Original Article

Effects of Folate Supplementation on Carotid Intima-Media Thickness, Biomarkers of Inflammation, and Oxidative Stress in Carbamazepine-Treated Epileptic Children

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Abstract

Background: This study was conducted to assess the effects of folate supplementation on carotid intima-media thickness (CIMT), biomarkers of inflammation, and oxidative stress in carbamazepine-treated epileptic children. **Methods:** This randomized, double-blind, placebo-controlled trial was carried out in 54 epileptic children aged 2–12 years old receiving carbamazepine monotherapy. Participants were randomly allocated into two groups to receive either 5 mg folate supplements or placebo (n = 27 in each group) for 12 weeks. **Results:** After the 12-week intervention, compared with the placebo, folate supplementation resulted in a significant reduction in plasma homocysteine (Hcy) (changes from baseline − 2.1 ± 2.5 vs. +0.1 ± 0.4 μmol/L, $P < 0.001$), serum high-sensitivity C-reactive protein (hs-CRP) (changes from baseline − 1.5 ± 3.5 vs. +0.4 ± 1.4 mg/L, $P = 0.01$), a significant increase in plasma nitric oxide (NO) (changes from baseline + 1.9 ± 5.8 vs. −2.0 ± 6.4 μmol/L, $P = 0.02$), and total antioxidant capacity (TAC) concentrations (changes from baseline + 88.6 ± 116.0 vs. +1.8 ± 77.4 mmol/L, $P = 0.002$). We did not observe any significant effects in mean levels of left and right CIMT, maximum levels of left and right CIMT, and total glutathione (GSH) and malondialdehyde (MDA) levels following the supplementation of folate compared with the placebo. **Conclusions:** Overall, folate supplementation at a dosage of 5 mg/day for 12 weeks among epileptic children receiving carbamazepine had beneficial effects on Hcy, hs-CRP, NO, and TAC levels, but did not affect CIMT, and GSH and MDA levels.

Keywords: *Carotid intima-media thickness, epilepsy, folate, inflammation, oxidative stress*

Introduction

Epilepsy is defined as a disorder of the brain characterized by an enduring predisposition to generate epileptic seizures which predominantly affects children and young adults.[1] The prevalence of epilepsy is 9.9 to 41 per 100,000/year.[2] Epilepsy can be due to genetic and acquired reasons with interaction of these factors in many cases.[3] Subjects with epilepsy have vascular risk factors, including insulin resistance, elevated biomarkers of inflammation, oxidative stress, and elevated levels of plasma homocysteine (Hcy).[4,5] In addition, elevated Hcy levels, a risk factor for atherosclerosis, is common in subjects on long-term antiepileptic drugs (AEDs) therapy due to vitamin B deficiencies, particularly folate and vitamin B6.[6]

Carotid intima-media thickness (CIMT), used as an early marker for atherosclerosis, was evaluated in different age groups of epileptic subjects on AEDs. The results of CIMT in epileptic children treated with old generation AEDs were reported inconsistent,[7,8] while adults showed significant increase in IMT compared with controls.[9] In addition, few studies have reported that folate supplementation may benefit on CIMT. In a meta-analysis conducted by Qin *et al*.,[10] it was observed that folate administration to subjects with high cardiovascular disease (CVD) risk and among studies with baseline levels of higher CIMT or a larger Hcy reduction was effective in reducing progression of CIMT. Furthermore, we have previously demonstrated that folate supplementation at a dosage of 5 mg/day to subjects with metabolic syndrome (MetS) had beneficial effects on CIMT and few metabolic profiles.[11] Folate administration at a dosage of 400 μg/day among subjects with coronary artery diseases (CAD) for 7 weeks improved vascular function

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through effects on endothelial nitric oxide (NO) synthase and vascular oxidative stress.[12] However, vitamin B12 (500 μg/day) and folic acid (400 μg/day) supplementation to elderly subjects with hyperhomocysteinemia for 2 years did not affect either endothelial function or low-grade systemic inflammation.[13]

