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Effects of folic acid supplementation on psychomotor performance and hemorheology in healthy elderly subjects

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Abstract

Cognitive impairment is associated with increased blood concentrations of homocysteine and high blood viscosity. Previous studies have shown that vitamin B supplementation reduces homocysteine and enhances cognitive function in patients with mild dementia and low serum folic acid. However, whether folic acid enhances cognitive function in elderly subjects without dementia and normal serum folic acid is unknown. Twenty-four healthy elderly subjects (age 73.0 ± 5.6 years, mean $\pm$ S.D.) with normal serum folic acid (6.3 ± 2.4 μg/l) and Mini Mental State Examination (MMSE) >27/30 were randomized to 4-week treatment with folic acid 5 mg/day or placebo in a randomized, placebo-controlled, parallel-group study. Continuous Attention Test (CAT), Four-Choice Reaction Time (FCRT), Digit-Symbol Substitution (DSS), Scanning Memory Sets (SMS), and blood viscosity for different shear rates were measured before and after treatment. Folic acid supplementation induced a significant increase in serum folic acid levels (+13.8 versus +1.6 μg/l, $p < 0.001$) and fall in homocysteine levels (-1.91 versus -0.41 μmol/l, $p = 0.05$) compared to placebo. However, there was no significant change in CAT, FCRT, DSS, SMS, and

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blood viscosity between the two groups. Short-term folic acid supplementation does not enhance psychomotor performance or reduce blood viscosity in healthy elderly subjects with normal serum folic acid levels and preserved cognitive function.

Keywords: Folic acid; Homocysteine; Psychomotor performance; Hemorheology

1. Introduction

Elevated plasma concentrations for the sulphur-containing amino-acid homocysteine and reduced concentrations of the B-vitamin folic acid, two common abnormalities in elderly subjects, have been associated with poor memory and cognitive performance as well as Alzheimer's and other forms of dementia (Rosenberg, 2001; Seshadri et al., 2002; Morris, 2003; Ellinson et al., 2004; Nilsson et al., 2004). There is an inverse relationship between folic acid and homocysteine concentrations through the methionine cycle, which is an essential part of methylation reactions in the brain (Rosenberg, 2001). Folic acid supplementation reduces homocysteine levels and homocysteine levels rise in states of folic acid deficiency (Mangoni and Jackson, 2002).

Elevated plasma homocysteine levels may be a more sensitive marker of functional folic acid deficiency in elderly subjects, in whom normal levels of serum folic acid may not always correspond to adequate cerebrospinal fluid concentrations (Selhub and Miller, 1992; Bottiglieri et al., 2000). Apart from its relationship with vitamin B deficiencies, homocysteine may exert detrimental effects on cognitive function through endothelial dysfunction, arteriosclerosis, thrombosis, and cellular damage (Rosenberg, 2001; Mangoni and Jackson, 2002).

Recent reports have shown that the neuro-psychological impairment of subjects with mild dementia and folic acid deficiency can be improved by folic acid replacement (Nilsson et al., 2001; Sommer et al., 2003). In subjects without vitamin deficiency, lowering homocysteine with short-term folic acid supplementation has been shown to improve endothelial function and reduce pro-thrombotic activity (Undas et al., 1999; Chambers et al., 2000; Mangoni et al., 2002). Therefore, lowering homocysteine concentrations with folic acid even in the absence of overt folic acid deficiency might have a beneficial effect on psychomotor performance. This may be particularly true of the healthy elderly subjects in whom there may be a degree of psychomotor impairment as a result of higher homocysteine levels and perhaps functional folic acid deficiency.

Hematocrit and blood viscosity rise with age and are independently associated with cognitive impairment (Elwood et al., 2001). Studies have also linked blood viscosity and fibrinogen with homocysteine (VonEckardstein et al., 1994). Moreover, folic acid has been shown to reduce fibrinogen, a major determinant of blood viscosity (VonEckardstein et al., 1994; Mangoni et al., 2003). In polycythemic patients the reduction of hematocrit by venesection improves cognitive performance (Wade, 1981). Therefore, it is possible that changes in folic acid and homocysteine blood levels have a positive effect on blood rheology and demonstrate a relationship with psychomotor performance.

