Influence of Oral S-Adenosylmethionine on Plasma 5-Methyltetrahydrofolate, S-Adenosylhomocysteine, Homocysteine and Methionine in Healthy Humans

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ABSTRACT

Elevated plasma homocysteine concentration is an independent risk factor for vascular disease in humans. In addition to nutritional and genetic factors, an interruption of the coordinate regulatory function of S-adenosylmethionine has been proposed to be involved in the occurrence of hyperhomocysteinemia. The effect of oral S-adenosylmethionine on homocysteine metabolism in humans is unknown. We investigated the effect of oral S-adenosylmethionine (400 mg) on plasma levels of 5-methyltetrahydrofolate, which is the active form of folate in the remethylation of homocysteine to methionine, S-adenosylhomocysteine, the demethylated product of S-adenosylmethionine, homocysteine and methionine over 24 hr in 14 healthy subjects. After oral administration, S-adenosylmethionine increased from 38.0 ± 13.4 to 361.8 ± 66.4 nmol/liter (mean ± S.E., P < .001) and returned to base-line values with a half-life of 1.7 ± 0.3 hr. Both S-adenosylhomocysteine and 5-methyltetrahydrofolate showed a significant transient increase (from 29.9 ± 3.7 to 51.7 ± 7.1 nmol/liter, and from 25.1 ± 2.5 to 36.2 ± 3.5 nmol/liter, respectively, P < .001), although homocysteine and methionine did not change over the time of measurement. These changes were not found in subjects without previous S-adenosylmethionine administration. The observed metabolic changes suggest that S-adenosylmethionine, at least in concentrations obtained in this study, does not inhibit 5,10-methylentetrahydrofolate reductase, the 5-methyltetrahydrofolate forming enzyme. Rather they indicate a positive effect on 5-methyltetrahydrofolate, a key cofactor in homocysteine metabolism, which should be considered in homocysteine lowering strategies for the prevention of vascular disease.

Elevated levels of plasma homocysteine, either postmethionine loading or fasting, have been reported repeatedly in patients with various forms of vascular disease (Boushey et al., 1995; Stampfer et al., 1992; Perry et al., 1995; den Heijer et al., 1996). These findings, in patients without a known inborn error of methionine metabolism, have established increased plasma homocysteine (hyperhomocysteinemia) as an independent risk factor for vascular events.

The cause of mild elevation of homocysteine in vascular disease has by no means been completely elucidated. Genetic factors, such as increased thermolability of methyleneTHF reductase (fig. 1), crucial for MeTHF synthesis, have been shown to cause hyperhomocysteinemia in some patients (Kang et al., 1991; Engbersen et al., 1995; Jacques et al., 1996). In addition, nutritional factors such as deficiencies of vitamin B_{12}, folate or vitamin B_{6} seem to play a role in the occurrence of hyperhomocysteinemia (Ubbink et al., 1993; Ueland et al., 1992) and treatment with folic acid and vitamin B_{6} can lower elevated homocysteine in vascular disease patients with normal vitamin status (Franken et al., 1994; Landgren et al., 1995). Additionally, an interruption of the coordinate regulatory function of AdoMet in homocysteine metabolism, has recently been proposed (Selhub and Miller, 1992).

AdoMet, a major methyl donor in many biochemical reactions, is formed in humans from the essential amino acid methionine by AdoMet synthetase (fig. 1). Further metabolism yields homocysteine that is either catabolized via transulfuration or recycled via remethylation to methionine. Little is known about the control of these pathways in vivo in humans, but in vitro studies on purified enzymes point to a

