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Molecules in focus

Methylthioadenosine

Matías A. Avila [a], Elena R. García-Trevijano [a], Shelly C. Lu [b], Fernando J. Corrales [a], José M. Mato [c,*]

[a] *Division of Hepatology and Gene Therapy, F.I.M.A., University of Navarra, Pamplona, Spain*
[b] *Division of Gastroenterology and Liver Diseases, USC Liver Disease Research Center, USC School of Medicine, Los Angeles, CA, USA*
[c] *CIC-Biogune, Metabolomics Unit, Building 801, Technological Park of Bizkaia, Zamudio, 48710 Bizkaia, Spain*

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Abstract

5′-Methylthioadenosine (MTA) is a naturally occurring sulfur-containing nucleoside present in all mammalian tissues. MTA is produced from *S*-adenosylmethionine mainly through the polyamine biosynthetic pathway, where it behaves as a powerful inhibitory product. This compound is metabolized solely by MTA-phosphorylase, to yield 5-methylthioribose-1-phosphate and adenine, a crucial step in the methionine and purine salvage pathways, respectively. Abundant evidence has accumulated over time suggesting that MTA can affect cellular processes in many ways. MTA has been shown to influence numerous critical responses of the cell including regulation of gene expression, proliferation, differentiation and apoptosis. Although most of these responses have been observed at the pharmacological level, their specificity makes it tempting to speculate that endogenous MTA could play a regulatory role in the cell. Finally, observations carried out in models of liver damage and cancer demonstrate a therapeutic potential for MTA that deserves further consideration.

Keywords: Methylthioadenosine; *S*-adenosylmethionine; MTAP

1. Introduction

The natural existence of the sulfur-containing nucleoside methylthioadenosine [5′-deoxy-5′-methylthioadenosine; adenine-9-β-D (5′-deoxy-5′-methylthio) ribofuranoside], commonly abbreviated as MTA and occasionally as MeSAdo, was realized almost a century ago (Williams-Ashman, Seidenfeld, & Galletti, 1982). Its molecular structure was reported in 1924, and the biological importance of MTA became apparent in 1952, 1 year before the discovery of its metabolic precursor *S*-adenosylmethionine (AdoMet, also abbreviated as SAM and SAMe), in studies on the metabolic interrelationship of methionine and 5-thiomethylribose (Williams-Ashman et al., 1982). MTA is present in small amounts in all cell types, including prokaryotes, yeast, plants and higher eukaryotes. In mammalian tissues, MTA is mainly produced during the biosynthesis of polyamines (Williams-Ashman et al., 1982; Pegg, 1988). For many years, this nucleoside has received by far much less attention than its precursor AdoMet. However, information accumulated over the past two decades evidences a wide variety of potent and specific effects of MTA upon its interaction with mammalian cells and tissues. The present review summarizes what is known about MTA synthesis and degradation, its biological functions and its potential therapeutic applications.

* Corresponding author. Tel.: +34-94-4061300; fax: +34-94-4061300.
E-mail address: director@cicbiogune.com (J.M. Mato).

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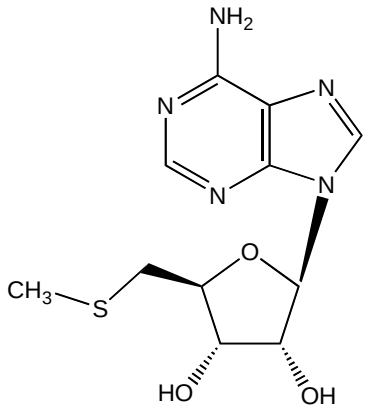

Fig. 1. Structure of 5′-methylthioadenosine (MTA).

2. Structure

MTA is a hydrophobic sulfur-containing adenine nucleoside in which the hydroxyl group in the 5′ position of the ribose is substituted by a methylthio moiety (Fig. 1). This methylthio moiety is derived from the amino acid methionine, while the rest of the molecule comes from ATP.

