The Effect of S-Adenosyl-L-Methionine Supplementation on Endothelial Function

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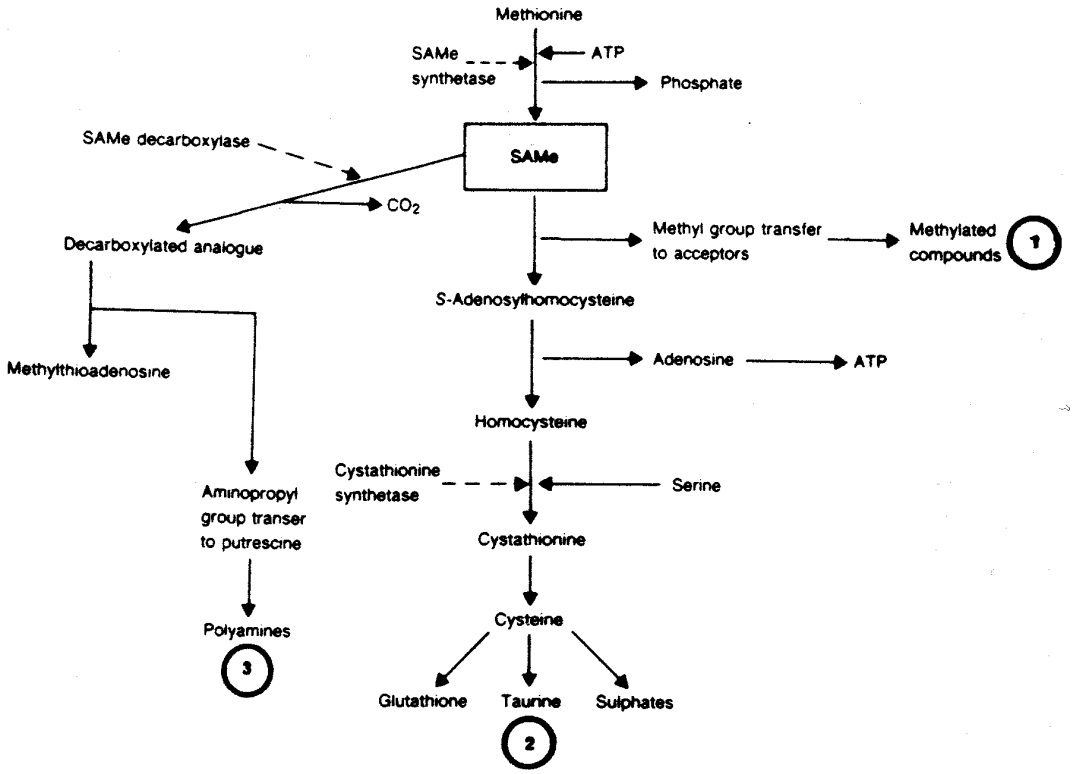
A. Study Purpose and Rationale

The vascular endothelium is a delicate structure, whose equilibrium is essential to the maintenance of vascular homeostasis. The normal endothelium secretes factors that regulate the vascular tone, decrease the adhesion of platelets and monocytes to the vessel wall and modulate smooth muscle growth. Damage to the endotheliurn and subsequent loss of these functions has important pathological implications. Endothelial dysfunction is now considered a precursor of atherosclerosis (1-2), and may contribute to the progression of disease. It has been found in patients with risk factors for the development of atherosclerosis, such as hypertension, hypercholesterolemia, smoking, diabetes mellitus and advanced age, but with no angiographic evidence of the disease (3). In patients with congestive heart failure, endothelial dysfunction limits flow-mediated vasodilation during exercise by reducing functional and structural changes in skeletal muscle circulation and is a determinant of peak aerobic capacity (4).

The pathophysiological mechanisms for endothelial dysfunction are not fully elucidated, but accumulating evidence suggests that decreased synthesis of endothelial-derived relaxing factor (NO), identified as nitric oxide, plays a pivotal role in reduced endothelial-dependent vasodilation (5). Vascular endothelial cells synthesize this powerful endogenous vasodilator from its amino acid precursor, L-arginine, by action of the constitutive form of the enzyme NO synthase (eNOS), in response to a diverse array of hormonal agonists, physiochernical and physical stimuli It is then rapidly converted to inactive nitrite and nitrates in the presence of water, oxygen, and superoxide species. A decrease in the bioavailability of endothelium-dependent NO has been demonstrated in patients with coronary artery disease risk factors, as well as in patients with CHF (4).

