ELSEVIER

Alcohol 27 (2002) 163–167

ALCOHOL

S-Adenosylmethionine revisited: its essential role in the regulation of liver function

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Received 4 February 2002; received in revised form 25 March 2002; accepted 28 March 2002

Abstract

Dietary methionine is mainly metabolized in the liver where it is converted into *S*-adenosylmethionine (AdoMet), the main biologic methyl donor. This reaction is catalyzed by methionine adenosyltransferase I/III (MAT I/III), the product of *MAT1A* gene, which is exclusively expressed in this organ. It was first observed that serum methionine levels were elevated in experimental models of liver damage and in liver cirrhosis in human beings. Results of further studies showed that this pathological alteration was due to reduced *MAT1A* gene expression and MAT I/III enzyme inactivation associated with liver injury. Synthesis of AdoMet is essential to all cells in the organism, but it is in the liver where most of the methylation reactions take place. The central role played by AdoMet in cellular function, together with the observation that AdoMet administration reduces liver damage caused by different agents and improves survival of alcohol-dependent patients with cirrhosis, led us to propose that alterations in methionine metabolism could play a role in the onset of liver disease and not just be a consequence of it. In the present work, we review the recent findings that support this hypothesis and highlight the mechanisms behind the hepatoprotective role of AdoMet.

Keywords: *S*-Adenosylmethionine; Liver damage; Cirrhosis; Hepatocarcinoma; Gene expression

1. Overview

Methionine is one of the nine essential amino acids for mammals. In addition to its incorporation in proteins, methionine is a precursor of *S*-adenosylmethionine (AdoMet). In this reaction, the adenosyl moiety of ATP is transferred to methionine, a process catalyzed by methionine adenosyltransferase (MAT) (Finkelstein, 1990). In the liver, MAT is the product of *MAT1A* gene, which encodes a catalytic subunit (α_1) that organizes into dimers (MAT III) and tetramers (MAT I) (Kotb et al., 1997). AdoMet is the methyl donor for essentially all known methylation reactions. In addition, AdoMet provides the propylamine group for the synthesis of polyamines and, in the liver, participates in the synthesis of glutathione (GSH) through the transsulfuration pathway (Finkelstein, 1990). Synthesis and metabolism of AdoMet take place mainly in the liver, where up to 85% of methylation reactions and almost 50% of methionine metabolism occur (Mudd & Poole, 1975). Other cell types express a different MAT gene, namely *MAT2A*, which codes for the catalytic subunit α_2 that associates to form MAT II (Kotb & Geller, 1993; Kotb et al., 1997). The kinetic and regulatory properties of MAT I/III and MAT II are different. The expression of MAT I/III results in elevated cellular levels of AdoMet for a wide range of physiological concentrations of methionine, whereas MAT II is inhibited by micromolar concentrations of AdoMet (Kotb & Geller, 1993; Mato et al., 2001). The inhibition by AdoMet of MAT II activity seems to be mediated by a regulatory subunit (β), which is the product of *MAT2B* gene (LeGros et al., 2000). In the fetal hepatocyte *MAT1A* is not expressed, whereas *MAT2A* is actively transcribed. This pattern of expression is rapidly switched after birth (Gil et al., 1996). Interestingly, when the adult hepatocyte proliferates, as occurs after partial hepatectomy, *MAT2A* expression is induced (Huang et al., 1998; Latasa et al., 2001). The fetal pattern of MAT gene expression is recovered on malignant transformation of the liver: *MAT1A* expression is silenced and *MAT2A* is induced

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Editor: T.R. Jerrells

PII: S0741-8329(02)00228-8

(Avila et al., 2000; Cai et al., 1996). This switch in MAT gene expression has been suggested to provide the transformed cell with a proliferative advantage (Cai et al., 1998).

