluate potential mechanistic effects of SAM treatment on methionine metabolism, and to provide results for potential changes in liver histopathology. However, the overall changes in aminotransferase levels, other liver function tests, methionine metabolites, vitamin levels, and histopathology were no different between the SAM and placebo groups (Tables 2 to 4).

The weakness of our study is the relatively small numbers of patients, which resulted from the combination of our stringent inclusion criteria and the high 30% drop-out rate secondary to alcoholic recidivism which is similar to other treatment trials in outpatients with ALD (Lieber et al., 2003). Ultimately, with 13 patients per group in the PP cohort, we could detect significant differences of 1.15 standard deviations between groups, which is marginally adequate for detection of clinically meaningful effects, while the relatively small numbers of patients who completed both liver biopsies limits the conclusiveness of the histopathology interpretation. Nevertheless, this negative study may be considered a valuable addition to the meta-analysis of clinical trials of SAM in ALD, and a guide for those who may plan larger and longer multicenter treatment trials.

There are several plausible reasons for the observed lack of efficacy of SAM in the treatment for our patients with ALD. First, although our patients were at a moderately severe baseline stage of ALD according to Child and Meld scores (Table 1), the baseline liver biopsies showed a high level of fibrosis (Table 4), which reduces the intrahepatic population of potentially responsive hepatocytes. Second, although prior experimental studies indicated that dietary SAM

supplementation can prevent the development of liver injury in previously normal ethanol-fed animals (Esfandiari et al., 2007; Lieber et al., 1990; Villanueva et al., 2007), the effective treatment for established clinical ALD with orally administered SAM would require its transport, retention, and metabolism by injured hepatocytes. The likelihood that hepatocyte retention of SAM was decreased is supported by findings in our baseline study that serum SAM levels were increased in ALD compared with healthy control subjects and to alcoholics without liver disease (Medici et al., 2010). We were unable to compare serum and hepatic SAM levels in the present study because the amount of tissue obtained from liver biopsies was insufficient for its measurement. Last, the potential therapeutic efficacy of SAM may require concurrent administration of supplemental vitamin B6 for production of the antioxidant GSH, as suggested by the findings of subnormal vitamin B6 levels at baseline (Medici et al., 2010) that were unchanged during the course of treatment in the present study (Table 2).

Finally, any clinical trial in patients with ALD presents excessive challenges owing to the poor compliance, high rates of relapse, and the social circumstances of the target population (Lieber et al., 2003). Although pill compliance was incomplete, it was similar in each group and therefore unlikely to influence the treatment outcome in either group. Sobriety cannot be assured by interval histories in a chronic alcoholic population, and the usefulness of %CDT as a measure of sobriety is limited in the presence of advanced liver disease (Berlakovich et al., 2004). Whereas our present study suggests that oral SAM is ineffective in treatment for ALD, subsequent studies with larger numbers of compliant patients and longer periods of treatment might produce a different outcome.

ACKNOWLEDGMENTS

Supported by R01AA14562 (CHH), R01AG09834 (S.P.S.), P50 011999 (SWF), R01DK072398 (JFG), and UL1 RR024146 from the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH), and NIH Roadmap for Medical Research. S-adenosyl-L-methionine was provided as a generous gift from Abbott Laboratories. The funding agencies had no role in the study design, data analysis and interpretation, nor in drafting the manuscript.

REFERENCES

Avila MA, Berasain C, Torres L, Martín-Duce A, Corrales FJ, Yang H, Prieto J, Lu SC, Caballería J, Rodés J, Mato JM (2000) Reduced mRNA abundance of the main enzymes involved in methionine metabolism in human liver cirrhosis and hepatocellular carcinoma. J Hepatol 33:907–914.

Berlakovich GA, Soliman T, Freundorfer E, Windhager T, Bodingbauer M, Wamser P, Hetz H, Peck-Radosavljevic M, Muehlbacher F (2004) Pretransplant screening of sobriety with carbohydrate-deficient transferrin in patients suffering from alcoholic cirrhosis. Transpl Int 17:617–621.

Bush K, Kivlahan DR, McDonell MB, Fihn SD, Bradley KA (1998) The AUDIT alcohol consumption questions (AUDIT-C): an effective brief screening test for problem drinking. Ambulatory Care Quality Improvement Project (ACQUIP). Alcohol Use Disorders Identification Test. Arch Intern Med 158:1789–1795.

Esfandiari F, Villanueva JA, Wong DH, French SW, Halsted CH (2005) Chronic ethanol feeding and folate deficiency activate hepatic endoplasmic reticulum stress pathway in micropigs. Am J Physiol Gastrointest Liver Physiol 289:G54–G63.

