Importance of a deficiency in *S*-adenosyl-L-methionine synthesis in the pathogenesis of liver injury[1–4]

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ABSTRACT
One of the features of liver cirrhosis is an abnormal metabolism of methionine—a characteristic that was described more than a half a century ago. Thus, after an oral load of methionine, the rate of clearance of this amino acid from the blood is markedly impaired in cirrhotic patients compared with that in control subjects. Almost 15 y ago we observed that the failure to metabolize methionine in cirrhosis was due to an abnormally low activity of the enzyme methionine adenosyltransferase (EC 2.5.1.6). This enzyme converts methionine, in the presence of ATP, to *S*-adenosyl-L-methionine (SAMe), the main biological methyl donor. Since then, it has been suspected that a deficiency in hepatic SAMe may contribute to the pathogenesis of the liver in cirrhosis. The studies reviewed here are consistent with this hypothesis. *Am J Clin Nutr* 2002;76(suppl):1177S–82S.

KEY WORDS Methionine, *S*-adenosyl-L-methionine, SAMe, methionine adenosyltransferase, liver injury, liver regeneration, cirrhosis, oxidative stress, protein *S*-nitrosylation

BIOSYNTHESIS AND METABOLISM OF *S*-ADENOSYL-L-METHIONINE

S-Adenosyl-L-methionine (SAMe) is synthesized from L-methionine and ATP in a 2-step reaction where the complete tripolyphosphate moiety is cleaved from ATP as SAMe is being formed. This rather unusual reaction is catalyzed by the enzyme methionine adenosyltransferase (MAT, also known as SAMe-synthetase; EC 2.5.1.6) (1, 2). In the first step, SAMe is formed by a direct attack of the sulfur atom of methionine on the C-5′ atom of ATP, with cleavage of the complete tripolyphosphate moiety from ATP. In the second step, the tripolyphosphate thus generated is hydrolyzed asymmetrically to orthophosphate and pyrophosphate. The 3 products of the reaction (orthophosphate, pyrophosphate, and SAMe) are then released simultaneously. MAT can also hydrolyze exogenously added tripolyphosphate. The cloning and sequencing of the structural genes or complementary DNA encoding for a large variety of MATs in different organisms and tissues (including *Mycoplasma genitalium*, *Escherichia coli*, *Saccharomyces cerevisiae*, *Arabidopsis thaliana*, and mice, rat, and human kidney and liver tissue) have shown that MAT is an exceptionally well-conserved enzyme through evolution (3). Thus, MAT from *E. coli* and humans share 59% sequence identity (4).

Analyses of genome sequences from archaea, eubacteria, fungi, plants, and animals indicate that all organisms synthesize SAMe and that a surprisingly large fraction of all genes are SAMe-dependent methyltransferases. The crucial importance of the liver in the regulation of blood methionine concentrations was first established by Kinsell et al in 1947 (5). These authors showed a marked impairment of methionine metabolism in patients with liver cirrhosis. Later work, mainly in the laboratories of Mudd and Poole (6) and Finkelstein (7), showed that under normal conditions, both in men and rats, ≤85% of all methylation reactions and ≈50% of methionine metabolism occur in the liver (6, 7). In addition, these authors estimated that the half-life of SAMe in the liver is ≈5 min. In normal liver, most SAMe is used in methylation reactions because the SAMe decarboxylation pathway accounts for < 10% of the available SAMe (**Figure 1**). In humans, the fraction of available homocysteine converted to cystathionine during each cycle is 53% and this percentage drops to ≈20% when the dietary content of methyl groups is restricted, as a way to preserve methionine consumption. A central role of SAMe in the regulation of hepatic methionine metabolism was first proposed by Finkelstein et al (8–10) on the basis of their findings that SAMe activates cystathionine β-synthase (EC 4.2.1.22) and betaine–homocysteine *S*-methyltransferase (EC 2.1.1.5) and on the earlier observation by Kutzbach and Stokstad (11, 12) that SAMe inhibits methylenetetrahydrofolate reductase (EC 1.7.99.5). Thus, during each cycle, hepatic SAMe regulates the fraction of homocysteine that is methylated to form methionine and the fraction that is taken into the transsulfuration pathway. A high hepatic SAMe content, usually the result of a high blood methionine concentration, activates the flow of homocysteine through the