The aim of this study was to test the hypothesis that lowering homocysteine levels by folic acid replacement could improve psychomotor performance in healthy elderly subjects with normal serum folic acid levels. The secondary aim was to study the effect of folic acid supplementation on blood rheology.

2. Methods

2.1. Subjects

Twenty-four subjects received folic acid or placebo in a randomized, double-blind, parallel-group study. Subjects were recruited from our volunteers' database. Written consent was obtained in accordance with approval from the local research ethics committee. The subjects were aged $\geq$65 years; had no previous history of vascular disease, hypertension, diabetes, or smoking; a Mini Mental State Examination (MMSE) score >27/30 (Folstein et al., 1975); were not deficient in folic acid or vitamin B_{12}; had normal renal function; and were not taking any vitamin supplements or drugs known to affect homocysteine of folic acid levels.

2.2. Protocol

Each subject was trained to perform the psychomotor performance tests before the study and practised on three separate occasions to exclude any learning bias. During the first training day, the following were also performed: physical examination, blood pressure (BP), MMSE, and electrocardiogram.

On Day 0, psychomotor tests were performed and the results represented the baseline values. Blood samples were also collected for the assessment of homocysteine, folic acid, vitamin B_{12}, creatinine, and cholesterol concentrations, full blood count (FBC), blood viscosity, and mean platelet volume (MPV). Each subject then received 4-week treatment with either folic acid (5 mg/day) or identically appearing placebo. The allocation sequence was generated by software located in the Pharmacy Department. On Day 28, each subject had their psychomotor performance reassessed and repeat blood samples withdrawal.

2.3. Psychomotor tests

Assessment of psychomotor performance was performed using the touch-screen of the Newton Message Pad 2000 (Apple Computers Inc, Cupertino, CA, USA) and the following standard tests, whose sensitivity and validity had been previously established (Kalra et al., 1993; Bryant et al., 1998). (1) Continuous Attention Test (CAT): subjects were shown a series of 240 geometric shapes with 40 repetitions built up from a random pattern of four light and five dark squares arranged in a 3 $\times$ 3 grid. The duration of each stimulus was 0.1 s and the interval between successive stimuli was 2–4 s. The subject's task was to respond by touching the response box on the screen with the indicator pen whenever two consecutive patterns were the same. The number of repetitions identified correctly (cat-c) and incorrect

responses (cat-i) were scored. The error index was calculated by expressing the total number of false negative and false positive responses as a proportion of the total number of responses [(40 − cat-c) + cat-i/240]. This test assessed attention, visuo-spatial memory, and response speed (Kalra et al., 1993; Bryant et al., 1998); (2) Four-Choice Reaction Time (FCRT): this test consisted of four circles that could potentially be lit arranged in the shape of a square. The subject placed the indicator pen lightly on an equivalent series of circles arranged in a square below on the same screen. At random intervals, one of the circles on the monitor screen was illuminated, whereupon the subject responded by touching the equivalent circle on the lower part of the screen as quickly as possible. Three measures of accuracy and reaction time were obtained: responses to random stimuli, fixed stimuli, and transition between the two. The results were recorded as the number of correct (fc-c) and incorrect (fc-i) responses and the mean reaction time of the correct responses (fc-rtc) and incorrect responses (fc-rti) for each category of stimuli. This test assessed attention, reaction time, and visuo-motor coordination (Kalra et al., 1993; Bryant et al., 1998); (3) Digit-Symbol Substitution (DSS): this test is derived from the Wechsler Adult Intelligence Scale. The subject was shown a series of 10 digits on the top of the screen with corresponding symbols. On starting the test, a digit and symbol appeared in a box in the middle of the screen, and the subject responded by touching ''yes'' or ''no'' on the screen as to whether it was the correct combination. The subject was expected to make as many responses as possible over a 90-s period. The digits and the symbols corresponding to them were automatically randomised before each presentation. The total number of correct (dsyn-c) and incorrect responses (dsyn-i) was scored. The mean reaction times to the correct (dsyn-rtc) or incorrect responses (dsyn-rti) were also recorded. This test assessed attention, associative memory, and reaction time (Kalra et al., 1993; Bryant et al., 1998); and (4) Scanning Memory Sets (SMS): a set of three numbers appeared on the screen for a minute then disappeared. Then single digits appeared and the subject had to touch ''yes'' or ''no'' at the bottom of the screen to indicate if the number was in the set or not. The following set consisted of four numbers to be memorized and the final set of five. The total number of correct (sms-c) and incorrect responses (sms-i) and the mean reaction time to the correct (sms-rtc) and incorrect responses (sms-rti) were scored. This test assessed attention, memory, logical reasoning, and reaction time (Kalra et al., 1993; Bryant et al., 1998).

