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The effects of high-dose folic acid on blood pressure of hypertensive adults with hyperhomocysteinemia: A randomized double- blind placebo controlled clinical trial (Tehran Homocysteine Survey)

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Abstract

Background: An elevated homocysteine concentration is associated with increased risk of hypertension. Although both low- and high-dose folate reduce blood homocysteine levels significantly, the relative effects of different doses of folate on blood pressure is not well established yet. In this study we investigated the effects of administrating high-dose folic acid on homocysteine and blood pressure of hyperhomocysteinemic persons.

Methods: Forty-two individuals with hypertension and moderate/intermediate hyperhomocysteinemia received either 5 mg/day folic acid or placebo for six weeks. Baseline and 6th week blood pressures, homocysteine, folate and Vitamin B12 levels were measured.

Results: Systolic blood pressure reduced significantly while decrease in diastolic blood pressure was not significant in folic acid compared to the placebo group (P=0.001 and P=0.17, respectively; ANOVA). Homocysteine decreased while folate increased significantly in folic acid relative to placebo group (P=0.04 and P=0.002, respectively; ANOVA]. Changes in folic acid concentrations were significantly and negatively correlated with changes in systolic blood pressure (P=0.02, r = -0.51, Pearson's correlation coefficient).

Conclusion: Short-term high-dose supplementation with folic acid is effective in reduction of systolic blood pressure in hypertensive hyperhomocysteinemic subjects.

Keywords: Folic acid, Hypertension, Homocysteine, Blood Pressure

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Introduction

Hypertension is an important public health challenge and the leading mortality risk factor worldwide (1-5). About 80% of the burden of blood pressure-attributable disease occurs in low- and middle-income economies (1). The incidence of hypertension has increased in recent decades in Iran and we are currently facing a high prevalence of hypertension (30%) among Iranian adults (6).

Concurrently, cardiovascular events such as myocardial infarction and stroke are on escalating trend in our country (7).

Various genetic and environmental factors are involved in the pathogenesis of hypertension (8). These factors mainly do their effects through injury to endothelium or altering its function (9).

Elevated plasma homocysteine concentration is considered as a risk factor for cardiovascular disease and is associated with hypertension (10-12). Epidemiological studies have shown that each 5 µmol/l increase in plasma homocysteine is associated with an increase in systolic and diastolic blood pressure of 0.7/0.5 mmHg in men and 1.2/0.7 mmHg in women, independent of renal function and vitamin B status (13).

The hypothesis of homocysteine accumulation in the vascular endothelium resulting in endothelial injury via oxidative stress has been an incentive for conducting several investigations on the interrelation between folate, homocysteine and endothelial dysfunct-ion (14-17); but, there have been few studies regarding the effect of different doses of folic acid supplementation in hypertensive hyperhomocysteinemic subjects. The present study was conducted to investigate the effects of high dose folate supplementation on blood pressure in a group of hypertensive volunteers with moderate/ intermediate yperhomocysteinemia.

Methods

Participants

The Tehran Homocysteine Survey was a population based study performed on 1214 adults and apparently healthy residents of downtown Tehran (428 men and 786 women of 25-64 years old) in 2003-2004. Details of this study have been described elsewhere (18, 19). The present trial began in October 2006. Baseline blood pressure, physical examination and blood sampling for laboratory measurements were performed in the beginning and at the end of our study. Hypertension was determined according to JNC-7 criteria (20). For this purpose, blood pressure were determined in seated position after at least 5 minutes of relaxed and arm supported at heart level. An appropriate-sized cuff encircling 80 percent of the arm was used. At least two discrete measurements performed with 2 weeks interval. Twenty-three persons were initially excluded due to incomplete available data. Of remained 1191 participants, 120 men (29%) and 345 women (34.8%) were hypertensive. Of these 465 subjects, 205 had moderate/intermediate hyperhomocysteinemia and addressed as eligible subjects for our trial (21). In adults, the normal plasma total homocysteine (tHcy) level is 5-15 µmol/L, with a mean concentration of about 10 µmol/L. Hyperhomocysteinemia is defined as a plasma tHcy>15 µmol/L, and is denoted moderate (15-30 µmol/L), intermediate (30-100 µmol/L) or severe hyperhomocysteinemia (>100 µmol/L) (22).