Reduction in NO bioavailability may be due to impaired synthesis or increased degradation of the molecule.A number of mechanisms are involved in the regulation of eNOS, including posttranslational modification (N-myristolation and palmitoylation), protein phosphorylation,, and binding to calcium-calmodulin, and calveolin- 1. Recently, a circulating endogenous eNOS inhibitor, N ,N -dimethylarginine (asymmetric dimethylarginine; ADMA) has been characterized, which may play a role in impairment of the nitric oxide synthase pathway. ADMA is synthesized from L-arginine by the action of methylase-I, a ubiquitous enzyme which catalyzes methylation of arginine at its guanido nitrogen. The ratio of the concentrations of ADMA and the normal substrate for NOS, L-arginine, regulate NOS activity It has been recently observed that ADMA levels are increased in patients with peripheral arterial disease and hypercholesterolemia, that elevation of ADMA is associated with impaired endothelium-dependent vasodilation and decreased urinary nitrate excretion (a reflexion of systemic NO production), and that this abnormality is reversed by administration of L-arginine (6). Studies of isolated vessels and cultured endothelial cells suggest that ADMA concentrations between I and 10 umoUL inhibit endothelial-dependent vasodilation and vascular NOS activity.

Hyperhomocysteinernia has emerged as a major and independent risk factor for vascular disease. This metabolic disturbance may promote atherosclerosis by causing endothelial damage affecting platelet function and coagulation factors, and promoting LDL oxidation. Recent studies report acutely impaired endothelium-dependent NO-mediated vasodilation during acute hyperhomocysteinernia in normal subjects which is dose and time dependent, and suggest that these adverse effects are mediated through oxidative stress mechanisms (7). In vitro studies show that continued exposure of culured endothelial cells to homocysteine leads to the generation of superoxide anion radicals and hydrogen peroxide, resulting in reduced production and/or inactivation of NO. Deactivation of NO may lead to vasoconstriction, platelet aggregation and monocyte adhesion, all of which promote atherosclerosis.



SAMe, S-adenosyl-L-methionine, a naturally occurring metabolite of the amino acid methionone, has recently appeared in synthetic form as a dietary supplement in U.S. drugstores, and may, by virtue of its role in metabolic processes, affect endothelial function. SAMe is distributed to virtually all body tissues and fluids and has a complex role in cellular biochemistry. It acts as a methyl-group donor, contributing to the synthesis, activation, and/or metabolism of neurotransmitters, nucleic acids, proteins, and phospholipids. It also provides a source of cysteine for the production of glutathione, a major endogenous hepatoprotective agent. SAMe has been studied as a pharmacologic agent and has been shown to restore normal hepatic function in the presence of various chronic liver diseases, to prevent or reverse drug hepatotoxicity, and to effectively treat osteoarthritis and depression. (8). In these clinical trials, SAMe was administered as a stable salt, in doses of 400-1600 mg/ day orally, 200-800 mg/ day parentally As the effects of SAMe in cellular biochemistry are incompletely characterized, the mechanism of its actions in various disease states remains speculative. While SAMe is sold by prescription in Europe, it is available as an over-the-counter supplement in the U.S. Overt therapeutic are avoided, and SAMe is labeled only as a promoter of "joint health" and "emotional well-being" in recommended daily doses of 400 mg.

The biologic effects of SAMe in normal subjects is unknown. Some of the known biochemical pathways of SAMe are relevant to the endothelium. As a methyl-donor, SAMe participates in the formation of ADMA via protein methylase 1. Having donated its methyl group to a variety of acceptors, SAMe is converted to S-adenosylhomocysteine, which is then hydrolized to adenosine and homocysteine. Homocysteine then either enters the transsulfaration pathway, where it is metabolized to cysteine (Vit 136-dependent) or the remethylation cycle, where it is recycled to methionine (folate and Vit B 12-dependent) (Figure-(8)). A hypothesis can be made that the administration of supraphysiologic doses of SAMe may result in elevated levels of metabolic products and intermediates known to be detrimental to endothelial function, namely homocysteine and ADMA

The purpose of this study is to determine whether administration of SAMe in normal persons, in doses currently recommended for dietary supplementation, is associated with elevated plasma levels of ADMA and hornocysteine, and to determine the functional significance of such levels with physiological measurements of endothelial function, flow-mediated vasodilation. This study aims to serve as a pilot study for future studies involving patients with coronary artery disease and CHF, who may represent groups particularly susceptible to these adverse affects.

