Effect of folic acid supplementation on the change in plasma S-adenosylhomocysteine level in Chinese hypertensive patients: a randomized, double-blind, controlled clinical trial

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The relationship between folic acid and S-adenosylhomocysteine (SAH) is controversial. This study aims to explore the effect of different doses of folic acid supplementation on SAH levels in hypertensive patients and the modification of methylene-tetrahydrofolate reductase (MTHFR) C677T gene polymorphism. A randomized, double-blind, controlled clinical trial was conducted. Hypertensive patients aged 45–75 years without a history of stroke and cardiovascular disease were selected, who were randomly assigned to one of 8 dose groups. This trial has been registered with Trial Number: ChiCTR1800016135. In the total population, folic acid supplementation of 0.4–2.0 mg/day had no effect on SAH level (β = 0.47, 95% CI: −0.86–1.79, p = 0.491), while folic acid supplementation of 2.4 mg/day significantly increased SAH level (β = 1.93, 95% CI: 0.22–3.64, p = 0.027). Stratified analysis found that MTHFR C677T genotype CT supplemented with 2.4 mg/day folic acid had no effect on SAH level (β = 0.30, 95% CI: −2.74–3.34, p = 0.847), while CT and TT genotype supplemented with 2.4 mg/day folic acid showed a significant increase in SAH level (CT: β = 2.98, 95% CI: 0.34–5.62, p = 0.027; TT: β = 3.00, 95% CI: −0.51–6.51, p = 0.095; CT combined with TT: β = 2.99, 95% CI: 0.90–5.09, p = 0.005). In conclusion, supplementation of 2.4 mg/day folic acid can lead to increased SAH levels, especially in MTHFR C677T genotype CT and TT.

Key Words: hypertension, folic acid, S-adenosylhomocysteine, methylation, cardiovascular diseases

Folic acid, also known as vitamin B9, is a water-soluble vitamin that exists in natural foods and can also be artificially synthesized.¹² Studies have shown that folic acid deficiency is related to a variety of diseases such as neural tube defects and cardiovascular diseases.¹³–¹⁵ Based on this, North American countries strengthen folic acid supplementation. However, folic acid supplementation, especially high-dose folic acid supplementation, may also bring some harm. High concentrations of folic acid may increase the risk of death. A prospective cohort study found that compared with people with plasma folic acid levels of 26.1–54.6 nmol/L, the risk of death was increased by 58% when the plasma folic acid level was ≥54.6 nmol/L.⁶ Toole et al.⁷ conducted the Vitamin Intervention for Stroke Prevention study. After 1.8 years of folic acid treatment with 2.5 mg/day, the plasma folate level was 80.0 nmol/L (baseline folate level was 27.8 nmol/L), which was much higher than 54.6 nmol/L.

S-adenosylhomocysteine (SAH), as the intermediate product of methionine cycle, is the product of S-adenosylmethionine (SAM),⁸ and is also the precursor of homocysteine, which is reversible hydrolyzed to homocysteine and adenosine by S-adenosylhomocysteine hydrolase (SAHH).⁹ Elevated SAH levels may result in decreased DNA methylation and increased risk of cardiovascular disease. A cross-sectional study found that plasma SAH level was positively correlated with lymphocyte DNA hypomethylation level (r = 0.74, p = 0.001),¹⁰ indicating that the increase of plasma SAH level would lead to the decrease of DNA methylation level. A prospective cohort study also found that the higher plasma SAH level was significantly correlated with the increased risk of cardiovascular events in patients with coronary angiography, and it could predict the risk of cardiovascular events better than homocysteine. High SAH level increased the risk of cardiovascular diseases independently of homocysteine.¹¹

