

# Importance of a deficiency in S-adenosyl-L-methionine synthesis in the pathogenesis of liver injury<sup>1-4</sup>

M Luz Martínez-Chantar, Elena R García-Trevijano, M Ujue Latasa, Isabel Pérez-Mato, Manuel M Sánchez del Pino, Fernando J Corrales, Matias A Avila, and José M Mato

## 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 triphosphosphate 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 triphosphosphate moiety from ATP. In the second step, the triphosphosphate 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 triphosphosphate. 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

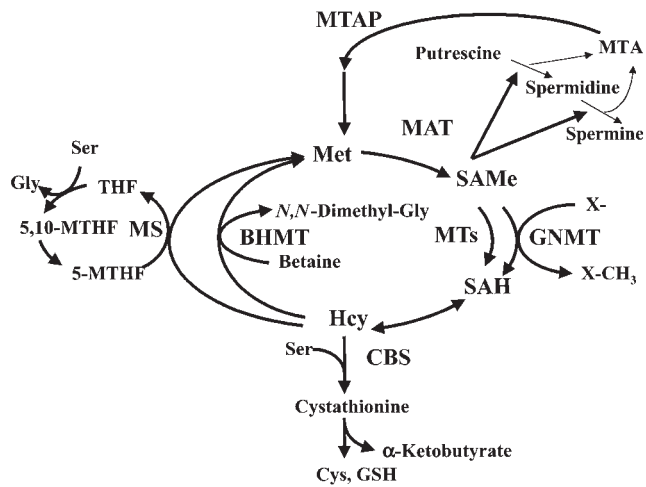
and that a surprisingly large fraction of all genes are SAME-dependent methyltransferases. The crucial importance of the liver in the regulation of blood methionine concentrations was first established by Kinsell et al in 1947 (5). These authors showed a marked impairment of methionine metabolism in 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,  $\leq 85\%$  of all methylation reactions and  $\approx 50\%$  of methionine metabolism occur in the liver (6, 7). In addition, these authors estimated that the half-life of SAME in the liver is  $\approx 5$  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  $\approx 20\%$  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  $\beta$ -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

<sup>1</sup> From the Division of Hepatology and Gene Therapy, School of Medicine, University of Navarra, Pamplona, Spain.

<sup>2</sup> Presented at the symposium S-Adenosylmethionine (SAME): from Molecular Mechanism to Clinical Implications, held in Santa Barbara, CA, March 7–10, 2001.

<sup>3</sup> Supported by grant 99/0038 from Plan Nacional de I+D, Ministerio de Educación y Ciencia, Knoll grant ROI AA-12677 from the National Institute on Alcohol Abuse and Alcoholism, grant FIS 01/0712 from the Ministerio de Sanidad y Consumo, and grants 5697/1999, 681/2000, and 349/2001 from Gobierno de Navarra.

<sup>4</sup> Address reprint requests to JM Mato, Division of Hepatology and Gene Therapy, School of Medicine, University of Navarra, 31008 Pamplona, Spain. E-mail: jmmato@unav.es.



**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  $\beta$ -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<sub>3</sub>, 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  $\alpha_1$  that organizes into dimers (MAT III) and tetramers (MAT I), whereas *MAT2A* encodes a catalytic subunit  $\alpha_2$  that associates to form MAT II (13). The  $\alpha_1$  and  $\alpha_2$  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).

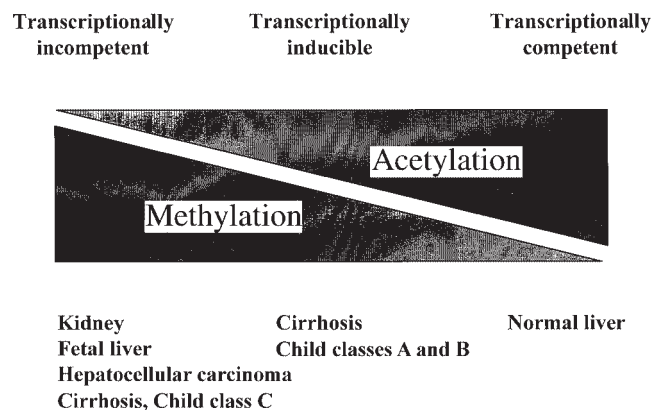
### REGULATION OF MAT I AND MAT III ACTIVITIES BY REACTIVE OXYGEN SUBSTANCES AND NITRIC OXIDE