B. Study Design and Statistical Analysis

This will be a randomized, double-blind, placebo-controlled study. Twelve healthy volunteers will be randomly assigned to receive SAMe (400 mg/day) (n=6), or matched placebo tablets (n=6) for 2 weeks. Studies will be performed on 2 occasions, 2 weeks apart, in order to examine the effects of single-dose and long-term use of SAMe on endothelial function.

Subjects will be instructed not to eat or drink on the morning of each visit. On the I' visit, vital signs will be taken and fasting blood samples will be obtained for total plasma homocysteine ADMA, folate and vitamin B 12. Physiological assessment of endothelial regulation of peripheral vascular tone will be determined by non-invasive measurement of the vasodilatory response of the brachial artery to endothelium-dependent (increased flow induced by metabolic hyperemia) and independent (sublingual nitroglycerin, NTG) stimuli, with Doppler Ultrasound. These measurements will be taken at 0 hours (baseline), then 3 hours, and 5 hours after the ingestion of oral SAMe or placebo.

Subjects will be instructed to continue their assigned treatment for the following 2 weeks. Upon their return visit, vital signs will be recorded, blood samples will be collected for the biochemical measurements above, and the noninvasive assessment of flow-mediated dilation and NTG-mediated dilation will be performed again.

C. Statistical analysis

To test the primary hypothesis that SAMe is associated with a 50% reduction in endothelial response following brachial artery occlusion, we assume that the untreated response is 4% with an intra-individual standard deviation of repeated measures of I%. A dependent T-test with 80% power and a 5% type I error rate requires 6 subjects to detect a 2.0 standard deviation change in endothelial response from 4 to 2%. To accommodate planned secondary analyses, 12 subjects will be enrolled. Direct administration of homocysteine is associated with a 3.5 standard deviation change in endothelial response from 4% toO% (7). Secondary exploratory analyses will attempt to identify the impact of chronic SAMe administration on ADMA and homocysteine using descriptive, graphical and analysis of covariance methods. The first day repeated sampling of endothelial response, ADMA and homocysteine levels following single-dose administration of SAMe will be analyzed by analysis of area-under-the-curve for total response, repeated measures analysis of variance to determine the temporal shape of the responses to SAMe, and regression analysis to model the metabolism of SAMe to homocysteine and ADMA.

D. Study Procedures

a. Doppler Ultrasonography

The vasodilatory responses of the brachial artery will be determined with a TL Apogee 800 Plus duplex ultrasound imaging system connected to a I I MHz high resolution transducer. The axial resolution of the I I MHz transducer is capable of detection of of changes in the brachial artery diameter of <0. I mm. Hyperemia will be induced by a cuff that will be inflated on the proximal portion of the upper arm to occlude arterial flow (>200mm Hg) for 5 minutes and then rapidly deflated. Brachial artery diameter will be measured at rest, I minute after release of transient occlusion, and 5 minutes after the administration of 0.4 mg of sublingual NTG at end-diastole with a computer-assisted edge detection system (NIH image

analysis software) by 2 independent individuals blinded to clinical information. NTG will be omitted if the SBP is <I OOmn-tHg or if the subject had previously had a reaction to NTG. The percent change from baseline diameter to maximum diameter following release of arterial occlusion will be calculated as a index of endothelium-dependent flow-mediated vasodilation in the brachial artery This Ultrasound-derived index has been extensively used for the noninvasive assessment of flow-mediated, endothelium-dependent vasodilation in human subjects (9).

b. Biochemical Measurements

Phlebotomy will be performed at the time of initial screening of potential candidates as well as the I' and 2 d study visits

Total plasma homocysteine levels and ADMA levels will be determined by high pressure liquid chromatograghy as previously described (6). These measurements will be performed at the ICCR Research Laboratory.

E. Study Drugs

SAMe is a naturally occurring intermediary of metabolism,and as a dietary supplement is currently consumed at a recommended dose of 400mg/day. Peak plasma concentrations obtained with an enteric-coated tablet formulation are dose related, with a peak plasma concentration which is 3.5- to 6-fold higher than baseline endogenous levels (0.5-1 mg/L), achieved 3 to 5 hours after single doses in the range of 400 to 1000 mg (8).

