S-Adenosyl-L-methionine (SAMe): from the bench to the bedside—molecular basis of a pleiotrophic molecule

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ABSTRACT
S-Adenosyl-L-methionine (SAMe), a metabolite present in all living cells, plays a central role in cellular biochemistry as a precursor to methylation, aminopropylation, and transsulfuration pathways. As such, SAMe has been studied extensively since its chemical structure was first described in 1952. Decades of research on the biochemical and molecular roles of SAMe in cellular metabolism have provided an extensive foundation for its use in clinical studies, including those on depression, dementia, vacuolar myelopathy, liver disease, and osteoarthritis. This article provides an overview of the biochemical, molecular, and therapeutic effects of this pleiotrophic molecule.

INTRODUCTION
S-Adenosyl-L-methionine (SAMe), first discovered in 1952 (1), is formed from the essential amino acid methionine and adenosine triphosphate. SAMe is found in every living cell, where it functions as a donor of methyl groups in more than 100 different reactions catalyzed by methyltransferase enzymes (2, 3). SAMe also serves as a precursor molecule to the aminopropylation pathway, which leads to the synthesis of polyamines, and the transulfuration pathway, which leads to the synthesis of glutathione.

In its native form, SAMe is labile and degrades rapidly. However, several patents for stable salts of SAMe have been granted. Among them, toluenedisulfonate and 1,4-butanedisulfonate forms have been chosen for pharmaceutical development, and as a result, preclinical and clinical studies have been performed. Numerous studies over the past 2 decades have shown that SAMe is effective in the treatment of depression (4–6), osteoarthritis (7–8), and liver disease (9–11). Moreover, SAMe has a very favorable side-effect profile, comparable with that of placebos. Thus, SAMe offers considerable advantages as an alternative to standard medications.

Since March 1999, SAMe has been available in the United States under the Dietary Supplement and Health Education Act as an over-the-counter supplement. Although SAMe is a relatively new product in the United States, it has been available in Italy since 1979, in Spain since 1985, and in Germany since 1989 as a prescription medication. SAMe was developed as a pharmaceutical or nutraceutical on the basis of an extensive foundation of basic research focused primarily on furthering our understanding of its biological importance and its effects on cellular metabolism and physiology.

BIOLOGICAL IMPORTANCE OF SAMe
SAMe occupies a central position in the metabolism of all cells as a precursor molecule to three main pathways: methylation, transsulfuration, and aminopropylation (Figure 1). To sustain the normal function of these pathways, a sufficient source of SAMe is essential. The amount of SAMe that is required by the body daily depends on the availability of methionine produced by de novo synthesis (involving methyltetrahydrofolate and vitamin B-12) and also methionine obtained from the diet, mainly from the breakdown of proteins.

Methylation
SAMe serves an important biological function as the sole methyl donor in a multitude of cellular methylation reactions (2, 3). Most cells contain numerous SAMe-dependent methyltransferases that can transfer the methyl group (CH₃) to the oxygen, nitrogen, or sulfur atoms of both small and large molecules. For example, the synthesis of creatine from guanidinoacetate, sarcosine from glycine, phosphatidylcholine from phosphatidylethanolamine, and epinephrine from norepinephrine, and the methylation of carboxyl residues of various proteins and cytosine residues of DNA all use SAMe as the methyl donor.

In prokaryotes and eukaryotes, enzymatic methylation of DNA cytosine residues at their C-5 position strongly affects the interaction with proteins, inhibits transcription, and generally leads to gene inactivation (13). DNA hypomethylation can lead to chromosomal instability and can cause mutations. The best-known relations between changes in DNA methylation and disease have been described for cancer. Abnormal methylation at the transcription site can affect expression of tumor suppressor genes such as the vitamin D receptor and the retinoic acid receptor β₂ (14).

1 From the Baylor University Medical Center, Institute of Metabolic Disease, Dallas.
2 Presented at the symposium S-Adenosylmethionine (SAMe): from Molecular Mechanism to Clinical Implications, held in Santa Barbara, CA, March 7–10, 2001.
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SAMe-dependent carboxyl methylation of proteins is an important element in the modification of protein function, particularly in the central nervous system (CNS). In mammals, concentrations of protein carboxymethyltransferase (EC 2.1.1.24) are highest in the brain (compared with other organs) and are specifically localized in the axon terminals, with a regional distribution of higher concentrations occurring in the substantia nigra, locus coeruleus, and paraventricular nucleus (15). Clarke (16) studied human erythrocyte protein carboxymethyltransferase, which is similar in its kinetic properties to protein carboxymethyltransferase from bovine brain, and they showed that carboxyl methylation is involved in the repair and degradation of proteins containing abnormal D-aspartyl residues that arise via spontaneous racemization of L-aspartyl residues.