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  $\alpha_1$  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



**MAT1A**

**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

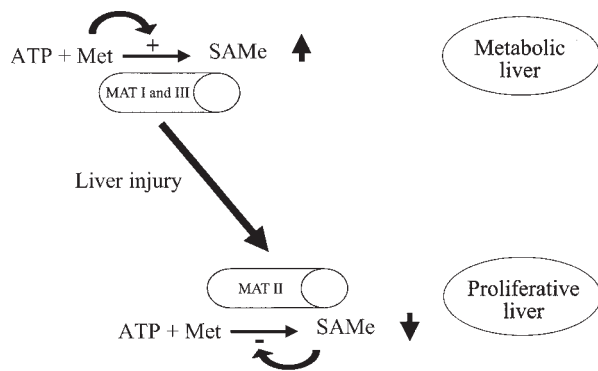
Functional analysis by transient transfection has shown that the rat *MAT1A* promoter is active not only in hepatocytes and liver-type cells, such as the human hepatoma cell line HepG2, but also in a nonhepatic cell such as Chinese hamster ovary cells (28). These findings suggest that the liver-restricted 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  $\approx 15$ -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 trichostatin 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



**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 (S-AMe) 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 S-AMe concentrations than did cells expressing *MAT1A*, and treatment of HuH-7 cells with S-AMe also led to reduced cell growth (44). These data suggest that S-AMe has a growth-modulatory effect. In fact, addition of S-AMe to culture hepatocytes has been found to markedly inhibit the mitogenic activity of HGF (43). Interestingly, S-AMe 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 S-AMe as a modulator of hepatocyte proliferation.

In summary, the available data indicate that the S-AMe 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 S-AMe 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 S-AMe 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  $\alpha$ , 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 S-AMe 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 S-AMe synthesis. The reduction in S-AMe 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 S-AMe 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 S-AMe 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 S-AMe SYNTHESIS IN THE PATHOGENESIS OF LIVER INJURY

To examine the hepatic consequences of a chronic reduction in S-AMe, 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 S-AMe 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,  $\alpha$ -fetoprotein, *MAT2A*, and orosomucoid) up-regulated (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 S-AMe synthesis, such as that observed in patients with liver cirrhosis, contributes to the pathogenesis of liver injury.

### USE OF S-AMe 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 S-AMe 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 S-AMe 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), S-AMe 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, S-AMe treatment was shown to be beneficial in severe cholestasis of pregnancy (54). These results clearly show that short-term administration of S-AMe improves the clinical and biochemical features of cholestasis. Moreover, in patients with alcoholic liver disease, oral administration of 1.2 g S-AMe/d for 6 mo resulted in a significant increase in hepatic glutathione (55). In animal models of

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  $\leq 2$  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. 