The relationship between SAH and folic acid is controversial. In one study of 97 Caucasians aged 60–85, SAH level was not related to folic acid.¹² In another cross-sectional study, 100 elderly patients with type 2 diabetes in China were included, SAH level was negatively correlated with folic acid,¹³ suggesting that folic acid supplementation may reduce SAH level. A randomized controlled trial found that 0.4 mg/day folic acid supplementation for 2 years can reduce the level of SAH in patients with mild cognitive impairment (MCI),¹⁴ while other randomized controlled trials did not find that folic acid supplementation has a significant effect on the level of SAH.¹⁵–¹⁸ Previous studies on the effect of folic acid supplementation on SAH level were inconsistent, and previous studies explored the effect of folic acid supplementation on SAH level at a fixed dose, lacking the effect of different doses. In addition, no study examined the modification effect of MTHFR C677T polymorphism, a key gene for folic acid metabolism. Therefore, this study will investigate the effect of different doses of folic acid supplementation on SAH in hypertensive population and explore the modification of MTHFR C677T gene polymorphism.

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Materials and Methods

Participants and ethics. This study is a randomized, double-blind, controlled clinical trial (Trial Number: ChiCTR1800016135), which was conducted in strict accordance with the Helsinki Declaration. From October 2018 to October 2020, 1,657 patients with mild to moderate hypertension were recruited in three centers in Wuyuan, Linyungang, and Anqing, China. Inclusion criteria: (1) Aged 45–75 years; (2) Patients with various types of primary hypertension that have been diagnosed, including those who are currently taking antihypertensive drugs. And for those who did not take antihypertensive drugs within two weeks, two sitting blood pressure (average value of three measurements), which were examined at least one day interval, met the following criteria: systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg; (3) Women of childbearing age agreed to use a reliable contraceptive method during the experiment; (4) To participate voluntarily and sign an informed consent. Exclusion criteria: (1) Pregnant and lactating women; (2) Allergic to enalapril, folic acid or other ingredients in compound drugs; (3) Patients who had taken enalapril or other angiotensin converting enzyme inhibitors, or drugs or nutrients containing folic acid, had obvious intolerance of adverse reactions; (4) Secondary hypertension is known or suspected; (5) Patients with known severe medical diseases; (6) There are obvious laboratory tests or signs of abnormalities, and according to the researchers’ judgment, this abnormality shows that patients have serious diseases, or according to the researchers’ judgment, may affect the observation and evaluation of drug efficacy or adverse events, not suitable for researchers; (7) Patients taking folic acid, vitamin B12 or B6, containing the compound preparation, and can not or unwilling to discontinue; (8) Take folic acid or compound preparations containing folic acid regularly in the past three months; (9) Patients who participated in clinical trials of any drug that had not been officially approved by the state within one month before the first visit.

The Human Subjects Committee at the Biomedical Institute of Anhui Medical University approved the study protocol. All patients provided written informed consent prior to data collection.

Trial design. The trial consisted of three stages: screening and recruitment, a 2-week run-in treatment period, and an 8-week treatment follow-up period.

Screening and recruitment. During the screening period, eligible patients who met the inclusion and exclusion criteria of this study were screened at each research center. Patients were registered and filed. Relevant examinations and questionnaires (physical examination, lifestyle, disease history, and medication history) required by the study were completed. Before implementing any particular research procedure, the purpose and procedures of the study should be explained to the patient and written informed consent should be obtained.

Run-in treatment. All patients were given 10 mg enalapril daily for 2 weeks. This stage was set to investigate the tolerance of patients to enalapril and the effect of cleaning the original drug treatment, and to confirm that the patients still fully met the inclusion criteria and no exclusion criteria at the end of the cleaning period. The MTHFR C677T genotype was also detected at this stage.

Treatment and follow-up. Eligible participants, stratified by MTHFR C677T genotypes (CC, CT, or TT), were randomly assigned to receive 1 of 8 treatments: a daily oral dose of 1 tablet containing 10 mg of enalapril only, a daily oral dose of 1 tablet containing 10 mg of enalapril and 0.4 mg folic acid, a daily oral dose of 1 tablet containing 10 mg of enalapril and 0.6 mg folic acid, a daily oral dose of 1 tablet containing 10 mg of enalapril and 0.8 mg folic acid, a daily oral dose of 1 tablet containing 10 mg of enalapril and 1.2 mg folic acid, a daily oral dose of 1 tablet containing 10 mg of enalapril and 1.6 mg folic acid, a daily oral dose of 1 tablet containing 10 mg of enalapril and 2.0 mg folic acid or a daily oral dose of 1 tablet containing 10 mg of enalapril and 2.4 mg folic acid, for 8 weeks. The patients were followed up at baseline, 2 weeks, 4 weeks, 6 weeks and 8 weeks, and the blood pressure of the patients was recorded. Blood samples were obtained at baseline and 8 weeks to detect blood SAH and folic acid levels.