Methylation also plays an important role in maintaining the integrity of cellular membranes. Phospholipid methylation is a reaction in which phosphatidylethanolamine is converted to phosphatidylcholine; this reaction involves the addition of 3 methyl groups to the amino moiety of a phosphatidylethanolamine molecule via SAMe, the methyl donor (17). During this conversion, phosphatidylethanolamine is translocated from the cytoplasmic side to the outside of the cell membrane. Furthermore, the ratio of phosphatidylethanolamine to phosphatidylcholine determines the fluidity or viscosity of the membrane (ie, the greater the amount of phosphatidylethanolamine, the more fluid or less viscous the cell membrane). In vitro studies showed that an increase in phospholipid methylation and the ensuing decrease in membrane viscosity create a more fluid environment, which may facilitate the lateral movement of proteins, such as receptors, within the lipid bilayer (18).

SAMe-dependent methylation reactions are also required in the synthesis and inactivation of neurotransmitter monoamines such as noradrenaline, adrenaline, dopamine, serotonin, and histamine. The methyltransferases involved have a selective substrate specificity and distribution in tissues, and their activity is finely regulated by a variety of cofactors. The administration of substrates such as L-dihydroyxphenylalanine, which stimulates dopamine synthesis, causes a marked decrease in SAMe concentrations in the brains of rats (19) and a decrease in whole blood (20) and cerebrospinal fluid (CSF) (21) SAMe concentrations in humans. Furthermore, various drugs that interfere with monoaminergic neurotransmission, such as the monoamine oxidase inhibitor paragmine and inhibitors of monoamine uptake (imipramine and desipramine), considerably reduce SAMe concentrations in the whole brain of mice (22).

Transsulfuration

The product of all SAMe-dependent methylation reactions is S-adenosylhomocysteine (Figure 1). S-Adenosylhomocysteine is metabolized rapidly to homocysteine, which may be converted to cystathionine in a reaction that requires pyridoxal phosphate (vitamin B-6) as a cofactor. This is the first step of the transsulfuration pathway that ultimately leads to the synthesis of glutathione, a major cellular antioxidant. Alternatively, homocysteine may function as the methyl acceptor for the betaine–homocysteine methyltransferase (EC 2.1.1.15) and methionine synthetase (EC 2.1.1.13) reactions. Much of the homocysteine skeleton is efficiently reutilized; it was calculated that the methyl group is conserved for an average of 1.9 turnovers in men and 1.5 turnovers in women (23).

Homocysteine concentrations are closely related to homocysteine metabolism. When methionine is needed, homocysteine is remethylated by methylenetetrahydrofolate, but when methionine is in excess, catabolism of homocysteine via cystathionine β-synthetase (EC 4.2.1.22) is accelerated (24). This regulatory mechanism has been studied in some detail. Womack and Rose (25) first showed that cysteine could replace ∼70% of the dietary requirement for methionine. Later, Finkelstein and Martin (26) studied the enzymatic basis for this methionine-sparing effect of cysteine. Replacement of methionine with cysteine resulted in a marked decrease in hepatic concentrations of cystathionine β-synthetase. In other studies, the same authors showed that feeding excess methionine to rats resulted in decreased hepatic concentrations of methionine synthetase and increased concentrations of betaine–homocysteine methyltransferase, methionine adenosyltransferase (MAT; EC 2.5.1.6), and cystathionine β-synthetase (26). These studies showed that the partitioning of homocysteine between the transsulfuration and methylation pathways is finely regulated by the tissue concentrations of substrates and effector metabolites.