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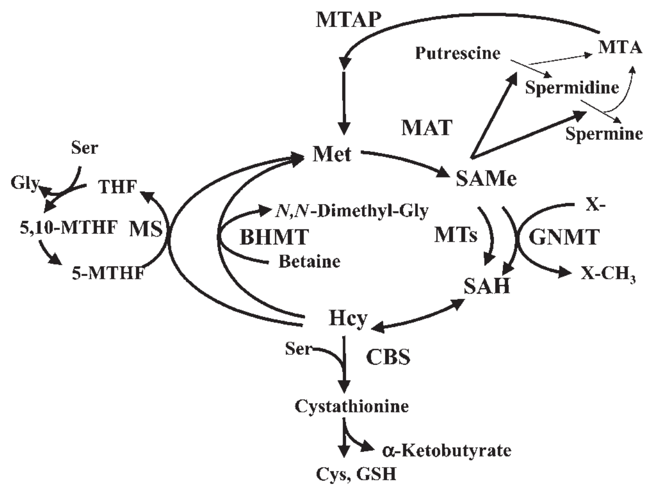

FIGURE 1. Hepatic methionine (Met) metabolism. Met is converted to homocysteine (Hcy) via *S*-adenosyl-L-methionine (SAMe) and *S*-adenosylhomocysteine (SAH). The conversion of Met into SAMe is catalyzed by the enzyme methionine adenosyltransferase (MAT; EC 2.5.1.6). After decarboxylation, SAMe can donate the remaining propylamino moiety attached to its sulfonium ion to putrescine [to form spermidine and methylthioadenosine (MTA)] and to spermidine (to form spermine and a second molecule of MTA). SAMe donates its methyl group in a large variety of reactions catalyzed by dozens of methyltransferases (MTs), the most abundant in the liver being glycine methyltransferase (GNMT; EC 2.1.1.20). The SAH thus generated is hydrolyzed to form Hcy and adenosine through a reversible reaction catalyzed by the enzyme SAH hydrolase (EC 3.3.1.1). Two enzymes catalyze the methylation of Hcy to form methionine: methionine synthase (MS; EC 2.1.1.13) and betaine methyltransferase (BHMT; EC 2.1.1.5). MTA can be recycled back to methionine in a methionine biosynthetic pathway initiated by the enzyme MTA-phosphorylase (MTAP; EC 2.4.2.28). Hcy, in the presence of serine, forms cystathionine in a reaction catalyzed by the enzyme cystathionine β-synthetase (CBS; EC 4.2.1.22). Cystathionine is then hydrolyzed to form cysteine, which is a precursor of the synthesis of glutathione (GSH). THF, tetrahydrofolate; 5,10-MTHF, 5,10-methylenetetrahydrofolate; 5-MTHF, 5-methyltetrahydrofolate; X, methyl acceptor molecule; X-CH_3, methylated molecule.

transsulfuration pathway, whereas low concentrations of SAMe enhance methionine conservation.

MAMMALIAN MATs

In mammals there are 2 genes encoding MATs: *MAT1A* and *MAT2A* (7). Whereas *MAT1A* is only expressed in the adult liver, *MAT2A* is expressed in all tissues, including fetal liver, hepatocellular carcinoma, and in small amounts in the adult liver (13). *MAT1A* encodes a catalytic subunit α_1 that organizes into dimers (MAT III) and tetramers (MAT I), whereas *MAT2A* encodes a catalytic subunit α_2 that associates to form MAT II (13). The α_1 and α_2 subunits share an amino acid sequence identity of 84% (13). The main difference between *MAT1A* and *MAT2A* is that MAT I and MAT III, the enzymes encoded by *MAT1A*, can maintain higher intracellular SAMe concentrations than can MAT II, the enzyme encoded by *MAT2A* (13). This is because of differences in the kinetic and regulatory properties of the enzymes. Although the combination of MAT I and MAT III provides the cell with a set of enzymes capable of efficiently metabolizing methionine over a large range of physiologic concentrations of this amino acid and without being inhibited by the intracellular accumulation of SAMe, the product of the reaction. MAT II has a high affinity but a low capacity to metabolize methionine and is strongly inhibited by SAMe at concentrations in the micromoles per liter range (13).