Alterations in liver methionine metabolism have been associated with liver damage for more than 50 years (Kinsell et al., 1947). Serum methionine levels are elevated in patients with alcoholic liver cirrhosis, and these patients display abnormal methionine clearance after an oral load of this amino acid and delayed sulfate excretion (Avila et al., 2000; Horowitz et al., 1981; Marchesini et al., 1992). Similarly, in experimental models of liver damage, such as in ethanol- and CCl_4-induced liver injury, impairment in methionine metabolism has been reported (Mato et al., 1997). The mechanisms behind this alteration in methionine metabolism began to be understood when it was observed that patients with cirrhosis had reduced (50%) liver MAT activity as compared with findings for healthy controls (Cabrero et al., 1988; Martín-Duce et al., 1988). These observations were also made in various experimental models of liver disease, including in ethanol- and CCl_4-treated rats and ethanol-fed baboons (Barak et al., 1987; Lieber et al., 1990; Lu et al., 2000; Trimble et al., 1993; Varela-Moreiras et al., 1995). Impairment in MAT activity compromises cellular AdoMet synthesis, and this situation may have an impact on many essential metabolic pathways in the liver in which AdoMet participates. In fact, it has been observed that alterations in homocysteine, carnitine, and phosphatidylethanolamine metabolism and DNA methylation develop in patients with cirrhosis and in experimental models of liver damage (Avila et al., 2000; Krahenbuhl, 1996; Krahenbuhl et al., 2000; Lieber et al., 1990, 1994; Mirpuri et al., 2002; Varela-Moreiras et al., 1995). These alterations are likely to influence the development of the disease. This view is supported in part by the protective effect of AdoMet (see discussion later) and AdoMet-dependent metabolites such as phosphatidylcholine (Lieber, 1999a; Lieber et al., 1990, 1994; Navder et al., 1997) in experimental models of liver damage and in patients with cirrhosis. It was subsequently observed that MAT I/III activity was dependent on the redox status of the cell, and that the prooxidant conditions that developed in the cirrhotic liver could be behind the inhibition of MAT I/III (Lieber, 1997). Experimental evidence for this was obtained when MAT I/III activity was measured in rat liver after administration of butionine sulfoximine (BSO), an inhibitor of GSH synthesis, and a reduction in MAT I/III activity was observed (Corrales et al., 1991). In addition, the administration of GSH–ethyl ester, which is hydrolyzed intracellularly to provide GSH, prevented the reduction in GSH levels and the inactivation of MAT I/III by BSO treatment (Corrales et al., 1992).

The molecular mechanism responsible for MAT I/III inactivation by free radicals has been characterized recently. Rat MAT I/III contains 10 cysteine residues and thus is very sensitive to thiol group modification (Mato et al., 2001). Nevertheless, functional interaction with reactive oxygen species seems to occur at a specific cysteine residue located at position 121; the oxidation of this cysteine residue would render the enzyme inactive (Sánchez-Góngora et al., 1997). This observation is based on site-directed mutagenesis studies, in which all 10 cysteine residues were individually changed by serines. All individual mutants were sensitive to inactivation by oxygen free radicals, with the exception of cysteine 121 mutant, which produced an enzyme that retained its catalytic activity but that was resistant to inhibition by reactive oxygen species (Sánchez-Góngora et al., 1997). Hence, this residue could represent a regulatory switch for MAT I/III, sensitive to environmental changes in the redox status of the hepatocyte. Interestingly, cysteine 121 is specific to human, rat, and mouse liver MAT and MAT I/III and is not present in MAT II; hence this mechanism of regulation would be restricted to the liver parenchymal cell. Similarly, MAT I/III can be inactivated by nitric oxide (NO) through the interaction of this free radical with the same cysteine residue at position 121, resulting in the *S*-nitrosylation of the thiol group (Avila et al., 1997; Ruiz et al., 1998). This has been demonstrated both in vitro with the purified rat liver enzyme and in vivo when rats were administered bacterial lipopolysaccharide (LPS), or in cultured rat hepatocytes under hypoxia, conditions known to trigger the synthesis of NO in the liver (Avila et al., 1997, 1998; Ruiz et al., 1998). Interestingly, MAT I/III inactivation by NO was reversible by incubation of the purified enzyme with thiol-reducing agents such as GSH (Avila et al., 1997) and was dependent on the hepatic levels of GSH in vivo (Corrales et al., 1999). Taken together these observations may be of clinical relevance because the development of oxidative stress in the liver, endotoxemia, and deficient oxygen supply to the pericentral region of the hepatic parenchyma are well recognized in individuals who are chronically dependent on alcohol (Avila et al., 1998; Bjarnason et al., 1984).