2.4. Biochemical and hematological analysis

Blood samples were collected into tubes containing disodium EDTA, citrate and tubes without anticoagulant. The samples were then centrifuged at $1800 \times g$ within 30 min, the plasma/serum separated and stored at $-20\ ^{\circ}C$. Plasma homocysteine was determined using a fluorescence polarization immunoassay on an IMX analyser (Abbott Diagnostics, Maidenhead, UK) (Ueland et al., 1993). Between batch imprecision was assessed at homocysteine concentrations of 7.0, 12.5, and 25.5 μmol/l and coefficients of variation of 2.4, 2.3, and 1.6%, respectively were obtained ($n = 19$). Serum folic acid and vitamin B_{12} were measured by competitive protein binding enzyme immunoassays on the Immuno 1 analyser (Bayer Diagnostics, Newbury, Berkshire, UK) (Mangoni et al., 2002). The coefficient of variation for folic acid measurements was 5.2%.

Creatinine and cholesterol were measured using manufacturer's standard methods on the DAX 48 analyser (Bayer Diagnostics, Newbury, Berkshire, UK). MPV, platelet count, and haematocrit were measured using Sysmex series 9000 analyser (Mallinckrodt Baker, London, UK).

2.5. Hemorheological tests

Blood samples were collected into 10 ml tubes containing potassium EDTA. Rheometric characterization was performed using a strain controlled ARES rheometer (Rheometric Scientific Ltd, Epsom, Surrey, UK), which equips a very sensitive 100FRTN1 transducer with a range of 0.004–100 g cm torque and 0.1–100 gm normal force, and a re-circulating fluid bath system (Thurston, 1990; Tseng et al., 2002). All tests were carried out at a constant temperature of 37 °C in a double wall Couette cell with the following dimensions: 27.95 mm (inside cup diameter), 29.5 mm (inside bob diameter), 32 mm (outside bob diameter), 34 mm (outside cup diameter), and a bob length of 38.05 mm (Thurston, 1990; Tseng et al., 2002). The steady state viscosities of the blood samples were obtained from the automated steady rate sweep test in the shear rate range of 0.02–1750 s^{-1} (ICSH Expert Panel on Blood Rheology, 1986).

2.6. Statistical analysis

Data are presented as mean ± S.D. Differences between the folic acid and placebo groups were assessed by using Student's unpaired t-test. The within-group changes in folic acid, B12, and homocysteine after intervention were compared using a paired t-test. The comparison of amount of change in folic acid, B12, and homocysteine after intervention between the two groups was done using a Mann–Whitney U-test. The changes in psychomotor performance in subjects receiving folic acid were compared to the changes in those receiving placebo using the Mann–Whitney U-test. Correlations between folic acid, B12, homocysteine, and psychomotor performance were calculated using Spearman's correlation coefficient (SPSS for Windows Version 11.0, SPSS Inc, Chicago, IL, USA). A p-value <0.05 indicated statistical significance.

3. Results

The groups receiving folic acid or placebo were matched for age, gender, systolic, diastolic and mean BP, cholesterol, hemoglobin, platelet count, and MPV (Table 1). Baseline homocysteine, folic acid, and vitamin B_{12} levels were also comparable (Table 2). Taking all subjects at baseline, folic acid, and homocysteine levels were negatively correlated ($r = -0.53$, $p = 0.008$). There was no significant correlation between baseline levels of folic acid, vitamin B_{12}, or homocysteine, and baseline psychomotor performance or hemorheology (Table 2).