Body measurement. A unified electronic sphygmomanometer was used for blood pressure measurement in this study. Before blood pressure measurement, the subjects should be at least seated and rested for 5 min. The sitting position was taken, and the feet were placed on the ground. The right upper arm was exposed, and the upper arm was at the same level as the heart. The measurement was repeated at intervals of 1–2 min. Three consecutive measurements were performed, and the average blood pressure was recorded for three times. Weight and height were measured in participants wearing light clothes and without shoes. Waist circumference was measured at 1 cm above the navel. Hip circumference measures the length of the most convex part of the hip. Body mass index (BMI) is calculated by weight/height square (kg/m²).

Measurement of biochemical indexes and biomarkers. Blood samples were collected from all patients at baseline and 8 weeks after random treatment (8 ml each time) and sent to the laboratory of Shenzhen Ausa Changqing Research Institute for unified detection. MTHFR C677T gene polymorphism was determined by blood samples collected at baseline, and Taqman probe was used for detection on ABI Prism 7900HT sequence detection system. Fasting blood glucose, total cholesterol, triglyceride, high density lipoprotein, low density lipoprotein, total homocysteine and other indicators were measured by biochemical analyzer. The total folic acid was measured by chemiluminescence method, and the instrument was Bccman Contler Access Immuno Assay System (Bccman-Coulter Canada, Inc., Mississauga, Canada). SAH and SAM were measured by liquid chromatography. Agilent 1100 liquid chromatograph was used and the chromatographic column was Hypersil C18 column (4.6 × 250 mm, 5 μm).

Outcome. The SAH change, which was defined as SAH concentration at week 8 minus SAH at baseline, was applied as the primary outcome.

Statistical analysis. The data conforming to the perprotocol set (PPS) is selected for analysis. In continuous variables, the mean ± SD is used to represent the normal distribution, and the median ± interquartile range (Median ± IQR) is used to represent the non-normal distribution. Classification variables are expressed as percentage (%), ANOVA tests and chi-square tests were used to compare the differences between different baseline dose groups. Generalized additive model (GAM) and a fitted smoothing curve (penalized spline method) were used to assess trends in changes of SAH levels with increasing doses of folic acid supplementation in total population. The black solid line is the fitting curve, and the grey area represents the 95% confidence interval. Multiple linear regression was used to compare the effects of folic acid supplementation at different doses on SAH changes. The interaction between MTHFR C677T genotype and folic acid supplement dose was tested by Wald test, which was used to evaluate interaction on a multiplicative scale. The statistical analysis was carried out using Empower 2.0 (http://www.empowerstats.com, X&Y Solutions, Inc., Boston, MA) and R 4.0 (http://www.R-project.org) software. Bilateral test was used and p<0.05 was considered statistically significant.

Results

Participant flow. A total of 1,657 participants were recruited for this trial. The baseline non-metabolized folic acid efficiency
In addition, we analyzed the relationship between each folic acid dose and SAH changes stratified by MTHFR C677T genotype (Supplemental Fig. 1*), with results consistent with previous analyses.

**Discussion**

This study is the first study to explore the effect of folic acid supplementation with different doses on SAH based on Chinese hypertensive population. We found that SAH increased significantly at high doses of folic acid supplementation (2.4 mg/day), especially in MTHFR C677T genotype CT and TT.