REGULATION OF MAT I AND MAT III ACTIVITIES BY REACTIVE OXYGEN SUBSTANCES AND NITRIC OXIDE

Nitric oxide (NO) and reactive oxygen substances (ROS) have been shown to switch both MAT I and MAT III to an inactive conformation through *S*-nitrosylation (formation of an SNO group) and oxidation (formation of an SOH group) of a single cysteine residue in position 121 (C-121), respectively. The α_1 subunit from which MAT I and MAT III are made contains 10 cysteine residues. It has been shown that only replacement of cysteine 121 by serine (Cys121→Ser) yields an active enzyme that is resistant to NO and ROS inactivation (14, 15). Replacement, one by one, of all other cysteine residues by serine yields active enzymes that are inactivated by both NO and ROS, just as the wild-type enzyme (14, 15). Cysteine 121 is located over the active site of the enzyme, in a flexible loop, suggesting that the covalent modification of this thiol group switches MAT I and MAT III into an inactive conformation (16). Protein *S*-nitrosylation involves an acid-base–catalyzed SNO-SH exchange reaction where the target cysteine is surrounded by basic and acidic amino acids that reduce the pKa of its thiol group and make it more nucleophilic (17). Accordingly, MAT *S*-nitrosylation has been shown to be regulated by the basic (Arg357 and Arg363) and acidic (Asp355) amino acids surrounding the target thiol, and replacement of any of these 3 residues by serine markedly reduces the capacity of NO to *S*-nitrosylate and inactivate liver MAT (16). In MAT II, Arg357, Arg363, and Asp355 are conserved, but the equivalent position to Cys121 in MAT I and MAT III is occupied by a residue of glycine (Gly120). Accordingly, MAT II is not inactivated by NO, but the replacement of Gly120 by cysteine (Gly120) yields an active enzyme that, in the presence of NO donors, is *S*-nitrosylated and inactivated (18). An alignment of a large variety of MATs, cloned from bacteria, yeast, plants, *Drosophila*, and mice, rat, and human kidney and liver tissue have shown that the presence of a cysteine residue in position 121 is a characteristic of the liver enzymes and that all other MATs have a different amino acid in this position, most frequently a residue of glycine.

Inactivation of MAT I and MAT III by NO or ROS is reversed by physiologic (millimolar) concentrations of glutathione (14, 19, 20). In isolated rat hepatocytes, incubation with NO donors or inhibition of glutathione synthesis induces the *S*-nitrosylation and inactivation of MAT and a reduction in SAMe content (21). Removal of the NO donor from the incubation media or restoration of the cellular glutathione concentrations leads to the denitrosylation and reactivation of liver MAT and to the rapid recovery of the SAMe content (21). The intraperitoneal injection of bacterial lipopolysaccharide to rats also results in the *S*-nitrosylation and inactivation of hepatic MAT (19). Similarly, inhibition in rats of glutathione H synthesis by intraperitoneal injection of buthionine sulfoximine leads to the *S*-nitrosylation and inactivation of hepatic MAT (21, 22).