Aminopropylation

SAMe plays another important metabolic role in the synthesis of polyamines, by way of a pathway known as aminopropylation. In this pathway, SAMe is metabolized to decarboxylated SAMe and the aminopropyl group is transferred to putrescine. Next, the polyamines spermidine and spermine are formed (Figure 1). In this process, methylthioadenosine is produced; it is then converted back to methionine. This pathway constitutes a salvage route for the conservation of methionine and also ensures the rapid removal of methylthioadenosine, which was shown to be a potent inhibitor of S-adenosylhomocysteine hydrolase (EC 3.3.1.1) (27). The polyamines spermidine and spermine are involved in the control of cell growth (28) and were also shown to have analgesic and anti-inflammatory properties (29).
Early studies in rats showed that the absorption of SAMe is much better after intraduodenal administration than after oral administration (30). In a phase I study, administration of enteric-coated capsules of SAMe in doses of 400, 600, and 1000 mg to 4 male subjects increased plasma concentrations in a dose-dependent manner to 30–50 times basal values (31). Despite substantial increases above physiologic concentrations, the systemic bioavailability of SAMe after oral administration appears to be low. However, oral administration of [methly-\(^{14}\)C]SAMe (200 mg; 0.02 μCi/μmol) to 3 adult volunteers showed that urinary excretion of radioactivity during the first 48 h was 15.5 ± 1.5% and feces contained 23.5 ± 3.5% of the radioactivity up to 72 h (32). These findings indicate that ~60% of the radioactivity is incorporated into stable pools. A comparison of SAMe metabolism after an intravenous dose and an oral dose of [methly-\(^{14}\)C]SAMe (50 mg; 0.4 mCi/mmol) was performed in 6 male volunteers (33). After intravenous administration, plasma radioactivity decreased rapidly, corresponding to the decay of the unmodified product. In contrast, after oral administration, plasma radioactivity increased over time, reaching a peak between 8 and 24 h after treatment. The higher radioactivity found at the later time points after oral administration compared with intravenous administration suggests that SAMe given orally is actively metabolized, with the methyl group being incorporated into stable pools such as proteins and phospholipids. These observations are supported by data that show that after oral administration of a single 100-mg dose of doubly labeled [methyl-\(^{3}\)H:35S]SAMe, 62% of \(^{3}\)H and 43.7% of \(^{35}\)S radioactivity still remained in the body after 5 d (33).

Several studies indicate that parenteral and oral administration of SAMe can increase CSF concentrations. In dogs, an intravenous injection of 8 mg SAMe/kg followed by an intravenous infusion of 12 mg SAMe/kg for 6 h was associated with a steady hourly increase in CSF SAMe concentrations; a 20–40-fold increase over basal values was measured after 6 h (32). CSF SAMe concentrations were also studied in a placebo-controlled trial after the parenteral administration of 200 mg SAMe/d for 14 d to depressed patients (34). At 2 and 24 h after the last injection, CSF SAMe concentrations were significantly increased, by 65% and 12%, respectively, compared with baseline values; this indicated that SAMe can cross the blood-brain barrier. Similarly, oral administration of SAMe (400 mg, 3 times/d for 4–8 mo) to 4 patients with Alzheimer’s dementia increased both plasma and CSF SAMe concentrations significantly (34). There is also additional evidence that SAMe crosses the blood-brain barrier intact. In one study, the parenteral administration of 800 mg SAMe-1,4-butanedisulphonate daily for 14 d to 7 HIV-infected patients was associated with marked increases in CSF SAMe (35).

In vitro studies show that the uptake of labeled SAMe into hepatocytes is about one-third that of labeled methionine (36). The positively charged sulfonium ion limits passage of the SAMe molecule into cells. This accounts for the relatively high doses of SAMe required to increase blood and tissue concentrations. Evidence of a carrier-mediated transport system has been found in isolated rat liver mitochondria, where approximately one-third of total hepatic SAMe resides in the absence of MAT in this organelle (37). The uptake of SAMe was shown to be saturable and not inhibited by methionine, adenosine, 5'-methylthioadenosine, carnitine, choline, betaine, quinine, or hemicholinium-3. SAMe uptake was inhibited by sinemetin and S-adenosylhomocysteine.

### BIOCHEMICAL BASIS FOR THE CLINICAL USE OF SAMe

Although Cantoni (1) first reported the chemical structure of SAMe in 1952, it was not until 1973 that a commercially available, stabilized, p-toluene sulfate form was tested in clinical trials. Intravenous preparations were used first, but this generally limited studies to a short duration of several weeks. However, in the past decade, the availability of stable enteric-coated forms, in particular SAMe-1,4-butanedisulphonate, has made it possible for clinical trials to be conducted over much longer periods of many months or years.

#### Depression

Clinical studies performed as early as 1973 indicated that SAMe had antidepressant effects (38). Over the next 2 decades, the efficacy of SAMe in treating depressive disorders was confirmed in ∼40 clinical trials. Several review articles that summarize these studies were published in 1988 (4), 1989 (5), 1994 (6), and 2000 (12). In a meta-analysis, Bressa (6) reviewed 25 controlled trials including a total of 791 patients. The outcome of this analysis showed that SAMe had a significantly greater response rate than did placebo and was comparable to tricyclic antidepressants. Brown et al (12) summarized the literature on the use of SAMe in depressive disorders up to the time of publication in 2000; they reported that SAMe had been studied in 16 open, uncontrolled trials (660 patients); 13 randomized, double-blind, placebo-controlled trials (537 patients); and 19 controlled trials comparing SAMe with other antidepressants (1134 patients). Significant antidepressant effects were observed in all 16 open trials. In 18 controlled trials, SAMe was as effective as was imipramine, chlorimipramine, nomifensine, and minaprine. An important observation from these studies is that SAMe had far fewer side effects than did standard medications.