To date, studies on the relationship between folic acid and SAH levels are inconsistent. A cross-sectional study showed that SAH was not associated with folic acid in the elderly Caucasian population. Other studies found that the level of SAH was negatively correlated with folic acid \((r = -0.32, p < 0.01)\) in Chinese elderly patients with type 2 diabetes. Furthermore, the level of folic acid was divided into three grades. When the folic acid level was lower and higher (\(<6.3 \text{ ng/ml} \) and \(\geq 9.1 \text{ ng/ml}\)), the level of SAH was not correlated with folic acid \((r = -0.12, p = 0.05; r = -0.30, p = 0.05)\). When the folic acid level was in the middle \((6.3 - 9.1 \text{ ng/ml})\), the level of SAH was negatively correlated with folic acid. This suggests that the level of SAH may be negatively correlated with folic acid in a certain range of folic acid levels. Studies on the effect of folic acid supplementation on SAH levels are also inconsistent. A randomized controlled trial of folic acid supplementation in patients with MCI in Tianjin, China, found that 0.4 mg/day folic acid supplementation had no significant change in the level of SAH at 6 months of follow-up, and the level of SAH decreased significantly at 2 years of follow-up, indicating that low-dose folic acid supplementation may reduce the level of SAH in patients with mild cognitive impairment. Other studies found no significant effect of folic acid supplementation on SAH levels. A randomized controlled trial, conducted in patients with Alzheimer’s disease (AD), given 0.8 mg/day folic acid supplementation for 6 months, found no effect on SAH levels.

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Table 1. Baseline characteristic of participants

| Characteristic | 0 mg | 0.4 mg | 0.6 mg | 0.8 mg | 1.2 mg | 1.6 mg | 2.0 mg | 2.4 mg | p value |
|----------------|------|--------|--------|--------|--------|--------|--------|--------|---------|
| n              | 216  | 218    | 171    | 215    | 160    | 209    | 163    | 215    |         |
| Male, No. (%)  | 97 (44.9) | 103 (47.2) | 86 (50.3) | 102 (47.4) | 75 (46.9) | 96 (45.9) | 87 (53.4) | 101 (47.0) | 0.823 |
| Age, year      | 65.5 ± 8.1 | 63.9 ± 7.7 | 65.1 ± 7.6 | 65.0 ± 7.9 | 64.5 ± 8.1 | 64.6 ± 7.4 | 64.0 ± 9.0 | 64.8 ± 9.0 | 0.527 |
| BMI, kg/m²     | 24.9 ± 6.9 | 24.9 ± 3.5 | 25.3 ± 1.1 | 24.9 ± 3.5 | 24.2 ± 3.5 | 24.4 ± 3.4 | 24.7 ± 3.2 | 24.6 ± 3.4 | 0.655 |
| SBP at baseline, mmHg | 153.9 ± 17.6 | 152.0 ± 17.4 | 152.8 ± 18.8 | 152.3 ± 15.8 | 154.7 ± 17.2 | 154.3 ± 16.6 | 153.9 ± 18.5 | 152.1 ± 17.4 | 0.635 |
| DBP at baseline, mmHg | 90.1 ± 10.1 | 90.1 ± 10.9 | 89.9 ± 11.5 | 89.9 ± 9.6 | 89.9 ± 10.1 | 90.7 ± 10.5 | 90.5 ± 11.0 | 90.1 ± 11.0 | 0.993 |
| Current smoking, No. (%) | 45 (20.8) | 51 (23.4) | 31 (18.1) | 58 (27.0) | 38 (23.8) | 45 (21.5) | 36 (22.1) | 47 (21.9) | 0.645 |
| Current drinking, No. (%) | 50 (23.3) | 60 (27.5) | 37 (21.6) | 52 (24.2) | 36 (22.5) | 45 (21.5) | 34 (20.9) | 46 (21.4) | 0.792 |