In summary, liver MAT I and MAT III activities seem to be precisely regulated by NO and ROS (which maintain the enzyme in an inactive conformation) and by glutathione (which reactivates the enzyme). The inactivation of MAT I and II by NO or ROS may

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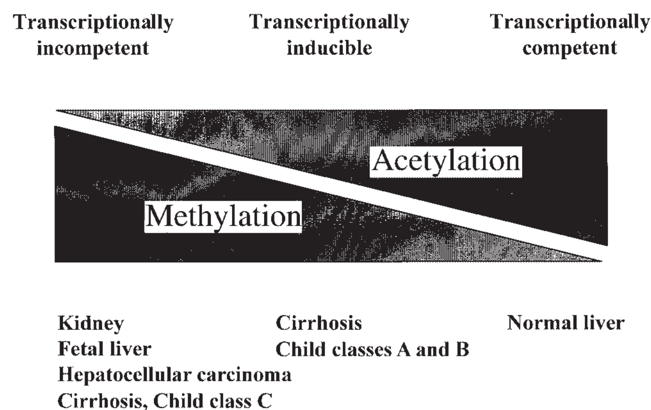

FIGURE 2. Regulation of the expression of *MAT1A* by methylation of the gene promoter and its association with nonacetylated histones. In the normal adult liver, *MAT1A* is transcriptionally competent and its promoter is demethylated and associated with acetylated histones. On the contrary, in extrahepatic tissues, fetal liver, hepatocellular carcinoma, and advanced states of liver cirrhosis (Child class C), *MAT1A* is transcriptionally incompetent and its promoter is methylated and associated with nonacetylated histones. In less advanced states of cirrhosis (Child classes A and B), *MAT1A* expression is partially silenced but can be induced (ie, by treatment with *S*-adenosyl-L-methionine).

be the mechanism by which a variety of conditions that induce oxidative stress (septic shock, ethanol, carbon tetrachloride, and hepatitis B– and hepatitis C–induced liver cirrhosis), or increase NO synthesis (hypoxia, liver regeneration, and cytokines), inactivate hepatic MAT I and III (14, 23–27). Moreover, these results suggest that liver MAT I and III inactivation may be a key signal to respond to injury.

REGULATION OF *MAT1A* AND *MAT2A* EXPRESSION

Functional analysis by transient transfection has shown that the rat *MAT1A* promoter is active not only in hepatocytes and liver-type cells, such as the human hepatoma cell line HepG2, but also in a nonhepatic cell such as Chinese hamster ovary cells (28). These findings suggest that the liver-restricted expression of the endogenous gene may not be mediated by the action of tissue-specific factors. It was then found that *MAT1A* promoter is hypermethylated at a CpG site in extrahepatic tissues and fetal liver but unmethylated in adult liver, where the gene is actively transcribed (29, 30). It has been shown that elevated concentrations of histone acetylation are critical to maintain a decondensed and active state of the chromatin, and the underlying pattern of CpG methylation modulates histone acetylation (31, 32). Accordingly, the degree of acetylation of histones associated with *MAT1A* promoter in the liver is ≈15-fold higher than that observed in the kidney (29, 30). In rat- and human-derived hepatoma cell lines and in human hepatocellular carcinoma, *MAT1A* is expressed at reduced or undetectable concentrations, whereas *MAT2A* expression is markedly induced (33, 34). Regarding the mechanisms behind the silencing of *MAT1A* expression during liver neoplastic transformation, it has been found that *MAT1A* is hypermethylated in HepG2 cells and that the treatment of these cells with the demethylating agent 5′-aza-2-deoxycytidine or the histone deacetylase inhibitor tricostatin A results in the induction of *MAT1A* expression (30). Taken together, these observations indicate that mechanisms involving DNA methylation and histone deacetylation may be the main factors responsible for the liver-restricted expression of *MAT1A* and for its silencing in neoplastic transformation (**Figure 2**).

In human liver cirrhosis, independently of the etiology (alcohol, hepatitis B, hepatitis C, etc), *MAT1A* is expressed at reduced and in some cases undetectable concentrations, whereas the expression of *MAT2A* remains low (34). It has been shown that *MAT1A* promoter is hypermethylated in the liver of cirrhotic patients (34), suggesting that this and the increased production of ROS and NO may be responsible for the impaired hepatic MAT activity (33, 34), increased serum concentrations of methionine (24, 35), and abnormal clearance of this amino acid (5, 24, 36) in human liver cirrhosis (Figure 2). Understanding why *MAT1A* becomes hypermethylated in liver cirrhosis may have applications in the treatment of this disease.