Esfandiari F, You M, Villanueva JA, Wong DH, French SW, Halsted CH (2007) S-adenosylmethionine attenuates hepatic lipid synthesis in micropigs fed ethanol with a folate-deficient diet. Alcohol Clin Exp Res 31:1231–1239.

French SW, Nash J, Shitabata P, Kachi K, Hara C, Chedid A, Mendenhall C (1993) Pathology of alcoholic liver disease. VA Cooperative Study Group 119. Semin Liver Dis 13:154–169.

Halsted CH, Villanueva JA, Devlin AM, Niemelä O, Parkkila S, Garrow TA, Wallock LM, Shigenaga MK, Melnyk S, James SJ (2002) Folate deficiency disturbs hepatic methionine metabolism and promotes liver injury in the ethanol-fed micropig. Proc Natl Acad Sci USA 99:10072–10077.

Lee TD, Sadda MR, Mendler MH, Bottiglieri T, Kanel G, Mato JM, Lu SC (2004) Abnormal hepatic methionine and glutathione metabolism in patients with alcoholic hepatitis. Alcohol Clin Exp Res 28:173–181.

Lieber CS, Casini A, DeCarli LM, Kim CI, Lowe N, Sasaki R, Leo MA (1990) S-adenosyl-L-methionine attenuates alcohol-induced liver injury in the baboon. Hepatology 11:165–172.

Lieber CS, Weiss DG, Groszmann R, Paronetto F, Schenker S; Veterans Affairs Cooperative Study 391 Group (2003) Veterans Affairs Cooperative Study of polyenylphosphatidylcholine in alcoholic liver disease: effects on drinking behavior by nurse/physician teams. Alcohol Clin Exp Res 27:1757–1764.

Mato JM, Camara J, Fernandez dePaz J, Caballería L, Coll S, Caballero A, García-Buey L, Beltrán J, Benita V, Caballería J, Solà R, Moreno-Otero R, Barrao F, Martín-Duce A, Correa JA, Parés A, Barrao E, García-Magaz I, Puerta JL, Moreno J, Boissard G, Ortiz P, Rodés J (1999) S-adenosylmethionine in alcoholic liver cirrhosis: a randomized, placebo-controlled, double-blinded, multicenter clinical trial. J Hepatol 30:1081–1089.

Medici V, Peerson JM, Stabler SP, French SW, Gregory JF 3rd, Virata MC, Albanese A, Bowlus CL, Devaraj S, Panacek EA, Rahim N, Richards JR, Rossaro L, Halsted CH (2010) Impaired homocysteine transsulfuration is an indicator of alcoholic liver disease. J Hepatol 53:551–557.

Myrick H, Henderson S, Anton RF (2001) Utility of a new assay for carbohydrate-deficient transferrin (Biorad %CDT TIA) to monitor abstinence during a treatment outcome study. Alcohol Clin Exp Res 25:1330–1334.

O'Connor PG, Schottenfeld RS (1998) Patients with alcohol problems. N Engl J Med 338:592–602.

Pugh RN, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R (1973) Transection of the oesophagus for bleeding oesophageal varices. Br J Surg 60:646–649.

Rambaldi A, Gluud C (2006) S-adenosyl-L-methionine for alcoholic liver diseases. Cochrane Database Syst Rev 2:CD002235.

Rockey DC, Caldwell SH, Goodman ZD, Nelson RC, Smith AD; American Association for the Study of Liver Diseases (2009) Liver biopsy. Hepatology 49:1017–1044.

Ubbink JB, Serfontein WJ, de Villiers LS (1985) Stability of pyridoxal-5-phosphate semicarbazone: applications in plasma vitamin B6 analysis and population surveys of vitamin B6 nutritional status. J Chromatogr 342:277–284.

Vendemiale G, Altomare E, Trizio T, Le Grazie C, Di Padova C, Salerno MT, Carrieri V, Albano O (1989) Effects of oral S-adenosyl-L-methionine on hepatic glutathione in patients with liver disease. Scand J Gastroenterol 24:407–415.

Villanueva JA, Esfandiari F, White ME, Devaraj S, French SW, Halsted CH (2007) S-adenosylmethionine attenuates oxidative liver injury in micropigs fed ethanol with a folate-deficient diet. Alcohol Clin Exp Res 31:1934–1943.

Wiesner R, Edwards E, Freeman R, Harper A, Kim R, Kamath P, Kremers W, Lake J, Howard T, Merion RM, Wolfe RA, Krom R; United Network for Organ Sharing Liver Disease Severity Score Committee (2003) Model for end-stage liver disease (MELD) and allocation of donor livers. Gastroenterology 124:91–96.