Fifty-two person of the eligible ones were not inclined to take part in the trial and we did not have access to 78 others due to migration; another 33 were excluded due to uncontrolled severe hypertension (diastolic blood pressure ≥105 mmHg), hypertensive complications such as impaired renal function (serum creatinine >150 mmol/l), secondary hypertension, alcohol consumption, cigarette smoking, depression and non-compliance to therapy.

Finally, 42 hypertensive individuals (23 men and 19 women) with moderate/intermediate hyperhomocysteinemia (15.7-42 µmol/L) were participated in this trial (22).

Study protocol

This study was a randomized, double blind placebo-controlled trial. Randomization of participants to treatment groups was done after all eligible ones had been identified for screening. All of the participants were in the hypertensive range at the time of baseline measurement after randomization. They were

asked to take either 5 mg/day folic acid or placebo for 6 weeks. The pharmacist prepared placebo capsules which contained a blend of microcrystalline cellulose as a filler and di calcium phosphate; which had the same appearance and taste to folate capsules. The treatment assignment was performed by the same pharmacist. Investigators and participants were blinded to randomization code. The participants were asked to continue their routine dietary intake during the trial. All participants gave written informed consent. Research protocol was approved by the ethics committee of Endocrinology and Metabolism Research Center of the Tehran University of Medical Sciences and study was conducted according to the Declaration of Helsinki.

At baseline a general questionnaire containing data on antihypertensive medicate-ons, cigarette smoking status and history of high blood pressure was collected by a trained nurse. Weight and height were measured with estimation of 0.1 kg and 0.1 cm, respectively using an electronic scale and a stadiometer. BMI was calculated as weight (kg) divided to height squared (m^2).

Baseline and 6^{th} week blood pressures of participants were measured in a seated position using a standard mercury column sphygmomanometer with appropriate cuff size. Blood samples were collected at baseline and at 6^{th} week after an overnight fasting in evacuated tubes containing 0.1% EDTA and were immediately placed on ice. Within 2 hours of collection, all samples were centrifuged at 1800 g for 20 minutes. After centrifugation, the plasma samples were divided into aliquots in microtubes and stored at $-80^{°C}$ for Hcy measurement. Plasma total homocysteine concentration (sum of homocysteine and homocysteine-cysteine mixed disulfides, free and protein bound) was determined on frozen samples by high performance liquid chromatography (HPLC) with fluorometric detection. Serum folic acid and vitamin B12 were measured by radioimmunoassay kit, Simultrac (ICN Pharmaceuticals, 2007). Analysis of serum folate and B12 levels were undertaken at the same day to minimize the changes between samples.

Statistical analysis

Data are expressed as mean±SD. Normality of variables is tested by Kolmogrov-Smirnov test. Values of total homocysteine, folate and vitamin B12 were log-transformed (ln) to normalize their right- skewed distribution. A repeated measures analysis of variance (ANOVA) was performed (SPSS v. 17 Inc., Chicago, Illinois, USA) to determine the significance of differences of initial and final biochemical variables and blood pressures between folate versus placebo groups. Correlation between changes in folic acid, homocysteine and blood pressure values was performed by calculating Pearson's correlation coefficient. Multivariate analysis was used to investigate the effects of other variables on the means of systolic and diastolic blood pressure. P-value less than 0.05 considered as statistical significance.