With respect to *MAT2A*, when the methylation pattern of *MAT2A* promoter was analyzed, no differences were observed between the liver and other tissues where the gene is actively transcribed, such as the kidney and spleen (37). Moreover, this analysis showed the same degree of methylation in all 3 tissues. However, differences in the degree of methylation have been observed between normal liver and in human liver cancer; the human *MAT2A* promoter is hypomethylated in hepatocellular carcinoma but hypermethylated in normal liver (38). The reason for these differences is not clear and the exact role of promoter methylation in *MAT2A* expression remains to be established. Histones associated with *MAT2A* promoter are hyperacetylated in kidney and hypoacetylated in liver (31), suggesting that the low activity of *MAT2A* in liver results from the inaccessibility of transcription factors to binding sites within the condensed chromatin.

Liver regeneration is a fundamental feature of the response of this organ to injury. Liver mass loss, caused by hepatotoxins or partial hepatectomy, initiates a cellular response—involving a vast number of growth factors, cytokines, and transcription factors—that has positive and negative effects on liver until the restoration of normal hepatic architecture and function (39). In regenerating liver after partial hepatectomy, a switch in MAT gene expression has been observed: *MAT2A* is induced and *MAT1A* messenger RNA (mRNA) concentrations decrease (40–42). The induction of *MAT2A* expression is accompanied by a marked increase in the acetylation of the histones associated with this gene (42). In culture hepatocytes, *MAT2A* expression is induced and *MAT1A* mRNA concentrations decrease (43), a situation reminiscent of that found in fetal and regenerating liver and in hepatocellular carcinoma. Addition of hepatocyte growth factor (HGF, a potent mitogen for hepatocytes that plays a key role in liver regeneration) to culture hepatocytes markedly stimulates the acetylation of histones associated with *MAT2A* and the expression of the gene (43). The effect of HGF on *MAT2A* expression is prevented by SAMe (43). On the other hand, the reduction in *MAT1A* expression in hepatocytes in culture is suppressed by SAMe through a mechanism that remains unclear but that may involve protein methylation (43). In HepG2 and HuH-7 cells differing only in the type of MAT gene that is expressed, *MAT2A* expression associates with

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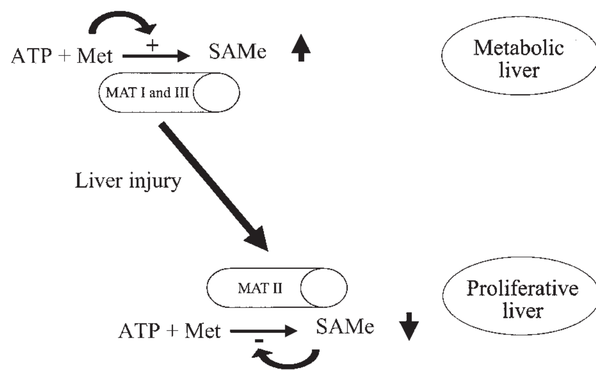

FIGURE 3. In response to liver injury, both the expression and activity of methionine adenosyltransferase (MAT; EC 2.5.1.6) I and III are switched off and MAT II expression is switched on. As a consequence of this switch in MAT expression, the hepatic content of *S*-adenosyl-L-methionine (SAMe) changes to a new lower steady state concentration, which facilitates the progression of the cell division cycle and hepatocyte growth.

more rapid cell growth, whereas the opposite situation is observed for *MAT1A* (44). Cells expressing *MAT2A* had lower SAMe concentrations than did cells expressing *MAT1A*, and treatment of HuH-7 cells with SAMe also led to reduced cell growth (44). These data suggest that SAMe has a growth-modulatory effect. In fact, addition of SAMe to culture hepatocytes has been found to markedly inhibit the mitogenic activity of HGF (43). Interestingly, SAMe therapy has been shown to be effective in preventing the growth of rat hepatocellular carcinoma (45, 46). These findings provide a new and unexpected view of SAMe as a modulator of hepatocyte proliferation.