Besides MAT I/III activity, the expression of *MAT1A* is also compromised in the diseased liver. This has been observed in the experimental model of CCl_4-induced liver injury in rats (Torres et al., 2000) and in the liver of patients with cirrhosis of different causes (alcoholic and viral cirrhosis) (Avila et al., 2000). The oxidative stress that develops in these experimental models and in pathological conditions seems not to be related directly to the reported alterations in *MAT1A* gene expression. This is based on both the absence of oxidant-sensitive response elements in *MAT1A* promoter (Mato et al., 2001) and the direct experimental evidence obtained in cultured rat hepatocytes treated with oxidative stress–promoting agents (Carretero et al., 2001). Interestingly, the reduction in *MAT1A* mRNA levels was accompanied by the hypermethylation of its promoter, a covalent modification of DNA that plays a role in the silencing of gene expression and that could be involved in the above-mentioned loss of *MAT1A* expression on malignant transformation of the liver (Avila et al., 2000; Torres et al., 2000).

The main consequence of all these defects in MAT I/III expression and activity is the impaired synthesis of AdoMet in alcoholic liver disease, which is a clear example of how

ethanol may interfere with the activation of an essential nutrient (Holm et al., 1999; Lieber, 1999b). The relevance for the liver of a deficient supply of AdoMet may be inferred from the key role played by this metabolite in cellular function. The most extreme example of the essential role of preserving adequate hepatic AdoMet levels would be the observation that administration of methionine–choline-deficient diets induced hepatocyte de-differentiation and the development of liver tumors in rodents (Newberne & Rogers, 1986; Wainfan et al., 1989). Thus it has been proposed that reduced AdoMet availability plays an important role in the development of liver damage and that AdoMet supplementation could have hepatoprotective effects and a therapeutic value devoid of unwanted side effects (Chawla et al., 1990; Lieber, 1999b). Compelling evidence supporting this view has been provided by numerous experimental models of liver damage, both in rodents and in non-human primates, in which the administration of AdoMet partially restored liver function, including prevention of liver steatosis, fibrosis, and chemically induced hepatocarcinogenesis (Chawla et al., 1990; Garcea et al., 1989; Mato et al., 1997; Pascale et al., 1992). There are also studies with human subjects in which AdoMet administration to patients suffering from cholestasis induced by chronic liver disease resulted in the improvement of serum markers of cholestasis (Frezza et al., 1990). AdoMet treatment also has been shown to be beneficial in severe cholestasis of pregnancy (Frezza, 1993). However, the best evidence of the therapeutic potential of AdoMet was provided recently by a long-term randomized, placebo-controlled, double-blind, multicenter clinical trial of AdoMet (1.2 g/day, orally, over a 24-month period) in 123 patients with alcoholic liver cirrhosis, in whom it improved survival or delayed the need for liver transplantation (Mato et al., 1999).

The mechanisms through which AdoMet exerts its hepatoprotective actions are likely to be multifaceted, given the numerous reactions in which this molecule participates, including phospholipid, protein, and DNA methylation. Counteracting the oxidative stress developed with ethanol metabolism or viral infection may be one key mechanism (Mato et al., 1997). As previously mentioned, AdoMet is a precursor for cysteine, one of the three amino acids of GSH, the major biologic antioxidant molecule. It has been shown in patients with alcoholic liver disease that administration of AdoMet (1.2 g/day, orally) for 6 months significantly increased hepatic GSH (Vendemiale et al., 1989). Similar observations have been made in experimental models of ethanol- and CCl_4-induced liver injury, in which AdoMet treatment improved hepatic GSH and attenuated hepatocellular injury (Corrales et al., 1992; García-Ruiz et al., 1995; Lieber et al., 1990). Among the beneficial effects of improving GSH levels in the hepatocyte is the restoration of MAT I/III activity and thus of the ability of the cell to convert dietary methionine into AdoMet, therefore preserving the essential metabolic flow of methyl groups (Corrales et al., 1992; Finkelstein, 1990).