Results

Total number of 42 eligible persons was recruited who 19 subjects from placebo and 18 subjects from folic acid group completed the study. At baseline, there was no significant difference between two groups (Table 1). Four persons in the placebo group and four persons in the 5 mg folic acid group reported using antihypertensive drugs. The type and dosage of these drugs were not altered during the study period. Although diastolic blood pressure decreased in the folate group after 6 week, the difference score of diastolic blood pressures between folate and placebo groups did not reach to the level of significance (P= 0.17); However, the difference score of systolic blood pressures were significantly lower (P=0.001) in folate compared to placebo group (Table 2).

The difference score of homocysteine reduction and folate increase were significant in folic acid relative to placebo group (P=0.04 and P=0.002, respectively) (Table 2). Changes in folic acid concentrations were significantly and negatively correlated with changes in systolic blood pressure (P=0.02, r = -0.51, Pearson's correlation coefficient).

The difference scores of homocysteine were also significantly lower (P=0.008) in folate compared to placebo group; while, those of vitamin B12 were not significantly different between the two groups (P= 0.67).

Table 1- Baseline clinical and biochemical characteristics of the study subjects [a]

	Placebo (n=19)	Folic acid (n=18)
Age (years) [b]	41±2	42±3
Male [b]	6 (16%)	4 (11%)
Female [b]	13 (35%)	14 (38%)
BMI (kg/m^2) [b]	30± 4	29± 9
Plasma homocysteine (µmol/L) [b]	29.6± 5.6	30±5.7
Serum folate (ng/ml) [b]	7.6± 4.2	7.1± 3.1
Serum B_{12}(pg/ml) [b]	309.3 ± 103.5	288±101.8
Systolic blood pressure [b] **(mmHg)**	146 ± 17	148 ± 19
diastolic blood pressure [b] **(mmHg)**	92 ± 8	94 ± 9

a. data are presented as mean±SD
b. difference between placebo and folate groups were not significant

Table 2- baseline and post-intervention values along with difference scores in the serum homocysteine, folate, vitamin B12 and blood pressures after 6 weeks of 5 mg/d folate supplementation compared to placebo*

	Placebo	5 mg/d Folic acid
Homocysteine(µmol/L)§		
Initial	29.6 ± 1.6	30.53 ± 1.74
Final	26.1 ± 2.1 †	24.02 ± 1.51
Difference	3.46 ± 0.64-	6.50 ± 0.18‡-
Folic acid(ng/ml)§		
Initial	7.6± 4.2	7.1 ± 3.1
Final	10.9 ± 3.5	25.3 ± 3.1
Difference	3.26 ± 0.63	18.17 ± 0.09‡
Vitamin B12(pg/ml)§		
nitial	309.3 ± 83.5	288± 69.1
Final	295.6 ± 79.4	301.9 ± 74.3
Difference	14.26 ± 4.08-	13.80 ± 5.12
Systolic Blood Pressure (mmHg)		
Initial	146.3 ± 17.2	148.2 ± 14.3
Final	143± 17.1	128.1 ± 12.6
Difference	3.30 ± 0.1-	20.10 ± 1.70‡-
Diastolic Blood Pressure (mmHg)		
Initial	92.6± 6.5	94.7± 9.3
Final	89.1± 9.3	86.5 ± 10.4†
Difference	3.50 ± 0.63-	-8.20 ± 1.20

* Data are presented as Mean ±SD
§ Values are log-transformed
‡ P-value < 0.05 (ANOVA)
† P-value < 0.05 (paired t-test)

Discussion

In this study, we found that supplementation with 5 mg/day folic acid for 6 weeks led to significant reduction of systolic blood pressure in hypertensive subjects with moderate and intermediate hyperhomocysteinemia. Our findings are in accordance with three previous trials on this issue (21, 23, 24).