In summary, the available data indicate that the SAMe content in hepatocytes regulates the type of MAT gene that is expressed and that the type of MAT gene expressed strongly influences the rate of cell growth and DNA synthesis. As mentioned above, the main difference between *MAT1A* and *MAT2A* is that MAT I and MAT III, the enzymes encoded by *MAT1A*, can maintain higher intracellular SAMe concentrations than can MAT II, the enzyme encoded by *MAT2A*. Under normal conditions, MAT I and MAT III are the main enzymes present in the liver and the hepatic SAMe content is maintained high. Consequently, *MAT1A* is highly expressed and *MAT2A* mRNA concentrations remain very low. Liver mass loss, caused by hepatotoxins or partial hepatectomy, initiates a cellular response involving a vast number of growth factors and cytokines (eg, HGF, tumor necrosis factor α, and interleukin 6) and the generation of NO and ROS (39, 47, 48). This leads to the inactivation of MAT I and MAT III and a concomitant reduction in the SAMe content, which in turn produces a reduction in *MAT1A* expression and a further decrease in the activity of MAT I and MAT III and in SAMe synthesis. The reduction in SAMe content releases the inhibition that this molecule exerts on the mitogenic activity of HGF as well as on the expression of *MAT2A*. As a whole, in response to liver injury, the expression and activity of MAT I and MAT III is switched off and MAT II expression is switched on and the hepatic content of SAMe changes to a new lower steady state concentration; this facilitates the progression of the cell division cycle and hepatocyte growth (**Figure 3**). During restoration of the original liver mass, the inhibitory effect that NO and ROS exert on the activity of MAT I and MAT III is released, *MAT1A* expression is induced, the SAMe content increases, and *MAT2A* expression is again switched off, which may help the liver to recover its normal nonproliferative metabolic phenotype.

IMPORTANCE OF A DEFICIENCY IN SAMe SYNTHESIS IN THE PATHOGENESIS OF LIVER INJURY

To examine the hepatic consequences of a chronic reduction in SAMe, we recently generated a knockout mouse deficient in hepatic MAT synthesis (49). As expected, *MAT1A* null (MATO) mice have a marked elevation in blood methionine and a low hepatic SAMe content with respect to wild-type animals (49). Three-month-old mice, although histologically normal, have hepatic hyperplasia and are more prone to develop liver steatosis after being fed a diet deficient in choline than are wild-type mice (49). Moreover, carbon tetrachloride–induced injury of the liver, determined histologically and on the basis of serum concentrations of alanine transaminase (EC 2.6.1.2) and aspartate transaminase (EC 2.6.1.1), was much more intense in MATO mice than in wild-type mice. At 8 mo of age, MATO mice develop steatohepatitis (49). Three-month-old MATO mice, although histologically normal, display a phenotype that resembles that observed in liver injury or during stress with a vast array of growth, dedifferentiation, and acute phase response genes (eg, proliferating cell nuclear antigen, α-fetoprotein, *MAT2A*, and orosomucoid) upregulated (49). This indicates that, at the molecular level, the hepatic lesion has started long before it is histologically evident. Because hepatic hyperplasia may precede the appearance of liver cancer (50), it is important to know whether the risk of hepatocellular carcinoma is increased in MATO mice. These results clearly indicate that a chronic deficiency in SAMe synthesis, such as that observed in patients with liver cirrhosis, contributes to the pathogenesis of liver injury.