According to existing evidence, folate may improve CIMT, biomarkers of inflammation, oxidative stress through Hcy-linked effects,[14] and decreased production of reactive oxygen species.[15] As Hcy concentrations are the cornerstone of this condition, folate supplementation through influencing Hcy levels might be useful in the management of CIMT and metabolic profiles of children receiving carbamazepine. However, whether folate supplementation has direct benefits on CIMT, biomarkers of inflammation, and oxidative stress in epileptic children receiving carbamazepine has to date not been evaluated. The current study was, therefore, conducted to determine the effects of folate supplementation on CIMT, biomarkers of inflammation, and oxidative stress in epileptic children receiving carbamazepine.

Methods

Trial design and participants

This randomized, double-blind, placebo-controlled clinical trial, registered in the Iranian website for registration of clinical trials (http://www.irct.ir) as IRCT201704225623N110, was conducted on 54 epileptic children aged 2–12 years old receiving carbamazepine monotherapy from March 2015 to December 2016 who were referred to the Naghavi Hospital in Kashan, Iran. This trial was performed in accordance with the Declaration of Helsinki and informed consent was taken from all patient's parents. The ethical committee of the Kashan University of Medical Sciences (KAUMS) approved the study (no. 1393.6011). Taking folate supplements or other therapies such as vitamins or antioxidants within the last 3 months, unwillingness to cooperate, metabolic diseases, chronic medical conditions including anemia and gastrointestinal disease associated with malabsorption, neuropsychological diseases, and neurodegenerative disorders were excluded from the study.

Study design

At first, to decrease potential confounding effects, all participants were stratified randomization according to age and body mass index (BMI). Then, participants in each block were randomly allocated into two treatment groups (27 participants in each group) to take either 5 mg/day folate as tablet (Tehran Darou, Tehran, Iran) or placebo (Barij Essence, Kashan, Iran) for 12 weeks. Both folate supplements and placebo tablets had similar packaging. Compliance to the folate intake was evaluated through quantification of plasma Hcy values. The use of folate supplementation and the placebo during the study was checked by asking subjects to return the medication containers and receiving brief daily cell phone reminders to take the supplements. All participant's parents completed three-dietary records (two week days and one weekend) at baseline, Week 3, 8, and 11 of the trial. To obtain nutrient intakes of patients according to 3-day food records, we applied the Nutritionist IV software (First Databank, San Bruno, CA) adopted for the Iranian food pattern.

Randomization

Randomization assignment was conducted using a computer-generated random numbers. Randomization and allocation concealment were conducted by the researchers and participants and were carried out by a trained staff at the pediatrics clinic. Another person, who was not involved in the trial and not aware of random sequences, assigned the subjects to the numbered bottles of tablets.

Assessment of anthropometric measures

Weight (kg) and height (cm) (Seca, Hamburg, Germany) were determined at baseline and after the intervention without shoes in a minimal clothing condition in the pediatrics clinic by a trained staff. BMI was calculated as weight in kilograms divided by height in meters squared.

Outcomes

We considered CIMT as primary outcome variable and biomarkers of inflammation and oxidative stress as secondary outcome variables.

Clinical assessment

Measurement of the CIMT levels was conducted in the participants at the 2-cm distance of the common carotid bifurcation, by the same sonographist, at baseline and after the 12-week intervention using a Doppler ultrasonography device (Samsung Medison V20, Korea) with linear multifrequencies of 7.5- to 10-MHz probe. Automated systems provide the mean value of 150 measurements performed on a 10-mm segment of common carotid artery instantaneously. The physician was blinded to any clinical information of the subjects.