## REFERENCES

- Cantoni GL. S-adenosylmethionine: a new intermediate formed enzymatically from L-methionine and adenosine triphosphate. *J Biol Chem* 1953;204:403–16.
- Kotb M, Mudd HS, Mato JM, et al. Consensus nomenclature for the mammalian methionine adenosyltransferase genes and gene products. *Trends Genet* 1997;13:51–2.
- Mato JM, Alvarez L, Ortiz P, Pajares MA. S-Adenosylmethionine synthesis: molecular mechanisms and clinical implications. *Pharmacol Ther* 1997;73:265–80.
- Alvarez L, Asunción M, Corrales FJ, Pajares MA, Mato JM. Analysis of the 5' non-coding region of rat liver S-adenosylmethionine synthetase mRNA and comparison of the Mr deduced from the cDNA sequence and the purified enzyme. *FEBS Lett* 1991;290:142–6.
- Kinsell LW, Harper HA, Marton HC, Michael GD, Weiss HA. Rate of disappearance from plasma of intravenously administered methionine in patients with liver damage. *Science* 1947;106:589–90.
- Mudd SH, Poole JR. Labile methyl balances for normal humans of various dietary regimens. *Metabolism* 1975;24:721–35.
- Finkelstein JD. Methionine metabolism in mammals. *J Nutr Biochem* 1990;1:228–36.
- Finkelstein JD, Kyle WE, Martin JJ, Pick AM. Activation of cystathionine synthase by S-adenosylmethionine and S-adenosylethionine. *Biochem Biophys Res Commun* 1975;66:81–7.
- Finkelstein JD, Martin JJ. Inactivation of betaine-homocysteine methyltransferase by S-adenosylmethionine and S-adenosylhomocysteine. *Biochem Biophys Res Commun* 1984;118:14–9.
- Finkelstein JD, Martin JJ. Methionine metabolism in mammals: distribution of homocysteine between competitive pathways. *J Biol Chem* 1984;259:9508–13.
- Kutzbach C, Stokstad ELR. Feedback inhibition of methylene-tetrahydrofolate reductase in rat liver by S-adenosylmethionine. *Biochim Biophys Acta* 1967;139:217–20.
- Kutzbach C, Stokstad ELR. Mammalian methylene-tetrahydrofolate reductase: partial purification, properties and inhibition by S-adenosylmethionine. *Biochim Biophys Acta* 1971;250:459–77.
- Mato JM, Avila MA, Corrales FJ. Biosynthesis of S-adenosylmethionine. In: Carmel R, Jacobsen DW, eds. *Homocysteine in health and disease*. Cambridge, United Kingdom: Cambridge University Press, 2001:47–62.
- Avila MA, Mingorance ML, Martinez-Chantar ML, et al. Regulation of rat liver S-adenosylmethionine synthetase during septic shock: role of nitric oxide. *Hepatology* 1997;25:391–6.
- Sánchez-Góngora E, Pasrtorino JG, Alvarez L, et al. Increased sensitivity to oxidative injury in Chinese hamster ovary cells stably transfected with rat liver S-adenosylmethionine synthetase cDNA. *Biochem J* 1996;319:767–73.
- Pérez-Mato I, Castro C, Ruiz FA, Corrales FJ, Mato JM. Methionine adenosyltransferase S-nitrosylation is regulated by the basic and acidic amino acids surrounding the target thiol. *J Biol Chem* 1999;274:17075–9.