USE OF SAMe AS A THERAPEUTIC AGENT FOR LIVER DISEASE

In cirrhotic patients, the progressive silencing of MAT I and MAT III (expression and activity) caused by this condition may expose the liver to an additional oxidative stress that will contribute to the progression and complications of the disease. Preventing SAMe deficiency could be a major therapeutic target for the treatment of human liver diseases. As reviewed elsewhere (51), a variety of clinical studies indicate that SAMe treatment, given either orally or parenterally, is beneficial in intrahepatic cholestasis. Thus, in a double-blind, placebo-controlled, multicenter clinical trial performed in 220 patients with chronic liver disease (chronic active hepatitis and cirrhosis), SAMe treatment significantly improved serum markers (bilirubin and alkaline phosphatase) and subjective symptoms (eg, pruritus and fatigue) of cholestasis (52). Similar findings were obtained in a variety of blind and open studies, and a meta-analysis of the results of these studies confirmed the efficacy of the drug (53). In addition, SAMe treatment was shown to be beneficial in severe cholestasis of pregnancy (54). These results clearly show that short-term administration of SAMe improves the clinical and biochemical features of cholestasis. Moreover, in patients with alcoholic liver disease, oral administration of 1.2 g SAMe/d for 6 mo resulted in a significant increase in hepatic glutathione (55). In animal models of

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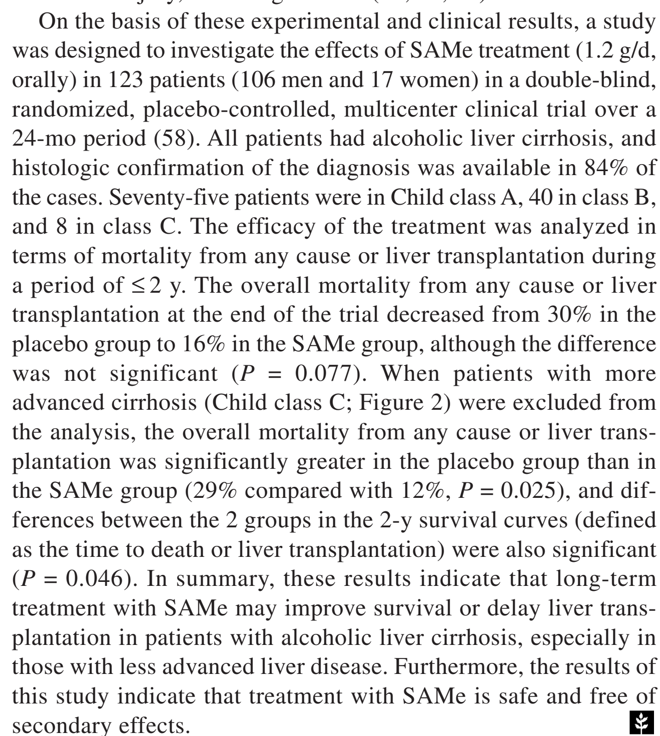

alcoholic liver disease and carbon tetrachloride hepatotoxicity, exogenous administration of SAMe prevented the depletion of SAMe and glutathione concentrations and significantly ameliorated liver injury, including fibrosis (25, 56, 57).

On the basis of these experimental and clinical results, a study was designed to investigate the effects of SAMe treatment (1.2 g/d, orally) in 123 patients (106 men and 17 women) in a double-blind, randomized, placebo-controlled, multicenter clinical trial over a 24-mo period (58). All patients had alcoholic liver cirrhosis, and histologic confirmation of the diagnosis was available in 84% of the cases. Seventy-five patients were in Child class A, 40 in class B, and 8 in class C. The efficacy of the treatment was analyzed in terms of mortality from any cause or liver transplantation during a period of ≤2 y. The overall mortality from any cause or liver transplantation at the end of the trial decreased from 30% in the placebo group to 16% in the SAMe group, although the difference was not significant ($P = 0.077$). When patients with more advanced cirrhosis (Child class C; Figure 2) were excluded from the analysis, the overall mortality from any cause or liver transplantation was significantly greater in the placebo group than in the SAMe group (29% compared with 12%, $P = 0.025$), and differences between the 2 groups in the 2-y survival curves (defined as the time to death or liver transplantation) were also significant ($P = 0.046$). In summary, these results indicate that long-term treatment with SAMe may improve survival or delay liver transplantation in patients with alcoholic liver cirrhosis, especially in those with less advanced liver disease. Furthermore, the results of this study indicate that treatment with SAMe is safe and free of secondary effects.

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