The antidepressant effect of SAMe is of particular interest because of the close metabolic relation between folate and SAMe (Figure 1) and the association of a high incidence of folate deficiency with depression (39, 40). Experimental animal studies showed that folate deficiency can reduce brain SAMe concentrations (41), and folate-deficient patients were shown to have significantly lower concentrations of SAMe and metabolites of dopamine and serotonin in CSF (42). The restoration of CSF SAMe concentrations after parenteral or oral SAMe treatment, and by inference the replenishment of CNS tissue methyl groups, may in some way be related to the therapeutic effect.

The exact mechanism of the antidepressant effect of SAMe is not clear, although preclinical studies indicated that SAMe has stimulatory effects on monoamine metabolism, monoamine turnover, or both. In agreement with this, SAMe treatment reportedly increased rat brain concentrations of norepinephrine (43, 44) and serotonin (45, 46). In humans, SAMe treatment increased the concentrations of 5-hydroxyindole acetic acid (47), a marker for serotonergic activity. It has been theorized that the stimulatory effect of SAMe on central monoaminergic neurotransmitters is the mechanism underlying its antidepressant effect. Alternative mechanisms may exist in which increased or restored membrane phospholipid methylation plays a role in the antidepressant effect. SAMe, by virtue of its ability to act as a methyl donor, may increase the fluidity of cell membranes by stimulating phospholipid methylation. An increase in cell membrane fluidity was linked to an increase in β receptor density (48) and muscarinic (M1) receptor density (49). The effect of SAMe on receptor systems is of particular interest because evidence suggests that age
changes in the membrane environment, especially those resulting in increased viscosity, may be responsible for G protein–receptor coupling-uncoupling dysfunction (50). The beneficial effects of SAMe treatment suggest a possible strategy for various systems that exhibit G protein–receptor coupling-uncoupling dysfunction.

Neurologic disorders

Several studies indicate that a CNS methyl group deficiency may play a role in the etiology of Alzheimer disease (AD). Reduced SAMe concentrations were found in CSF (34) and in several different brain regions (51) of patients with AD. In addition, reduced phosphatidylcholine concentrations were found in postmortem brain tissue from AD patients (52), and significant changes in brain phospholipids that are dependent on SAMe metabolism were detected in vivo with 31P magnetic resonance spectroscopy in the early stages of AD (53). Deficiencies of folate and vitamin B-12 are common in the elderly (39, 40) and can lead to decreased CNS SAMe concentrations. Several studies indicate that elevated blood homocysteine concentrations, considered to be a marker for folate deficiency, vitamin B-12 deficiency, and impaired methylation, may be a risk factor for AD (54–56). It is therefore important to note that preliminary studies using either SAMe (57) or alternative methyl group donors [such as betaine (58) or folate and vitamin B-12 (59, 60)] can improve measures of cognitive function. These treatments may be able to restore methyl group metabolism and normalize blood homocysteine concentrations. Reduced SAMe concentrations in CSF were also reported in patients with subacute combined degeneration of the spinal cord resulting from folate or vitamin B-12 deficiency (39) and in children with inborn errors of the methyl-transfer pathway who had demyelination (61). In these cases, treatment with methyl-group donors such as SAMe, methyltetrahydrofolate, betaine, and methionine was associated with remyelination and a clinical response (61).

In 3 independent studies, reduced SAMe concentrations in CSF were found in HIV-infected patients; one study was conducted in children (62) and the other 2 studies were conducted in adults (35, 63). Although the cause of the decreased CSF SAMe concentrations in HIV infection is not known, it was proposed that the resulting methyl-group deficiency may be a pathogenic mechanism involved in the etiology of the vacuolar myelopathy that is often part of the AIDS dementia complex (64). Furthermore, HIV-related vacuolar myelopathy bears a striking histologic resemblance to subacute combined degeneration of the spinal cord, which can accompany folate and vitamin B-12 deficiencies (65). Contrary to the studies discussed above, Goggin et al (66) reported that the amount of radiolabelled SAM incorporated into carboxymethyl and N-methylation sites within brain proteins from cortical white matter in vitro was significantly lower in samples from 9 HIV-positive patients than in samples from 16 control subjects; only 4 of the 9 patients had HIV encephalitis. These data suggest that cortical brain proteins are hypermethylated in HIV-positive patients.