Biochemical assessment

About 5 ml blood samples were collected at baseline and after the intervention at the Kashan Reference Laboratory. Plasma Hcy values were assessed by the method of enzyme immunoassay by Hcy kit (Axis-Shield Diagnostics, UK). Serum hs-CRP levels were determined by an enzyme-linked immunosorbent assay (ELISA) kit (LDN, Nordhorn, Germany) with intra- and inter-assay coefficient variances (CVs) of 3.1 and 5.5%, respectively. The plasma NO values were assessed using the Griess method.[16] Plasma total antioxidant capacity (TAC) levels were evaluated by the use of ferric reducing antioxidant power developed by Benzie and Strain,[17] total

glutathione (GSH) by the method of Beutler and Gelbart,[18] and malondialdehyde (MDA) concentrations by the thiobarbituric acid reactive substances spectrophotometric test[19] with inter- and intra-assay CVs below 5%.

Statistical methods

To determine the normal distribution of the variables, we applied the Kolmogorov–Smirnov test. The intention-to-treat (ITT) analysis of the primary study endpoint was applied for all of the randomly allocated participants. Anthropometric measures and dietary intakes were compared between intervention groups, using the independent samples *t*-test. To determine the effects of folate supplementation on CIMT, biomarkers of inflammation, and oxidative stress, we used the independent samples *t*-test. To control confounding variables, including baseline values, age, and baseline BMI, we used analysis of covariance (ANCOVA). Differences in proportions were evaluated by the Fisher's exact test. *P* values <0.05 were considered statistically significant. All statistical analyses were done by the use of the Statistical Package for Social Science version 18 (SPSS Inc., Chicago, Illinois, USA).

To determine sample size, we used a randomized clinical trial sample size calculation formula where type one (α) and type two error (β) were 0.05 and 0.20 (power = 80%), respectively. According to a previous trial,[20] we used 0.006 mm as SD and 0.005 mm as the change in mean (d) of CIMT as a main variable. Based on the formula, we needed 23 participants in each group; after considering of 4 dropouts in each group, the final sample size was 27 participants in each group.

Results

Among patients in folate and placebo groups, two subjects withdrawn due to personal reasons [Figure 1]. However, all 54 participants (27 participants in each group) were included in the final analysis using ITT principle. Overall, the compliance rate was high, such that higher than 90% of tablets were consumed throughout the study in both groups.

The mean age, height, baseline weight, BMI, as well as their means after the 12-week intervention were not significant between folate supplements and placebo groups [Table 1].

Based on the 3-day dietary records obtained at baseline, Week 3, 8, and 11 of the study, we observed no significant difference in mean dietary macro- and micronutrient intakes between the two groups (data not shown).

After the 12-week intervention, compared with the placebo, folate supplementation resulted in a significant reduction in plasma Hcy (changes from baseline −2.1 ± 2.5 vs. +0.1 ± 0.4 μmol/L, $P < 0.001$), serum hs-CRP (changes from baseline −1.5 ± 3.5 vs. +0.4 ± 1.4 mg/L, $P = 0.01$), significant increases in NO (changes from baseline +1.9 ± 5.8 vs. −2.0 ± 6.4 μmol/L, $P = 0.02$), and TAC concentrations (changes from baseline +88.6 ± 116.0 vs. +1.8 ± 77.4 mmol/L, $P = 0.002$) [Table 2]. We did not observe any significant effects in mean levels of left and right CIMT, maximum levels of left and right CIMT, and GSH and MDA levels following the supplementation of folate compared with the placebo.

There was a significant difference in baseline levels of maximum of left CIMT ($P = 0.01$), mean of left CIMT ($P = 0.04$), and plasma TAC ($P < 0.001$) between the two groups. Therefore, we adjusted the analysis for baseline values of biochemical parameters, age, and baseline BMI. When we adjusted the analysis for baseline values of biochemical parameters, age and baseline BMI, findings did not change [Table 3].