ABBREVIATIONS: methyleneTHF, 5,10-methylentetrahydrofolate; MeTHF, 5-methyltetrahydrofolate; AdoMet, S-adenosylmethionine; AdoHcy, S-adenosylhomocysteine; Cbl, cobalamine; PLP, pyridoxal phosphate; THF, tetrahydrofolate; MS, 5-methyltetrahydrofolate-homocysteine methyltransferase (methionine synthase); CO, base-line concentration; Cmax, peak or trough concentration; tmax, time to reach Cmax; ΔC, difference between CO and the concentration at a specific time of sampling; AUC, area under the concentration-time curve; BW, body weight.
regulatory role of AdoMet. At micromolar concentrations AdoMet acts as an activator of cystathionine β-synthase (Finkelstein et al., 1975) and as an allosteric inhibitor of methyleneTHF reductase that is crucial for MeTHF synthesis and therefore for homocysteine remethylation (Jencks and Matthews, 1987). Moreover, in a previous study, low whole blood AdoMet values were found in a significant proportion of coronary artery disease patients (Loehrer et al., 1996a), giving evidence for a potential protective role of AdoMet in the pathogenesis of vascular diseases. We further showed in healthy subjects after methionine loading, not only an increase in AdoMet simultaneous to the expected increase showed in healthy subjects after methionine loading, not only AdoMet in the pathogenesis of vascular diseases. We further showed in healthy subjects after methionine loading, not only an increase in AdoMet simultaneous to the expected increase of homocysteine, but also a marked decrease in MeTHF concentrations (Loehrer et al., 1996b). We suggested that this effect could be due to either an enhanced remethylation, caused by the increase of homocysteine or to an inhibition of methyleneTHF reductase by the increase of AdoMet as found in vitro (Jencks and Matthews, 1987). Although AdoMet has been given therapeutically for liver disease (Lieber and Williams, 1990), rheumatoid arthritis (Di Padova, 1987) and neurological disorders (Bottiglieri et al., 1994), no data exist so far about the effect of AdoMet on methionine metabolism in humans. In this study we determined the effect of oral AdoMet on critical metabolites of homocysteine metabolism in vivo.

Methods

Subjects. A total of 14 healthy subjects (7 female, 7 male; age 22–44; weight 48–86 kg) participated in the study after giving written informed consent. All had fasting plasma total homocysteine concentrations within our own healthy population normal range [2.2–13.2 μmol/liter, n = 50 (Loehrer et al., 1996a)]. Biochemical and hematological parameters were determined in each subject to exclude hematological disorders as well as abnormal liver and kidney function. None of these subjects had a family history of premature vascular disease. The protocol for this study was approved by the Ethical Committee of the Department of Medicine of the University Hospital Basel, Switzerland.

AdoMet loading and sample preparation. After an overnight fast, two enteric-coated tablets (GUMBARAL from Asta Medica AG, Frankfurt, Germany), each of which contained 384 mg AdoMet bis(sulfate)-p-toluenesulfonate (corresponding to 200 mg AdoMet) were given orally. Subjects fasted for another 6 hr after AdoMet administration. Blood was collected immediately before and 2, 3, 4, 5, 6, 7, 8, 9, 12 and 24 hr after AdoMet intake. All subjects received a standardized diet excluding methionine and folate-rich foods from the evening before, until 24 hr after AdoMet intake. Three months before this loading test, three control subjects (two males, one female) underwent the same procedure but without AdoMet intake.

As previously described, blood samples for AdoMet, MeTHF, AdoHcy, homocysteine and methionine determination were placed on ice after collection and processed within 0.5 hr. For lymphocyte isolation, blood was kept at room temperature until isolation of the cells (Loehrer et al., 1996b).

HPLC determination of AdoMet, MeTHF, AdoHcy, homocysteine, cystine and methionine in plasma. Plasma AdoMet, MeTHF, AdoHcy and total homocysteine were measured by reversed phase chromatography with fluorescence detection as previously described (Loehrer et al., 1996b), except for the use of a 4.0 × 200 mm Hypersil RP-18 (3 μm) column for the AdoHcy determination, which produced a better and more reliable separation of AdoHcy (detection limit: 1 nmol/liter in plasma, signal to noise ratio ≥ 5).

Methionine and cystine were determined in plasma, deproteinised immediately with sulphosalicylic acid, by ion-exchange column chromatography using ninhydrin detection as previously described (Fowler and Sardharwalla, 1981).

MethyleneTHF reductase activity was measured as previously described (Loehrer et al., 1996b). For determining heat lability, activity was measured without preheating (specific activity) and after preheating at 42°C for 10 min (heat stable). These conditions were selected after investigations were conducted in control cells at temperatures between 42 and 46°C for different times.

Statistical analysis and calculations. The significance of the change of a particular analyte over 24 hr was tested by nonparametric analysis of variance (Friedman test). Differences between baseline and postloading values were compared by Wilcoxon signed rank test and gender differences by Mann-Whitney U test. The relationship between pairs of variables was tested by linear regression analysis. If an outlier was detected [indicated by a Cooks distance > 4, pointing to a possible crucial influence on the regression results (Glantz, 1990)] regression analysis was repeated without that particular value. The interrelationship between the different parameters measured was performed by multiple regression analysis. P < .05 were considered significant. All tests were performed by the software package Student SYSTAT 1.0 for windows (1990–1994 by SYSTAT Inc., Evanston, IL). Unless indicated otherwise values are expressed as mean ± S.E.