In the folic acid supplementation group there was a rise in serum folic acid concentrations, which was greater than placebo (Table 3). This was associated with a corresponding fall in homocysteine concentrations (Table 3).

Table 1
Baseline data for the study groups

	Placebo ($n = 12$)	Folic acid ($n = 12$)
Age (years)	73.8 ± 5.3	72.3 ± 6.0
Gender (Females)	10	11
Systolic BP (mmHg)	137 ± 16	136 ± 21
Diastolic BP (mmHg)	74 ± 10	75 ± 9
Mean arterial pressure (mmHg)	95 ± 11	95 ± 12
Total cholesterol (mmol/l)	6.2 ± 0.6	6.0 ± 0.7
Hemoglobin (g/dl)	13.0 ± 0.9	13.2 ± 0.9
Platelet count (10^9)	255 ± 33	230 ± 42
MPV (dl)	9.4 ± 0.8	10.0 ± 0.1
Hematocrit (%)	0.40 ± 0.02	0.39 ± 0.02

Table 2
Correlations between baseline folic acid, homocysteine, and vitamin B_{12} concentrations, psychomotor performance, and blood viscosity in the whole study group

	CAT	FCRT	DSS	SMS	Viscosity
Folic acid					
r	−0.103	−0.152	−0.142	−0.071	0.162
p	0.631	0.478	0.507	0.740	0.483
Homocysteine					
r	0.122	−0.194	0.237	0.132	−0.067
p	0.569	0.363	0.264	0.539	0.773
Vitamin B_{12}					
r	−0.033	0.135	−0.024	0.157	−0.181
p	0.879	0.528	0.911	0.464	0.433

r: correlation coefficient; *p*: *p*-value.

Table 3
Biochemical and hematological parameters at baseline and after treatment in the folic acid and placebo group

	Placebo ($n = 12$)		Folic acid ($n = 12$)	
	Baseline	Post-treatment	Baseline	Post-treatment
Homocysteine (μmol/l)	11.6 ± 1.8	11.2 ± 3.1	10.8 ± 2.2	8.8 ± 2.2*
Folic acid (μg/l)	6.6 ± 2.8	8.4 ± 3.6	6.0 ± 2.3	19.9 ± 0.6**
Vitamin B_{12} (ng/l)	355 ± 170	364 ± 128	415 ± 141	434 ± 91
MPV (dl)	9.4 ± 0.8	9.5 ± 0.6	10.0 ± 0.7	10.0 ± 1.0
Platelets count (10^9)	255 ± 33	260 ± 31	230 ± 42	231 ± 55

* $p < 0.05$.
** $p < 0.01$.

There were no significant differences in psychomotor performance and blood viscosity at baseline and after supplementation with folic acid compared to placebo (Tables 4–7, Fig. 1).

Table 4
CAT

	Placebo ($n = 12$)	Folic acid ($n = 12$)	p
Correct pre	34.72 ± 4.84	37.08 ± 3.17	
Correct post	33.45 ± 8.08	37.75 ± 2.83	
Difference	−1.27 ± 4.73	+0.67 ± 2.15	0.29
Incorrect pre	1.73 ± 1.19	2.17 ± 1.75	
Incorrect post	0.90 ± 1.53	1.17 ± 1.03	
Difference	−0.81 ± 1.32	−1.00 ± 2.01	0.97
Error index pre	0.15 ± 0.12	0.09 ± 0.11	
Error index post	0.17 ± 0.20	0.06 ± 0.08	
Difference	0.02 ± 0.11	−0.02 ± 0.06	0.11