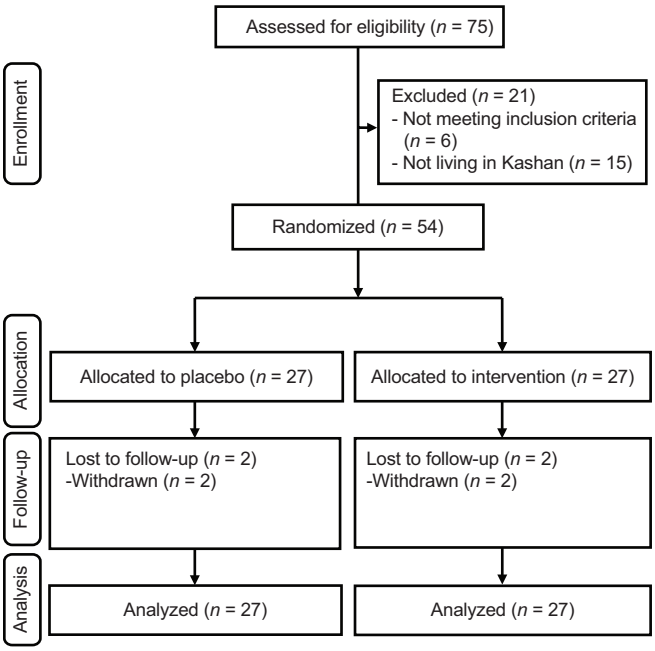

Figure 1: Summary of patient flow diagram

Table 1: General characteristics of study participants

	Placebo group (*n*=27)	Folate group (*n*=27)	*P*[1]
Gender			
Male	19 (70.4)	18 (66.7)	0.77[†]
Female	8 (29.6)	9 (33.3)	
Age (years)	8.7±1.5	9.4±2.1	0.16
Height (cm)	131.1±8.2	135.1±11.0	0.13
Weight at study baseline (kg)	28.9±7.4	32.4±10.5	0.16
Weight at end-of-trial (kg)	29.0±7.4	32.6±10.4	0.14
Weight change (kg)	0.1±0.4	0.2±0.3	0.19
BMI at study baseline (kg/m^2)	16.6±2.7	17.2±3.3	0.43
BMI at end-of-trial (kg/m^2)	16.5±2.7	17.4±3.3	0.37
BMI change (kg/m^2)	0.1±0.2	0.1±0.2	0.18

Data are means±SDs. [1]Obtained from the independent samples *t*-test, [†]Obtained from the Fisher's exact test

Table 2: Carotid intima-media thickness, biomarkers of inflammation, and oxidative stress at baseline and after the 12-week intervention in children receiving carbamazepine who received folate supplements or placebo

	Placebo group (*n*=27)			Folate group (*n*=27)			*P*[1]	*P*[2]
	Baseline	End-of-trial	Change	Baseline	End-of-trial	Change		
Maximum left CIMT (mm)	0.58±0.06	0.59±0.06	0.007±0.05	0.62±0.05	0.63±0.05	0.002±0.04	0.01	0.74
Mean left CIMT (mm)	0.48±0.05	0.49±0.04	0.01±0.04	0.51±0.05	0.52±0.05	0.004±0.03	0.04	0.61
Maximum right CIMT (mm)	0.59±0.06	0.58±0.06	−0.006±0.03	0.61±0.05	0.60±0.06	−0.01±0.05	0.27	0.84
Mean right CIMT (mm)	0.50±0.06	0.50±0.06	0.003±0.03	0.50±0.05	0.50±0.05	−0.004±0.03	0.89	0.37
Hcy (μmol/L)	16.3±2.9	16.4±2.9	0.1±0.4	16.1±3.1	14.0±3.0	−2.1±2.5	0.79	<0.001
hs-CRP (mg/L)	6.0±4.2	6.4±4.3	0.4±1.4	6.3±4.7	4.8±4.3	−1.5±3.5	0.79	0.01
NO (μmol/L)	43.5±9.4	41.5±8.5	−2.0±6.4	45.3±5.2	47.2±5.3	1.9±5.8	0.40	0.02
TAC (mmol/L)	733.6±65.0	735.4±67.0	1.8±77.4	850.4±98.3	939.0±111.9	88.6±116.0	<0.001	0.002
GSH (μmol/L)	604.1±79.6	620.6±70.7	16.5±83.1	587.0±98.2	592.3±118.5	5.3±122.6	0.48	0.69
MDA (μmol/L)	2.9±0.2	2.6±0.3	−0.3±0.3	3.1±0.7	2.6±0.3	−0.5±0.8	0.25	0.22