SAMe, in the formulation of 200 mg tablets, will be purchased from GNC stores. The CPMC Research Pharmacy will modify their appearance to form unmarked tablets, as well as provide identical placebo tablets.

The dose of SAMe administered will be 400 mg/day. SAMe is very well tolerated. At oral doses of up to 1600 mg daily no adverse effects have been noted other than occasional mild gastrointestinal effects (8).

F. Medical Devices

None

G. Study Questionnaires

None

H. Study Subjects

Healthy volunteers in the 21 to 40 age-range will be recruited to participate in this study. During an initial screening visit, a complete medical history will be obtained, in addition to vital signs, an ECG and fasting blood samples for glucose, total cholesterol, HDL, triglycerides, serum creatinine and liver function tests. Subjects with conditions known to be associated with endothelial dysfunction-hypertension, hypercholesterolemia, diabetes mellitus, vascular disease, a history of smoking, will be excluded from the study. Additional exclusion criteria will be the use of antioxidant therapy, vitamins C and E, within one- month time, in order to eliminate potential effects on endothelial reactivity. As estrogen has been shown to have significant effects on the endothelium, women in the follicular phase of their menstrual cycle, as well as women taking estrogen or estrogen-containing compounds will be excluded.

Due to the effects of liver and kidney disease on metabolism and clearance of the tested substances, subjects with abnormal liver function tests (elevations 2x that of normal) and elevated serum creatinine levels (>1.5) will be excluded in this study.

I. Recruitment of Subjects

Subjects will be recruited for this study by flyer and advertisement posted on bulletin boards located in the hallways and lobbies of Columbia Presbyterian Medical Center.

J. Confidentiality of Study Data

All data will be coded and the identity of study subjects kept confidential. Data will be stored in a locked office/ laboratory, accessible only to the investigators.

K. Potential Conflict of Interest

Not applicable

L. Location of the Study

Studies will be conducted at the Vascular Research Laboratory, located at the Division of Circulatory Physiology, PH-9 at CPMC.

M. Potential Risks

Ultrasonography There are no known risks to short-term exposure to high frequency ultrasound.
Phlebotomy-Phlebotomy may induce some local temporary discomfort and a risk of ecchyrnosis.

N. Potential Benefits

Patients are not expected to benefit from this study.
Potential benefit to society is the identification of an adverse effect of a dietary supplement.

O. Compensation to Subjects.

Subjects will compensated $ 100 for participation in this study. Payment will be in the form of cash given at the end of the end session.

P. Costs to Subjects

The subjects will not incur any additional costs as a result of participation in the study.

Q. References

1. Celer-mayer DS. Endothelial dysfunction: does it matter? Is it reversible ? J Am Coll Cardio. 1997;39:287-324.
2. Vanhouette PM. Endothelial dysfunction and atherosclerosis. Eur Heart J. 1997; 18(suppI E); E 19-E29.
3. Quyyumi AA, Dakak N, Andrews NP, Husain S et al. Nitric Oxide activity ion the human coronary circulation: impact of risk factors for coronary atherosclerosis. J Clin Invest. 1995;95:1747-1755.
4. Katz SD. Mechanisms and implications of endothelial dysfunction in congestive heart failure. Current Opinions in Cardiology. 1997; 12:259-264.
5. Moncada, S, Higgs, A. The I-arginine-nitric oxide pathway. N Eng J Med. 1993;27:2002-2012.

6. Boger, R.,Bode-Boger, S., Szuba, A. et al. Asymmetric dimethylarginine (ADMA): a novel risk factor for endothelial dysfunction. Circulation. 1998;98:1842-1847.
7. Chambers, J., McGregor, Ajean-Marie, J.et al. Demonstration of rapid onset vascular endothelial dysfunction after hyperhomocystelnemia; an effect reversible with vitamin C. Circulation. 1999;99:1156-1160.
8. Friedel, H. Goa, K., Benfield, P. S-adenosyl-l-methionine; a review of its pharmacologic properties and therapeutic potential in liver dysfunction and affective disorders in relation to its physiological role in cell metabolism. Drugs. 1989;38(3):389-416.
9. Celermayer DS., Sorensen KE., Bull C., Robinson J. et al. Endothelium-dependent dilation in the systemic arteries of asymotomatic patients relates to coronary risk factors and their interaction. J Am Coll Cardiol. 1994;24:1468-1474.