3. Synthesis and degradation

MTA is produced from AdoMet by different processes in a variety of organisms (Williams-Ashman et al., 1982). MTA can be produced by the cleavage of one of the AdoMet S–C bonds with the concomitant production of homoserine lactone. This process is catalyzed by AdoMet cyclotransferase, an enzyme present in yeast, certain bacteria and mammalian liver (Williams-Ashman et al., 1982). However, little is known about the quantitative contribution of this pathway to MTA synthesis. The major source of MTA in cells is formed from AdoMet during the synthesis of the polyamines spermine and spermidine (Pegg, 1988; Williams-Ashman et al., 1982). Polyamine biosynthesis occurs in all mammalian cells, and starts with the decarboxylation of AdoMet by the enzyme AdoMet decarboxylase (AdoMetDC). Decarboxylated AdoMet is a substrate for the aminopropyltransferases. These enzymes transfer the aminopropyl group of decarboxylated AdoMet to putrescine forming spermidine (spermidine synthase), and subsequently to spermidine forming spermine (spermine synthase) (Fig. 2). Therefore, MTA is produced in stoichiometric amounts to the aminopropyl groups in spermidine and spermine. AdoMetDC together with ornithine decarboxylase (ODC) conform the rate-limiting steps in polyamine biosynthesis, and thus in MTA production (Pegg, 1988). In most cells, MTA does not accumulate in significant amounts. Its concentrations are around 3 nmol/g of tissue in rat liver, heart, lung and kidney, being in the same order of magnitude as those reported for decarboxylated AdoMet and adenosine (Williams-Ashman et al., 1982). MTA is rapidly metabolized by 5′-methythioadenosine phosphorylase (MTAP) to yield adenine (Ade) and 5-methylthioribose-1-phosphate (MTR1P) (Fig. 2). MTAP is ubiquitously expressed in mammalian tissues, and the reaction it catalyses represents the first and rate-limiting step in the salvage pathways in which MTR1P is finally converted into methionine, and Ade is salvaged to ultimately replenish the AMP and ATP pools. Consequently, the two metabolites from which AdoMet and MTA are formed, namely methionine and ATP, are thus recovered (Williams-Ashman et al., 1982). Interestingly, MTAP activity has been found in human and fetal calf serum, but not in sera from other species such as horse serum (Riscoe & Ferro, 1984). The conversion of MTR1P into methionine involves a complex set of oxidations via the intermediate 4-methylthio-2-oxobutanoic acid (MTOB) (Fig. 2). This efficient cycle sustains the high rate of polyamine synthesis that occurs during cellular proliferation and provides methionine for AdoMet and protein synthesis. In addition, the removal of accumulating MTA by MTAP is necessary for the cell to carry out polyamine metabolism, since MTA is a strong inhibitor of spermine synthase, and to a lesser extent of spermidine synthase and of ODC (Pegg, 1988; Pascale, Simile, De Miglio, & Feo, 2002). The inhibition of ODC by MTA can be mediated in part by its metabolite MTOB, as has been recently demonstrated in yeast and human tumor cells (Suhbhi et al., 2003).

Metabolism of MTA in tumor cells deserves further consideration. It has been extensively reported that many malignant cells lack MTAP activity, and that cultured MTAP-deficient cells secrete MTA instead of metabolizing it (Williams-Ashman et al., 1982; Nobori et al., 1996). Loss of MTAP activity in human tumors