Laboratory results

| tHcy, μmol/L | 14.4 (11.8–17.8) | 14.3 (12.3–17.2) | 14.6 (11.8–17.6) | 14.1 (12.1–17.9) | 13.2 (11.2–17.3) | 14.5 (11.9–17.5) | 14.3 (11.9–17.4) | 14.5 (12.2–17.4) | 0.884 |
| Folate, ng/ml | 12.3 (7.8–16.9) | 10.6 (7.2–16.6) | 11.2 (8.0–16.3) | 10.4 (7.1–14.5) | 12.4 (8.4–17.8) | 10.9 (7.7–16.4) | 11.3 (8.4–17.3) | 11.1 (7.3–16.5) | 0.311 |
| SAM, nmol/L | 71.9 (56.1–86.3) | 71.7 (55.4–86.6) | 81.0 (68.8–96.5) | 70.3 (56.8–89.8) | 83.0 (73.0–100.8) | 72.6 (54.8–87.4) | 84.5 (69.2–99.9) | 71.0 (57.1–89.1) | <0.001 |
| SAH, nmol/L | 39.4 (31.3–45.0) | 39.7 (30.2–44.3) | 33.8 (29.0–41.7) | 37.0 (31.5–44.3) | 33.2 (27.5–40.9) | 38.1 (30.9–44.6) | 34.9 (28.2–43.1) | 37.6 (31.6–44.8) | <0.001 |
| MTHFR genotypes, No. (%) | | | | | | | | | 0.861 |
| CC | 64 (29.6) | 64 (29.4) | 55 (32.2) | 67 (31.2) | 53 (33.1) | 57 (27.3) | 48 (29.4) | 75 (34.9) | |
| CT | 105 (48.6) | 105 (48.2) | 81 (47.4) | 98 (45.6) | 70 (43.8) | 96 (45.9) | 80 (49.1) | 85 (39.5) | |
| TT | 47 (21.8) | 49 (22.5) | 35 (20.5) | 50 (23.3) | 37 (23.1) | 56 (26.8) | 35 (21.5) | 55 (25.6) | |
| Center, No. (%) | | | | | | | | | 0.194 |
| Anqiong | 42 (19.4) | 39 (17.9) | 32 (18.7) | 42 (19.5) | 30 (18.8) | 42 (20.1) | 28 (17.2) | 48 (22.3) | |
| Lianyangang | 73 (33.8) | 79 (36.2) | 49 (28.7) | 77 (35.8) | 43 (26.9) | 76 (36.4) | 40 (24.5) | 73 (34.0) | |
| Wuyuan | 101 (46.8) | 100 (45.9) | 90 (52.6) | 96 (44.7) | 87 (54.4) | 91 (43.5) | 95 (58.3) | 94 (43.7) | |

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; tHcy, total homocysteine; SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine.

Another study in MCI patients who were given 1.25 mg/day folic acid for 6 months also found no effect on SAH levels.³⁷ Pizzolo et al.⁵⁰ observed seven patients with MTHFR C677T TT genotype in hyperhomocysteinemia (tHcy >30 μmol/L), supplemented with 5 mg/day folic acid for eight weeks, and found no significant effect on SAH level. However, this study has certain limitations. Firstly, the sample size of this study was too small, with only seven cases. Secondly, although the research object of this study was patients with hyperhomocysteinemia with MTHFR C677T TT genotype, it did not involve CT and TT genotypes. Moreover, two randomized controlled trials also found no significant effect of vitamin B supplementation on SAH levels.⁶² It is worth noting that most of the previous studies were carried out among people with cognitive impairment, and there may be no study on hypertension population, although the hypertension population may need folic acid supplement.