The elimination rate constant was obtained by linear regression of at least the last three data points of the natural logarithm transformed concentration-time curve. The half-life was calculated by dividing 0.693 by the elimination rate constant (slope). The AUC was calculated by the trapezoidal rule (Gibaldi and Perrier, 1982) after subtraction of the area below the baseline. To account for interindividual differences in BW data were adjusted for body weight by division of the values by the factor dose (mg/VB (kg)).

Results

AdoMet was well tolerated in all volunteers. The mean results of plasma concentrations of AdoMet, MeTHF, AdoHcy, homocysteine and methionine before and after
AdoMet administration are shown in table 1. At baseline, interindividual variability in plasma concentrations was particularly large for AdoMet and in one female the AdoMet level was 5-fold higher than the average of the whole group (212.9 nmol/liter). In females AdoMet base-line concentrations were significantly higher (59.0 ± 212.9 nmol/liter) than in men (17.1 ± 0.6) (P < .01). This difference remained significant (P < .01) even after exclusion of the female with the highest concentration. Because this was the only gender difference, values are reported for males and females together in one group (table 1). Preload levels of AdoMet in the different subjects were not correlated with preload levels of any of the other metabolites measured.

After oral administration, plasma concentrations of AdoMet increased in all but one subject reaching peak concentrations on average after 4.3 hr (table 1; fig. 2). AdoMet concentrations returned to baseline levels with a half-life of 3.5 hr (table 1; fig. 2). AdoMet increased in all but one subject reaching peak concentrations after 2 hr (table 1; fig. 2). This shows that methylation reactions are active in the small intestine. After oral administration, plasma concentrations of MeTHF increased significantly (P < .001) even after exclusion of the female with the highest concentration. Because this was the only gender difference, values are reported for males and females together in one group (table 1). Preload levels of MeTHF in the different subjects were not correlated with preload levels of any of the other metabolites measured.

Measurement of methyleneTHF reductase activity in lymphocytes, before and after heating, revealed levels of thermostable activity of this enzyme in 13 subjects ranging from 23 to 58% (43.6 ± 2.9, mean ± S.E.) of total enzyme activity. These values compare well with a range of 27 to 51% (37.6 ± 1.2) in healthy controls reported by Kang et al. (1988) and suggest
that none of these 13 subjects has the mutation leading to increased thermolability. However, in one male subject thermostable activity was only 17% of total activity. This subject also had the lowest MeTHF base-line level (13.2 nmol/liter), which is just below the 95th percentile of our own healthy population normal range (>13.3 nmol/liter, n = 50). However, homocysteine was not elevated (10.8 μmol/liter) in this subject and the increase in MeTHF after AdoMet administration was well within the range of this study (adjusted AUC: 5.24 nmol/liter*hr) as were all the other parameters measured. To evaluate the interrelationship between all base-line values, weight-adjusted differences between base-line and peak concentrations, and weight-adjusted AUCs for AdoMet, MeTHF, AdoHcy, homocysteine and methionine as well as the thermostable methyleneTHF reductase activity were studied in multiple regression analysis. The subject who showed no change in MeTHF concentrations was excluded from this correlation analysis. In this analysis, baseline values of AdoMet were correlated with adjusted AUCs of MeTHF (r = 0.88, P < .001, without the outlier with the highest AdoMet baseline level: r = 0.63, P < .03). Moreover, MeTHF base-line values correlated with the activity of the thermostable methyleneTHF reductase (r = 0.55; P < .05). After excluding the subject with the decreased heatstable methyleneTHF reductase activity this correlation was no longer significant (r = 0.4; P = .12). No other correlations between the remaining parameters were found.

Discussion

The purpose of this investigation was to study the effect on methionine metabolites of oral AdoMet in doses used for
pharmacotherapeutic purposes, in healthy humans. Mean AdoMet base-line concentrations measured in this study were similar to those reported in previous studies [40 ± 12 and 50 ± 10.8 nmol/liter (Giulidori and Stramentiniol, 1984; Castagna et al., 1995)]. However, we found a substantial gender difference, which was not observed in those earlier studies, and the range was much larger as indicated by higher S.E. values. This can be explained by the high interindividual variability of AdoMet pharmacokinetics and its low bioavailability of less than 5% (product information, Asta Medica AG, Frankfurt, Germany, 1991). In contrast to an earlier study by Stramentinoli (1987), there were no gender differences in the time to reach peak concentrations. These differences could well be due to the more frequent blood sampling and the more sensitive analytical method used in this study.