Liver disease

The potential benefit of SAMe in treating liver disease stems from several important aspects of SAMe metabolism. In mammals, as much as 80% of the methionine in the liver is converted into SAMe (23). Hepatic glutathione, which is dependent on methionine and SAMe metabolism, is one of the principal antioxidants involved in hepatic detoxification. Studies have shown that abnormal SAMe synthesis is associated with chronic liver disease, regardless of its etiology. Early studies indicated that patients with liver disease are unable to metabolize methionine, resulting in elevated blood concentrations (67). Subsequent studies in patients with liver disease showed that the defect resulted from decreased activity of a liver-specific isoenzyme, MAT I/III; this defect effectively blocks the conversion of methionine to SAMe (68). Several well-designed experimental studies indicated that MAT I/III is regulated by cellular concentrations of both nitric oxide and glutathione. Thus, increased nitric oxide concentrations and decreased glutathione concentrations were shown to inhibit MAT I/III via mechanisms involving S-nitrosylation and free radical damage to the enzyme protein (69, 70). Experimental studies and clinical trials showed that parenteral and oral SAMe administration can increase glutathione concentrations in red blood cells (71) and in hepatic tissue (72, 73) and can effectively replenish depleted glutathione pools in patients with liver disease. The literature on the clinical potential of SAMe in the treatment of liver disease (including cholestasis, hepatitis, and cirrhosis) has been the subject of several review articles (9–11, 74, 75).

Osteoarthritis

The potential benefit of SAMe in treating osteoarthritis was discovered when patients enrolled in clinical trials of SAMe for depression reported marked improvement in their osteoarthritis symptoms (76). Nine clinical trials in Europe (77) and 1 in the United States (7) with a total of >22 000 participants have confirmed the therapeutic activity of SAMe against osteoarthritis. SAMe has effects similar to those of the nonsteroidal antiinflammatory drugs, but its tolerability is higher.

Experimental studies indicate that SAMe increases chondrocyte proteoglycan synthesis (78) and proliferation rate (79). SAMe induces the synthesis of polyamines which might stabilize the polyamionic macromolecules of proteoglycans and protect them from attack by proteolytic and glycolytic enzymes (80). Furthermore, in vitro studies show that SAMe can antagonize the tumor necrosis factor-α–induced decreases in synovial cell proliferation and fibronectin mRNA expression (81). These findings indicate that in cultured synovial cells, SAMe restores basal conditions after cytokine-induced cell damage. In addition, oral administration of SAMe (400 mg for 7 d) to 4 subjects significantly increased SAMe concentrations in synovial fluid by 3–4-fold compared with pretreatment values (32).

CONCLUSIONS

Research studies over the past 5 decades have unequivocally established the importance of SAMe in the cellular metabolism and function of all living organisms. The diet cannot provide adequate quantities of SAMe; therefore, the body relies on de novo synthesis to sustain the required concentrations of this essential metabolite. Pharmaceutical preparations of SAMe are available mainly in Europe as intravenous, intramuscular, and oral forms. An over-the-counter nutraceutical oral form is available in the United States.

Studies have shown that SAMe, when given in high pharmacologic doses, is absorbed and is utilized by being incorporated into the endogenous cellular pathways inherent in all cells. In disease states in which SAMe concentrations are reduced (either as a result of genetic defects, drug effects, or the disease process itself), there is a rational basis for administering SAMe to restore
methyl-group metabolism. A sufficient number of clinical trials have supported the use of SAMe in disorders including depression, dementia, vascular myelopathy, and liver disease. Further studies should be performed, and it is of primary importance that the safety and tolerability of SAMe be assured. Reviews of clinical studies to date indicate that SAMe has a low incidence of side effects with an excellent record of tolerability. This is of significant clinical benefit, particular in the elderly, in whom conventional therapies are often not tolerated well.

Although a considerable amount of literature exists on the biochemical and molecular roles of SAMe in cellular metabolism, further studies are needed to better understand its pharmacologic action. Clinical studies have shown clearly that SAMe has considerable potential in the treatment of depression, other neurologic disorders, and liver disease. Further studies should explore the full clinical potential of SAMe, and a primary objective should be defining the optimal dosage. Adequate dose-escalation studies have not been performed for use of SAMe in depression, liver disease, or osteoarthritis, especially with the newer oral formulations of SAMe. This information is needed before additional large clinical trials can be designed. The lack of any significant side effects may allow the long-term use of SAMe for evaluation of any possible prophylactic effect for prevention of depression, liver disease, or osteoarthritis. It is probable that the therapeutic dose and prophylactic dose of SAMe would be different.

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