- Stamler JS, Toone EJ, Lipton SA, Sucher NJ. (S)NO signals: translocation regulation, and a consensus motif. *Neuron* 1997;18:619–26.
- Castro C, Ruiz FA, Perez-Mato I, et al. Creation of a functional S-nitrosylation site in vitro by single point mutation. *FEBS Lett* 1999;459:319–22.
- Ruiz F, Corrales FJ, Miqueo C, Mato JM. Nitric oxide inactivates rat hepatic methionine adenosyltransferase in vivo by S-nitrosylation. *Hepatology* 1998;28:1051–7.
- Sánchez-Góngora E, Ruiz F, Mingorance J, An W, Corrales JM, Mato JM. Interaction of liver methionine adenosyltransferase with hydroxyl radical. *FASEB J* 1997;11:1013–9.
- Corrales FJ, Ruiz F, Mato JM. In vivo regulation by glutathione of methionine adenosyltransferase S-nitrosylation in rat liver. *J Hepatol* 1999;31:887–94.
- Corrales FJ, Ochoa, P, Rivas C, Martin-Lomas M, Mato JM, Pajares MA. Inhibition of glutathione synthesis in the liver leads to S-adenosyl-L-methionine synthetase reduction. *Hepatology* 1991;14:528–33.
- Avila MA, Carretero MV, Rodriguez N, Mato JM. Regulation by hypoxia of methionine adenosyltransferase activity and gene expression in rat hepatocytes. *Gastroenterology* 1998;114:364–71.
- Cabrero C, Martin-Duce A, Ortiz P, Alemany S, Mato JM. Specific loss of the high molecular weight form of S-adenosylmethionine synthetase in human liver cirrhosis. *Hepatology* 1988;8:1530–4.
- Corrales F, Giménez L, Alvarez L, et al. S-Adenosylmethionine treatment prevents carbon tetrachloride induced S-adenosylmethionine synthetase inactivation and attenuates liver injury. *Hepatology* 1992;16:1022–7.
- Lu SC, Huang ZZ, Yang HP, Mato JM, Avila MA, Tsukamoto H. Changes in methionine adenosyltransferase and S-adenosylmethionine homeostasis in alcoholic rat liver. *Am J Physiol* 2000;379:G178–85.
- Sakata SF, Shelly LL, Ruppert S, Schutz G, Chou JY. Cloning and expression of murine S-adenosylmethionine synthetase. *J Biol Chem* 1993;268:13978–86.
- Alvarez L, Sánchez-Góngora E, Mingorance J, Pajares MA, Mato JM. Characterization of rat liver-specific methionine adenosyltransferase gene promoter. Role of distal upstream cis-acting elements in the regulation of the transcription activity. *J Biol Chem* 1997;272:22875–83.
- Torres L, Avila MA, Carretero MV, et al. Liver-specific methionine adenosyltransferase *MAT1A* gene expression is associated with a specific pattern of promoter methylation and histone acetylation: implications for *MAT1A* silencing during transformation. *FASEB J* 2000;14:95–102.
- Torres A, López-Rodas G, Latasa MU, et al. DNA methylation and histone acetylation of rat methionine adenosyltransferase 1A and 2A genes is tissue-specific. *Int J Biochem Cell Biol* 2000;32:397–404.
- Singal R, Ginder GD. DNA methylation. *Blood* 1999;93:4059–70.