However, AdoMet seems to have even more profound effects on cell function. We have shown recently that changes in the intracellular concentrations of this molecule can modulate hepatocyte gene expression (García-Trevijano et al., 2000). In the experimental model of cultured primary rat hepatocytes, the expression of liver-specific genes (e.g., *MAT1A*) is progressively lost, whereas that of some non-liver-specific genes (including *MAT2A*) is increased, reflecting a certain degree of de-differentiation (García-Trevijano et al., 2000; Schuetz et al., 1988). Addition of AdoMet to cultured hepatocytes potently stimulated the expression of *MAT1A* while it simultaneously prevented induction of the expression of the non-liver-specific *MAT2A* gene (García-Trevijano et al., 2000). Moreover, AdoMet treatment of cultured rat hepatocytes reversed the negative effect of certain hepatotoxins such as aromatic aryl hydrocarbons on the expression of *MAT1A* (Carretero et al., 2001). These observations suggested to us that AdoMet could play a role in the preservation of the differentiated pattern of gene expression in the liver and that the progressive loss in the AdoMet-synthesizing ability of the chronically injured liver could have a negative impact on gene expression independent of DNA methylation.

The essential functions of AdoMet in the liver seem to transcend its purely metabolic role as a methyl-group donor in transmethylation reactions. Evidence is accumulating indicating that this molecule can indeed modulate the responses of the hepatocyte to growth factors and cytokines, such as hepatocyte growth factor (HGF) and tumor necrosis factor-α (TNF-α). In these regards, we have shown recently that the proliferative response of cultured hepatocytes to HGF depends on the intracellular levels of AdoMet, suggesting novel implications for this molecule in the liver regenerative response (Latasa et al., 2001). These observations may also be of relevance regarding the antineoplastic effects of AdoMet in chemically induced hepatocarcinoma (Garcea et al., 1989; Pascale et al., 1992). Similarly, activation of the transcription factor nuclear factor-kappa B (NF-κB) and induction of NO synthase 2 by proinflammatory cytokines, including TNF-α, were also modulated in vivo and in vitro by AdoMet treatment (Majano et al., 2001).

All evidence summarized so far supports the notion of the likely involvement of impaired AdoMet synthesis in the development of liver injury. However, definitive proof was lacking until a *MAT1A* knockout mouse deficient in hepatic MAT (MATO mouse) became available (Lu et al., 2001). In this model of chronic hepatic AdoMet reduction (70% less hepatic AdoMet levels than in wild-type mice), marked hepatic alterations were evident by 3 months of age, including hepatic hyperplasia and a pronounced tendency for steatosis to develop after feeding of a choline-deficient diet (Lu et al., 2001). Genomic profiling of these animals versus wild-type mice by microarray analysis showed changes in a substantial number of genes involved in cell proliferation, differentiation, and acute phase response (Lu et al., 2001). In addition, 3-month-old MATO mice are much more sensitive to

CCl_4-induced liver damage, as determined histologically and by serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (unpublished observations, J. M. Mato, 2001). At 8 months of age, steatohepatitis developed in MATO mice in the absence of any liver-damaging treatment (Lu et al., 2001). These results clearly attest to the contribution of chronic deficiency in hepatic AdoMet synthesis, as evidenced in patients with liver cirrhosis, in the pathogenesis of liver injury and also confirm a role for AdoMet in the preservation of the quiescent and differentiated status of the hepatocyte.

2. Future directions

Future challenges in the understanding of AdoMet functions in liver biology and pathophysiology will include issues such as the delineation of the precise molecular events through which this compound regulates gene expression. Experiments carried out in cultured hepatic cell models will help to identify the subcellular location and targets of AdoMet interactions. It is also of interest to know whether such effects would be mediated through a methylation reaction or through binding of AdoMet to proteins in the absence of methyl-group transfer. Additionally, use of MATO mice will be instrumental in furthering our knowledge of the consequences of chronic hepatic AdoMet deficiency. Extensive genomic profiling and proteomic analysis will help to identify novel targets for AdoMet, illustrating at the same time the early molecular changes that precede and accompany the development of steatohepatitis. As suggested by the proliferative disorders observed in MATO mice, it will be important to know whether these animals display enhanced risk for the development of hepatocarcinoma.

Acknowledgments

This work was supported by grants 99/0038 from Plan Nacional de I+D, Ministerio de Educación y Ciencia and Knoll to JMM; grant ROI AA-12677 from the National Institute on Alcohol Abuse and Alcoholism to JMM and MAA; grant FIS 01/0712 from Ministerio de Sanidad y Consumo to MAA and FJC; grants 5697/1999, 681/2000, and 349/2001 from Gobierno de Navarra to FJC, JMM, and MAA, respectively; and a grant from Fundación Renal Iñigo Alvarez de Toledo, Spain, to JMM.

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