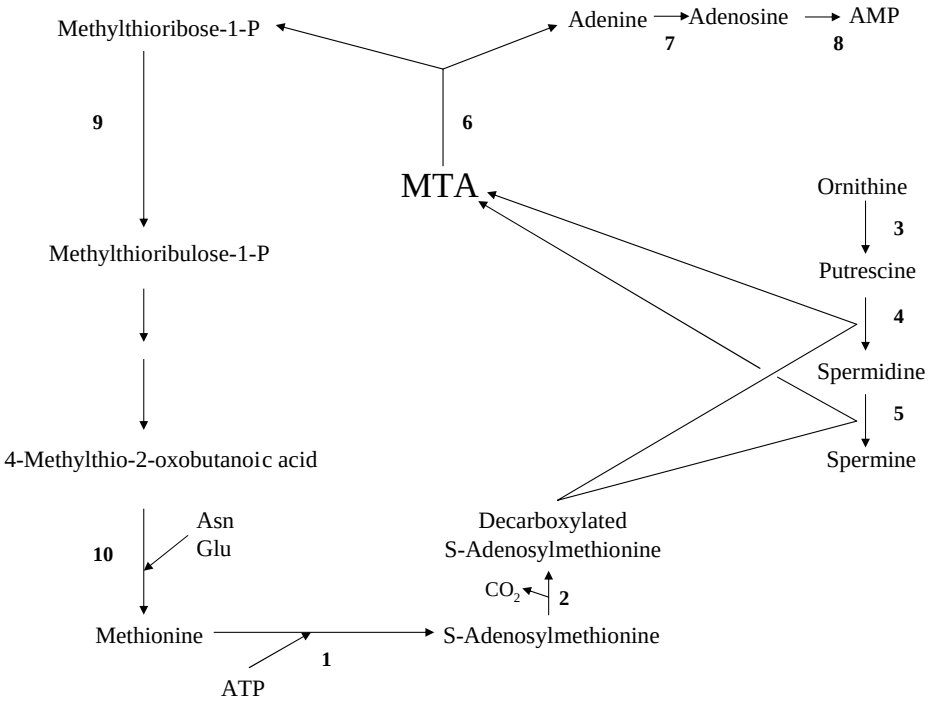

Fig. 2. Synthesis and metabolism of 5′-methylthioadenosine (MTA) in mammalian cells. The precursor of MTA, AdoMet, is synthesized from methionine and ATP in a reaction catalyzed by methionine adenosyltransferase (1). Subsequently, AdoMet is decarboxylated by AdoMet decarboxylase (2). Decarboxylated AdoMet participates in the synthesis of polyamines, which starts with the conversion of ornithine to putrescine, carried out by ornithine decarboxylase (3). Then, spermidine synthase (4) and spermine synthase (5) transfer the propylamine group from decarboxylated AdoMet to putrescine and spermidine, respectively, in two sequential reactions. In these two reactions MTA is formed. MTA is the substrate of 5′-methylthioadenosine phosphorylase (6), which catalyses the first step in methionine and Ade salvage pathways. Ade is converted into adenosine and then into AMP by the action of Ade-phosphoribosyltransferase (7) and adenosine kinase (8). The other reaction product of methylthioadenosine phosphorylase, methylthioribose-1-P, is isomerized by aldose-ketose isomerase (9) to methylthioribulose-1-P. This compound undergoes a complex set of oxidations to give 4-methylthio-2-oxobutanoic acid, which is finally transaminated to methionine (10).

has been mainly attributed to homozygous deletions and translocations at the chromosome 9p21 region (Nobori et al., 1996). As mentioned above, a primary consequence of the loss of MTAP activity in cellular metabolism is the impairment in Ade salvage to form AMP. Hitherto, the transformed cell relies only on the de novo purine biosynthesis pathway as a source of adenine nucleotides for DNA synthesis. This metabolic trait of tumoral cells has attracted much attention since it may be exploited chemotherapeutically. Blocking of the de novo purine biosynthesis pathway by antimetabolites such as methotrexate or L-alanosine would efficiently kill MTAP-deficient tumor cells, while normal cells are rescued due to their ability to produce Ade through the salvage pathway, and thus could be protected by MTA treatment. However, the in vivo efficacy of this strategy may be hampered due to the presence of MTAP in human serum as we previously mentioned. Another direct consequence of impaired MTAP activity is the intracellular accumulation of MTA. In spite of the ability of MTA to permeate the cell membrane, it has been observed that MTAP deficient cells accumulate sufficient amounts of endogenous MTA to modulate important cellular responses (see below). Whether the loss of MTAP plays a role in the tumorigenic process is not known at present. However, it has been recently reported that reintroduction of this gene in a human

mammary tumor cell line reversed in part its transformed phenotype (Suhbhi et al., 2003).

4. Biological functions

As can be inferred from above, MTA is located at a crossroad in cellular metabolism. MTA is a metabolic product of AdoMet in polyamine biosynthesis, and at the same time the starting point of two major salvage pathways. Besides its role as a metabolic intermediate, MTA may play relevant regulatory functions in the cell. Some of these functions, such as its inhibitory effects on spermidine and spermine synthase and on ODC, have been already outlined. In addition, a wide variety of biological responses to MTA administration have been reported both in vivo and in cultured cells. These effects range from the control of the expression of specific genes, the inhibition of cell proliferation, lymphocyte activation, tumor development and invasiveness, and the regulation of apoptosis (Ansorena et al., 2002; Law et al., 1992; Lee & Cho, 1998; Martínez-Chantar et al., 2003; Mato, Corrales, Lu, & Avil, 2002; Pascale et al., 2002; Williams-Ashman et al., 1982). Of special interest are the observations carried out in the liver as well as in normal and transformed cultured hepatic cells. This organ has a remarkable proliferative potential, and is able to quickly restore its mass after partial hepatectomy. This process, which is triggered by cytokines such as tumor necrosis factor-α (TNF-α) that make the hepatocyte responsive to growth factors like hepatocyte growth factor (HGF), needs to be tightly controlled and its fundamental mechanisms are not fully understood (Mato et al., 2002). Shortly after, partial hepatectomy MTA levels in the remaining liver parenchyma are significantly reduced, coinciding with the replicative response of the hepatocytes (Ferioli & Scalabrino, 1986). Interestingly, exogenous MTA administration to rats after partial hepatectomy inhibits hepatocyte DNA synthesis (Pascale et al., 2002), and a similar response has been observed in cultured rat hepatocytes treated with HGF in the presence of MTA (Mato et al., 2002). These observations suggest that intracellular fluctuations in MTA levels could participate in the regulation of the liver proliferative response. Furthermore, in experimental models of chemically induced hepatocarcinogenesis, where it has been observed that MTA levels are reduced (Ferioli & Scalabrino, 1986; Pascale et al., 2002), administration of MTA induces a dose-dependent inhibition of preneoplastic liver lesions and DNA synthesis (Pascale et al., 2002). The hepatoprotective effects of MTA have been extended to a model of CCl_4-induced chronic liver damage in rats. In this experimental setting, that reproduces the human lesions induced by alcohol and viral infections of the liver, replenishment of the hepatic pool of MTA showed strong anti-oxidant effects and reduced liver cell damage and fibrosis (Pascale et al., 2002). It is worth mentioning that normal and transformed liver cells display a differential response to MTA. While hepatocarcinoma cells undergo apoptosis when treated with MTA, normal hepatocytes remain viable and, furthermore, are protected from okadaic acid-induced programmed cell death (Ansorena et al., 2002). The mechanisms of this distinct response are not known at present. One could speculate that the different MTAP status, and thus the differential ability to metabolize MTA of normal and transformed hepatocytes, might have some significance. However, this different behavior of normal and transformed hepatocytes may be behind the chemopreventive and hepatoprotective effects of MTA.