All values are means±SDs. [1]*P* values represent the independent samples *t*-test to compare variables between the two groups in baseline levels, [2]*P* values represent the independent samples *t*-test to compare change in variables between the two groups. CIMT=Carotid intima-media thickness, GSH=Total glutathione, Hcy=Homocysteine, hs-CRP=High-sensitivity C-reactive protein, MDA=Malondialdehyde, NO=Nitric oxide, TAC=Total antioxidant capacity

Table 3: Adjusted changes in metabolic variables in children receiving carbamazepine

	Placebo group (*n*=27)	Folate group (*n*=27)	*P*[1]
Maximum left CIMT (mm)	0.0001±0.046	0.009±0.046	0.49
Mean left CIMT (mm)	0.005±0.036	0.01±0.036	0.57
Maximum right CIMT (mm)	−0.01±0.046	0.001±0.046	0.37
Mean right CIMT (mm)	0.001±0.031	−0.003±0.031	0.63
Hcy (μmol/L)	−0.02±1.5	−2.1±1.5	<0.001
hs-CRP (mg/L)	0.3±2.6	−1.4±2.6	0.01
NO (μmol/L)	−2.2±5.7	2.2±5.7	0.005
TAC (mmol/L)	−31.4±97.2	121.8±97.2	<0.001
GSH (μmol/L)	23.7±90.9	−2.0±90.9	0.30
MDA (μmol/L)	−0.3±0.5	−0.4±0.5	0.70

All values are means±SDs. Values are adjusted for baseline values, age and BMI at baseline, [1]Obtained from ANCOVA. CIMT=Carotid intima-media thickness, GSH=Total glutathione, Hcy=Homocysteine, hs-CRP=High-sensitivity C-reactive protein, MDA=Malondialdehyde, NO=Nitric oxide, TAC=Total antioxidant capacity

Discussion

In the current study, we evaluated the effects of folate supplementation on CIMT, biomarkers of inflammation, and oxidative stress in epileptic children receiving carbamazepine. We demonstrated that 5 mg/day of folate supplementation to epileptic children receiving carbamazepine for 12 weeks had beneficial effects on serum hs-CRP, plasma NO, and TAC levels; however, it did not affect CIMT, and plasma GSH and MDA levels.

We demonstrated that folate supplementation to epileptic children receiving carbamazepine for 12 weeks did not affect CIMT compared with the placebo. Few studies have evaluated the effects of folate supplementation on CIMT. Supporting our study, 3-month supplementation with 5 mg/day of folate had no effect on endothelial function or carotid artery IMT in renal transplant recipients.[21] In addition, treatment of hyperhomocysteinemia in hemodialysis subjects with daily 15 mg folate, 50 mg vitamin B6, and 1 mg vitamin B12 for 6 months significantly decreased Hcy concentrations, but did not affect IMT.[22] However, an 18-month supplementation with 5 mg/day of folate resulted in a significant decrease in IMT in subjects with at least one CVD risk.[23] Furthermore, supplementation with 2.5 mg of folate, 25 mg of vitamin B6, and 0.5 mg of vitamin B12 for 1 year significantly reduced IMT in subjects at risk to cerebral ischemia.[24] CIMT is an intermediate phenotype for early atherosclerosis[25] as well as for CHD and cerebral ischemic events.[24] The different findings of our study with others might be explained by different study designs, different duration of interventions, different dosages of folate used, as well as different participants of the study.