In one study, 24 healthy cigarette smokers consumed 5 mg folate/day or placebo for 4 weeks. This supplementation significantly enhanced endothelial function as measured by endothelium-dependent vasodilatation and reduced systolic and diastolic blood pressure (23). An earlier study by the same author in a group of elderly people with previous myocardial infarction or stroke who were supplied with 5 mg/day folic acid for 6 weeks had also shown significant reduction of systolic and pulse pressures after inducing a significant reduction of homocysteine to creatine ratio (24).

Another study showed that homocysteine-lowering treatment with 5 mg folic acid plus 250 mg pyridoxine over a 2-year period was associated with significant reduction of systolic and diastolic blood pressures in 158 clinically healthy siblings of patients with premature atherothrombotic disease (25). A recent study also found folic acid is a safe and

effective supplement to reduce homocysteine and blood pressure in children (26).
However, another study comparing the effect of daily supplementation with 1 mg folic acid plus 1500 μg vitamin B12 and 10 mg vitamin B6 with placebo in 276 healthy elderly subjects for 2 years found that systolic and diastolic blood pressures did not differ in the vitamin versus placebo group over the duration of the study (27). Also, in a large population-based study by VISP (Vitamin Intervention for Stroke Prevention) investigators, vitamin B supplementation with a formulation containing 25 mg pyridoxine, 0.4 mg cobalamin, and 2.5 mg folic acid had no significant effect on systolic and diastolic blood pressures over 2 years in subjects with a prior history of stroke (28). So, the blood pressure lowering effect of group B vitamins was not apparent in these two latter studies in which lower doses of folic acid were administered to the participants.
An important feature of studies which have demonstrated the effectiveness of folate in improving endothelial function and/or reducing blood pressure is the high folic acid dose (≥5 mg/day) used in these trials; While, low-dose (≤2.5 mg/day) folic acid is not effective in reducing blood pressure (27, 28) or cardiovascular morbidity and mortality (28, 29); higher doses of folic acid (≥5mg/day) can produce significant improvement in endothelial function in the absence of further homocysteine reduction (30-33).
In fact, several studies have demonstrated effectiveness of high-dose (≥ 5mg/day) folate supplementation in improvement of endothelial dysfunction (30-36). An well-designed study on 84 patients with coronary artery disease found that improvement in endothelial function as measured by brachial artery flow mediated dilatation was only achieved with high dose (5 mg/day) folic acid (15). This effect was largely independent of homocysteine reduction by folate. They showed a concentration dependent increase in the eNOS homodimer formation in the culture of porcine aortic endothelial cells by folic acid and suggested that promotion of stable nitric oxide forming dimer may underlie the beneficial effect of high dose folate; but not its anti-oxidant activity, and suggested that homocysteine may be considered as a marker rather than a risk factor for vascular disease.
Considerable homocysteine reduction in the placebo group (P=0.047 in paired t-test) may be related to increment of serum folate - although with non-significant difference - in these participants even without supplementation with folic acid capsules. This might be due to increased consumption of folate through their diet, as folate appears to be the most important dietary determinant of blood homocysteine concentration (37, 38). A performed meta-analysis reported that folic acid supplementation, irrespective of its daily dose between 0.5 and 5 mg, reduced homocysteine concentration significantly (37). Over the period of present study, food were not fortified with folic acid; so, the source of increased total folate intake in the placebo group may attribute to natural dietary sources such as increased intake of fruits and vegetables (39). Tries on life-style changes through mass media and educational activities of health professionals may have been effective in increased dietary consumption of folate containing food.
One of the limitations of this study was that dietary data were not available to examine whether changes in these endpoints were associated with dietary pattern of participants.
In conclusion, this study provides evidence that high- but not low- dose folic acid supplementation should be used to provide effective improvement in vascular endothelial function and reduce blood pressure. Further well-designed trials in this regard and deeper quest into the mechanism of beneficial effect of high-dose folic acid is of utmost importance because of the high burden of cardiovascular disease in urban societies and the potential beneficial effects of folate as a relatively safe and inexpensive therapeutic agent in prevention of vascular damage.

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