A common denominator to the development of liver damage and fibrosis, and the ultimate neoplastic transformation, is the presence of a chronic inflammatory response. Recent observations from our laboratory demonstrate that MTA is a powerful modulator of inflammation in vivo. The administration of MTA to mice challenged with lethal doses of bacterial lipopolysaccharide (LPS) completely prevented death induced by this toxin (Hevia et al., unpublished observations). This effect was associated with a substantial inhibition in the production of the pro-inflammatory cytokine TNF-α and with enhanced production of the anti-inflammatory cytokine interleukin-10.

Several molecular mechanisms have been proposed to explain the remarkable effects of MTA on cellular functions. The inhibition of polyamine synthesis could be responsible for the cytostatic effects of MTA or its downstream metabolite MTOB (Suhbhi et al., 2003). However, additional mechanisms must exist since the exogenous provision of spermidine does not always reverse the growth inhibitory effects of MTA (Williams-Ashman et al., 1982). MTA has been shown to interfere with key cell signaling pathways.

It is able to inhibit growth-factor induced protein tyrosine phosphorylation and to increase intracellular cAMP levels through the inhibition of cAMP phosphodiesterase (Maher, 1993; Riscoe et al., 1984). One of the best-characterized actions of MTA is the inhibition of protein methylation (Williams-Ashman et al., 1982). This post-translational modification may occur at arginine residues or at the carboxyl terminus of proteins, and is thought to modulate cellular signaling and gene expression (Law et al., 1992; Lee & Cho, 1998; Mowen et al., 2001). MTA may exert this effect either through the direct interaction with protein methyltransferases or indirectly through the inactivation of *S*-adenosylhomocysteine (AdoHcy) hydrolase (Williams-Ashman et al., 1982). Inactivation of AdoHcy hydrolase results in the intracellular accumulation of AdoHcy, which is also a potent inhibitor of many methyltransferases (Mato et al., 2002). To further complicate the understanding of the cellular responses to MTA, some of the effects of this molecule on the regulation of gene expression have been attributed to its ability to replenish the cellular AdoMet pool through the methionine salvage pathway (Martínez-Chantar et al., 2003).

The fact that most of the above-mentioned observations have been performed using high concentrations of MTA may question their physiological significance. Nevertheless, a recent report showed that the presence of excess MTA found in certain cancer cells is sufficient to inhibit arginine methylation of the STAT1 transcription factor and consequently to impair interferon-induced gene transcription (Mowen et al., 2001).

5. Possible medical applications

MTA is a natural compound with potent pharmacological effects. The administration in vivo of high doses of MTA in experimental models of acute and chronic liver damage and liver carcinogenesis has proved to be beneficial, and without major toxicological manifestations. Besides, our unpublished observations demonstrate a powerful anti-inflammatory profile for this molecule. Although systematic studies on the pharmacokinetics and toxicity of MTA are lacking, and its mechanism or mechanisms of action are not completely known (as occurs with many registered drugs), we believe that the potential therapeutic use of MTA in liver and inflammatory diseases deserves to be explored.

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