Our study has shown that folate supplementation to epileptic children receiving carbamazepine for 12 weeks resulted in a significant reduction in serum hs-CRP and a significant increase in plasma NO levels compared with the placebo. In a study conducted by Ishikawa *et al*.,[26] it was observed that daily generalized motor seizures resulted in elevated levels of inflammatory cytokines. Chronic increased inflammatory factors have been shown to play important roles in diverse chronic diseases such as CVD and neurological diseases.[27,28] There are some reports on the relationship between folate supplementation and inflammatory markers. We have previously shown that folate supplementation at a dosage of 5 mg/day to subjects with MetS for 12 weeks led to a significant decrease in serum hs-CRP and a significant elevation in plasma NO levels.[11] Furthermore, supplementation with B-group vitamins containing 5 mg/day folate resulted in a significant reduction in CRP concentrations among subjects with acute ischemic stroke for 14 days.[29] However, some research conducted by other authors showed

no effect of folate supplementation on CRP levels in chronic smokers for 4 weeks[30] and in populations with stable CAD for 24 months.[31] Increased levels of CRP are associated with an increased risk of diabetes and CVD.[32,33] Therefore, CRP levels is not only an inflammation marker, but is also considered as a risk indicator for CVD.[34] NO is a vasodilator that exerts antiplatelet, antioxidant, and antiproliferative effects.[35] In addition, endothelium-derived NO has been shown to be of crucial importance in cardiovascular protection.[35] It has been proposed that Hcy induces gene expression related to inflammatory factors, possibly by enhancing oxidative stress and subsequent nuclear factor kappa B activation[36] and increase of poly ADP ribose polymerase activation.[37] Therefore, decreased levels of Hcy may result in decreased concentrations of inflammatory factors.

The current study demonstrated that folate supplementation, compared with the placebo, to epileptic children receiving carbamazepine for 12 weeks was associated with a significant elevation in plasma TAC, but did not affect plasma GSH and MDA levels. The effects of AEDs on generation of oxidative stress have been investigated.[38,39] The membrane lipid peroxidation due to an increase in free radicals or activating decrease of antioxidant defense mechanisms has been suggested to be causally involved in some forms of epilepsy.[40] Racek *et al*.[41] reported that folate had an effective antioxidant role in subjects with hyperhomocysteinemia for 8 weeks due to decreased production of free radicals and Hcy levels. In addition, in an animal study, folate intake reduced intravascular oxidative stress.[42] In a study by Aghamohammadi *et al*.,[43] it was also seen that folate supplementation at a dosage of 5 mg/day to subjects with T2DM for 8 weeks lowered plasma Hcy and MDA, and improved TAC levels. Another study conducted by Shidfar *et al*.,[44] folate supplementation at dosage of 5 mg/day for 8 weeks in adults with hypercholesterolemia led to an improvement in TAC levels. Unlike, folate supplementation did not influence function or biomarkers of oxidant stress among subjects with T1DM and microalbuminuria for 2 months.[45] Increased biomarkers of oxidative stress are blamed for the pathogenesis of epilepsy as a potential mechanism.[46] Liang and Patel[47] have indicated that persistent seizures caused oxidative damage. Moreover, several animal and genetic studies have shown an increase in mitochondrial oxidative stress and subsequent cell damage after persistent seizures.[48,49] It has been reported that the increased amount of active oxygen species or reduced activity of antioxidative defense mechanisms may result in greater frequency of seizure.[50] Therefore, antioxidative systems inhibit oxidative stress.[51] Decreased Hcy levels following intake of folate decreases cellular and protein injury via oxidant mechanisms.[52] In addition, the antioxidant effect of folate was attributed, in part, to transcriptional regulation of NADPH oxidase.[53]

Our study had few limitations. Owing to funding limitations, we could not assess the effects of folate supplementation on plasma folate levels, and gene expression related to inflammation and oxidative stress. Furthermore, the current study was relatively of short duration of intervention. Long-term interventions might result in greater changes in CIMT levels. We did not find a similar study about folate supplementation in epileptic children for determining the sample size based on main outcome (CIMT); therefore, the sample size was calculated based on folate supplementation in older adults. This should be considered in the interpretation of our findings.

Conclusions

Overall, 5 mg/day of folate supplementation for 12 weeks among epileptic children receiving carbamazepine had beneficial effects on hs-CRP, NO, and TAC levels, but did not affect CIMT, and GSH and MDA levels.

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Conflicts of interest

There are no conflicts of interest.

Clinical trial registration number

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