This study explored the effects of different doses of folic acid supplementation on SAH in hypertensive population and proposed new insights. We found that compared with the control group, SAH level did not change significantly with supplementation of 0.4–2.0 mg/day folic acid, but SAH level increased significantly with 2.4 mg/day folic acid supplementation. Stratified analysis found that SAH increased not significantly in MTHFR C677T genotype CC with supplement 2.4 mg/day folic acid, while SAH increased significantly in CT and TT type. The following mechanism may explain. Interference with the efficient clearance of hcy and adenosine, the products of SAH hydrolysis, can lead to increased SAH.⁶¹,²¹ In addition, it has been reported that high levels of unmetabolized folic acid can inhibit the activity of MTHFR.²³ In this study, after 8 weeks of folic acid supplementation with 2.4 mg/day, the level of unmetabolized folic acid was relatively high (53.3 ± 44.5 ng/ml), which
may inhibit the activity of MTHFR and limit the synthesis of 5-methyltetrahydrofolate. Thus, the metabolic pathway of re-methylation of hcy and 5-methyltetrahydrofolate to methionine is limited, and hcy cannot be effectively eliminated, which eventually leads to the increase of its precursor SAH. Furthermore, the MTHFR activity of CT and TT genotypes of MTHFR C677T was impaired due to gene mutation, combined with the inhibitory effect of unmetabolized folic acid on the activity of MTHFR, so that the effect of elevated SAH level may be more significant.

SAH is the product of methylation reaction of SAM as a methyl donor, which can affect the methylation reaction of the body, and thus may affect the occurrence and development of some diseases. In addition, SAH may be better than homocysteine in predicting cardiovascular diseases. Liu et al.\(^{(24)}\) have shown that plasma SAH level is a more sensitive biomarker of atherosclerosis than homocysteine and is related to DNA hypomethylation in hyperhomocysteinemia mice. Several cross-sectional and case-control studies have also shown that plasma SAH may be a better indicator of cardiovascular disease than homocysteine.\(^{(25-27)}\)

The increase of SAH level will bring a series of hazards. Firstly, the increase of SAH level inhibits SAM-dependent methyl transfer reaction and leads to DNA hypomethylation. SAM is a key carbon donor in almost all biological methylation reactions.\(^{(23)}\) SAH, as the product of SAM methyl transfer reaction of methyl donor, has higher affinity with the active site of cell methyltransferase and is easier to combine with it than SAM. Therefore, SAH is an effective product inhibitor of SAM-dependent methyltransferase.\(^{(21,22)}\) The ratio of SAM to SAH is often used as an indicator of cell methylation potential, and SAH concentration may be a more sensitive biomarker for cell methylation status. An animal experiment showed that the decrease of SAM alone and the decrease of SAM/SAH ratio caused by SAM deficiency alone were not enough to affect DNA methylation. The increase of SAH, whether alone or related to the decrease of SAM, was closely related to DNA hypomethylation. The decrease of SAM/SAH ratio only predicted the decrease of methylation ability when related to the increase of SAH.\(^{(29)}\)

Secondly, elevated SAH may promote atherosclerosis and increase the risk of cardiovascular disease independently of homocysteine. In 2012, Luo et al.\(^{(30)}\) found that elevated SAH...
levels may promote early atherosclerosis through oxidative stress. In 2015, Xiao et al. proposed that the indirect mechanism of homocysteine toxicity was secondary to the accumulation of SAH. High SAH levels increased the risk of cardiovascular disease independently of homocysteine. Therefore, excessive folic acid supplementation may be harmful.

Several potential limitations of our research should be noted. First of all, our intervention time was eight weeks. The intervention time may be relatively short and the effect of folic acid supplementation on SAH may not be fully demonstrated within eight weeks. Secondly, our maximum dose of folic acid supplementation is 2.4 mg/day. The effect of folic acid supplementation above 2.4 mg/day on SAH level is not clear, which needs further exploration.

In conclusion, this study explored the effects of different doses of folic acid supplementation on SAH in hypertensive patients. For the first time, it was found that high-dose folic acid supplementation (2.4 mg/day) increased SAH levels, especially in MTHFR C677T genotypes CT and TT. Longer and larger doses of folic acid randomized, double-blind, placebo-controlled trials are required.

Author Contributions

R-SZ: analysis and interpretation of data, drafting of the manuscript; G-FT, YZ, X-LS, JS, and LW: study concept and design, administrative, technical, or material support; LT, interpretation of data, revised the manuscript for important intellectual content; XZ, Y-PX, J-FZ, RW, and HC: provided a critical review of the content of the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

No potential conflicts of interest were disclosed.
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