Oral administration of 400 mg AdoMet, the activated form of methionine, resulted in an increase in AdoMet plasma concentrations of the same order of magnitude to that observed after methionine loading in our previous study (Loehr et al., 1996b). In this earlier study the nonadjusted mean AUC of AdoMet above baseline was 2095 ± 975 nmol/liter*hr compared with 1223 ± 1052 in this study. However, although homocysteine concentrations increased 4-fold and MeTHF decreased by about 50% after methionine loading, homocysteine remained unchanged and MeTHF significantly increased by 50%, on average, after AdoMet. The small increase of AdoHcy concentration in this study compared with the lack of such a response after methionine loading, could be explained by the higher AdoMet peak concentration obtained after AdoMet loading (1554 ± 400% increase above baseline) with a short half-life (1.7 ± 0.3 hr) compared to those after methionine loading (729 ± 325%) with a much longer apparent half-life (7.1 ± 3.9 hr). Additionally we used in this study a more sensitive method for AdoHcy measurement, which is more reliable for the detection of small changes in AdoHcy at low concentrations.

The lack of change of homocysteine concentrations after AdoMet administration could be explained by sufficient capacity of homocysteine handling in contrast to the situation when excessive methionine was given. In fact no changes in plasma cystine concentration were observed as might be expected if transsulfuration increased. It must be borne in mind that the interpretation of the findings in such studies presume that plasma levels adequately reflect tissue levels and is based on the assumption that AdoMet is taken up into liver cells that still remains to be fully established (Chiang et al., 1996). However, oral administration of AdoMet, albeit at four times higher concentrations than used in this study, was effective in relieving the symptoms of cholestasis in adult women (Almasio et al., 1990). Also Muriel et al. (1994) demonstrated protection of liver damage secondary to biliary obstruction by AdoMet in rats. Marchesini et al. (1992) showed increased levels of cystine after long-term treatment with AdoMet at a dose of 1.2 g/day in patients with cirrhosis indicating stimulation of the transsulfuration pathway. A lower dose of 800 mg/day was effective in preventing estrogen induced hepatobiliary toxicity in women (Frezza et al., 1988). Furthermore the notion that AdoMet is not taken up from the blood stream into liver (Hoffman et al., 1980) has been thrown into doubt by more recent studies. An in vivo study in rats indicated uptake and metabolism of i.v. administered AdoMet by the liver (Giulidori et al., 1984) and Lieber et al. (1990) reported evidence for appreciable uptake of AdoMet into the liver in a primate model. Our study has shown a clear metabolic change whereby the plasma concentration of MeTHF increased significantly after AdoMet administration. This is not explained by circadian variation because there was no such change in subjects who received no AdoMet. Also food intake can be excluded as a cause, because peak concentrations of MeTHF were reached in all but one subject before the first meal, which was low in folate and was taken 6 hr after AdoMet administration. The increase is therefore likely to be caused directly or indirectly by AdoMet administration. The finding of a rise of MeTHF concentrations argues against a significant inhibitory effect of AdoMet on methyleneTHF reductase in humans at least at tissue concentrations reached after 400 mg oral AdoMet. It must also be borne in mind that, based on values reported in animals (Finkelstein et al., 1982; Lieber et al., 1990), the expected intracellular concentration of AdoMet in liver ranges from 80 to 110 μmol/kg. Therefore, liver concentrations of AdoMet may change only slightly after administration of approximately 1 mmol of this compound with an absorption rate of 51% of given doses (product information, Asta Medica AG, Frankfurt, Germany, 1991). Other possible causes of this increase of MeTHF need to be considered, such as extracellular changes or extracellular metabolism. Alteration of the intracellular/extracellular distribution of MeTHF, or moderation of renal tubular reabsorption might play a role, but further studies are needed to evaluate these possibilities. More information of the effects of AdoMet on methionine metabolism would likely be forthcoming from studies with longer-term oral or i.v. administration or higher doses.

The decreased heatstable methyleneTHF reductase activity in one subject is probably due to the recently described homozygous C677T mutation of this gene, which is supported by the low MeTHF value, although the plasma homocysteine concentration was not elevated as might be expected in the presence of decreased MeTHF values (Jacques et al., 1996). The increase of MeTHF was well within the range of the other subjects, showing no effect of decreased thermostability of methyleneTHF reductase on the response of MeTHF to AdoMet in this subject. Homocysteine has been established as an independent risk factor for vascular disease in numerous studies measuring methionine metabolites in plasma (Boushey et al., 1995). Therefore, investigation of the effect of AdoMet in plasma seems to be justified also in vascular disease patients using a similar approach to that which we described.

In summary, this study revealed an increase of plasma MeTHF after oral administration of AdoMet. This indicates that orally administered AdoMet could have a potentially beneficial effect on homocysteine metabolism, which should be considered in the development of prevention strategies for the lowering of homocysteine in vascular disease.

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References