32. Cheung P, Allis CD, Sassone-Corsi P. Signaling to chromatin through histone modifications. *Cell* 2000;103:263–71.
33. Cai J, Sun W-M, Hwang J-J, Stain SC, Lu SC. Changes in S-adenosylmethionine synthetase in human liver cancer: molecular characterization and significance. *Hepatology* 1996;24:1090–7.
34. Avila MA, Berasain C, Torres L, et al. Reduced mRNA abundance of the main enzymes involved in methionine metabolism in human liver cirrhosis and hepatocellular carcinoma. *J Hepatol* 2000;33:907–14.
35. Horowitz JH, Rypins EB, Henderson JM, et al. Evidence for impairment of transsulfuration pathway in cirrhosis. *Gastroenterology* 1981; 81:668–75.
36. Martín-Duce A, Ortiz P, Cabrero C, Mato JM. S-Adenosyl-L-methionine synthetase and phospholipid methyltransferase are inhibited in human cirrhosis. *Hepatology* 1988;8:65–8.
37. Avila MA, García-Trevijano ER, Torres L, et al. Methyl groups in the regulation of *MAT1A* and *MAT2A* gene expression. In: Mato JM, Caballero A, eds. Methionine metabolism: molecular mechanisms and clinical implications. Madrid: Master Line SL, 2000:91–104.
38. Yang H, Huang ZZ, Chen C, Selby RR, Lu SC. Role of promoter methylation in increased methionine adenosyltransferase 2A expression in human liver cancer. *Am J Physiol* 2001;280:G184–90.
39. Michalopoulos GK, DeFrances MC. Liver regeneration. *Science* 1997;276:60–6.
40. Horikawa S, Ozasa H, Ito K, Katsuyama I, Tsukada K, Sugiyama T. Expression of S-adenosylmethionine synthetase isoenzyme genes in regenerating liver after partial hepatectomy. *Biochem Mol Biol Int* 1996;40:807–14.
41. Huang ZZ, Mao Z, Cai J, Lu SC. Changes in methionine adenosyltransferase during liver regeneration in the rat. *Am J Physiol* 1999; 275:G14–21.
42. Latasa MU, Boukaba A, García-Trevijano ER, et al. Hepatocyte growth factor induces *MAT2A* expression and histone acetylation in rat hepatocytes: role in liver regeneration. *FASEB J* 2001;15:1248–50.
43. García-Trevijano ER, Latasa MU, Carretero MV, Berasain C, Mato JM, Avila MA. S-Adenosylmethionine regulates *MAT1A* and *MAT2T* expression in cultured rat hepatocytes: a new role for S-adenosylmethionine in the maintenance of the differentiated status of the liver. *FASEB J* 2001;15:1248–50.
44. Cai J, Mao Z, Hwang J, Lu SC. Differential expression of methionine adenosyltransferase genes influences the rate of growth of human hepatocellular carcinoma cells. *Cancer Res* 1998;58:1444–50.
45. Garcea R, Daino L, Pascale R, et al. Inhibition of promotion and persistent nodule growth by S-adenosyl-L-methionine in rat liver carcinogenesis: role of remodeling and apoptosis. *Cancer Res* 1989; 49:1850–6.
46. Pascale RM, Marras V, Simile MM, et al. Chemoprevention of rat liver carcinogenesis by S-adenosylmethionine: a long-term study. *Cancer Res* 1992;52:4979–86.
47. Hortelano S, Dewez B, Genaro AM, Díaz-Guerra MJM, Bosca L. Nitric oxide is released in regenerating liver after partial hepatectomy. *Hepatology* 1995;21:776–86.
48. Rai RM, Lee FYJ, Rosen A, et al. Impaired liver regeneration in inducible nitric oxide synthase-deficient mice. *Proc Natl Acad Sci U S A* 1998;95:13829–34.
49. Lu SC, Alvarez L, Huang Z-Z, et al. Methionine adenosyltransferase 1A knockout mice are predisposed to liver injury and exhibit increased expression of genes involved in proliferation. *Proc Natl Acad Sci U S A* 2001;98:5560–5.
50. Yang SQ, Lin HZ, Hwang J, Chacko VP, Diehl AM. Hepatic hyperplasia in noncirrhotic fatty livers: is obesity-related hepatic steatosis a premalignant condition? *Cancer Res* 2001;61:5016–23.
51. Lieber CS. Role of S-adenosylmethionine in the treatment of liver diseases. *J Hepatol* 1999;30:1155–9.
52. Frezza M, Surrenti C, Manzillo G, Fiaccadori F, Bortolini M, Di Padova C. Oral S-adenosylmethionine in the symptomatic treatment of intrahepatic cholestasis: a double-blind placebo controlled study. *Gastroenterology* 1990;99:211–5.
53. Frezza M, Pozzato G, Chiesa L, Stramentinoli G, Di Padova C. Reversal of intrahepatic cholestasis of pregnancy in women after high dose S-adenosyl-L-methionine administration. *Hepatology* 1984;4:274–8.
54. Frezza M. A meta-analysis of therapeutic trials with ademetionine in the treatment of intrahepatic cholestasis. *Ann Ital Med Int* 1993; 8(suppl):48S–51S.
55. Vendemiale G, Altomare E, Trizio T, Le Grazie C, Di Padova C, Salerno MT. Effect of oral S-adenosyl-L-methionine on hepatic glutathione in patients with liver disease. *Scand J Gastroenterol* 1989; 24:407–15.
56. Lieber CS, Casini A, DeCarli LM, et al. S-adenosyl-L-methionine attenuates alcohol-induced liver injury in the baboon. *Hepatology* 1990;11:165–72.
57. García-Ruiz C, Morales A, Colell A, et al. Feeding S-adenosylmethionine attenuates both ethanol-induced depletion of mitochondrial glutathione and mitochondrial dysfunction in periportal and perivenous rat hepatocytes. *Hepatology* 1995;21:207–14.
58. Mato JM, Cámara J, Fernandez P, et al. S-Adenosylmethionine in alcoholic liver cirrhosis: a randomized, placebo-controlled, double-blind, multicenter clinical trial. *J Hepatol* 1999;30:1081–9.