Table 5
FCRT

	Placebo ($n = 12$)	Treatment ($n = 12$)	p
Random			
Correct pre	105.33 ± 7.25	101.50 ± 10.44	
Correct post	99.75 ± 8.53	102.67 ± 8.06	
Difference	−5.58 ± 11.3	1.17 ± 14.6	0.20
Reaction time correct pre	0.86 ± 0.15	0.778 ± 0.08	
Reaction time correct post	0.84 ± 0.13	0.776 ± 0.10	
Difference	−0.02 ± 0.15	−0.002 ± 0.05	0.67
Incorrect pre	0.67 ± 0.98	0.50 ± 0.78	
Incorrect post	0.92 ± 1.16	0.00 ± 0.00	
Difference	+0.25 ± 1.71	−0.5 ± 0.80	0.18
Fixed			
Correct pre	109.75 ± 7.37	114.00 ± 10.02	
Correct post	114.91 ± 8.74	113.17 ± 8.01	
Difference	5.17 ± 11.62	−0.83 ± 14.0	0.26
Reaction time correct pre	0.81 ± 0.15	0.72 ± 0.08	
Reaction time post	0.78 ± 0.15	0.71 ± 0.08	
Difference	−0.03 ± 0.15	−0.006 ± 0.05	0.52
Incorrect pre	0.25 ± 0.45	0.00 ± 0.00	
Incorrect post	0.42 ± 0.67	0.17 ± 0.39	
Difference	0.17 ± 0.39	0.17 ± 0.39	1.0
Transform			
Correct pre	2.92 ± 0.29	2.92 ± 0.29	
Correct post	2.92 ± 0.29	3.00 ± 0.00	
Difference	0.00 ± 0.00	0.08 ± 0.28	0.32
Reaction time pre	0.96 ± 0.28	0.84 ± 0.18	
Reaction time post	0.91 ± 0.20	0.90 ± 0.17	
Difference	−0.02 ± 0.31	0.06 ± 0.25	0.46
Incorrect pre	0.08 ± 0.30	0.08 ± 0.29	
Incorrect post	0.09 ± 0.30	0.00 ± 0.00	
Difference	0.00 ± 0.00	−0.08 ± 0.29	0.35

Table 6
DSS

	Placebo ($n = 12$)	Treatment ($n = 12$)	p
Correct pre	46.67 ± 0.65	46.25 ± 1.48	
Correct post	46.33 ± 0.98	46.58 ± 0.51	
Difference	−0.33 ± 1.23	0.33 ± 1.61	0.52
Reaction time correct pre	2.63 ± 0.72	2.06 ± 0.29	
Reaction time correct post	2.47 ± 0.64	2.12 ± 0.39	
Difference	−0.16 ± 0.30	0.05 ± 0.20	0.05
Incorrect pre	0.33 ± 0.65	0.75 ± 1.48	
Incorrect post	0.67 ± 0.98	0.42 ± 0.51	
Difference	0.33 ± 1.23	−0.33 ± 1.61	0.51

Table 7
SMS

	Placebo ($n = 12$)	Treatment ($n = 12$)	p
Three digits			
Correct pre	119.25 ± 1.42	119.50 ± 0.67	
Correct post	119.75 ± 0.49	119.42 ± 0.67	
Difference	0.50 ± 1.44	−0.08 ± 1.08	0.59
Reaction time pre	1.07 ± 0.23	0.93 ± 0.12	
Reaction time post	1.04 ± 0.20	0.95 ± 0.14	
Difference	−0.02 ± 0.13	+0.02 ± 0.10	0.20
Four digits			
Correct pre	118.73 ± 1.56	118.42 ± 1.50	
Correct post	118.81 ± 1.76	118.50 ± 1.73	
Difference	0.09 ± 1.92	0.08 ± 2.27	0.88
Reaction time pre	1.11 ± 0.22	1.03 ± 0.20	
Reaction time post	1.12 ± 0.20	1.03 ± 0.21	
Difference	0.01 ± 0.13	0.005 ± 0.18	0.88
Five digits			
Correct pre	117.36 ± 1.91	117.50 ± 2.24	
Correct post	118.45 ± 2.10	118.75 ± 1.54	
Difference	1.1 ± 1.64	1.25 ± 3.36	0.74
Reaction time pre	1.24 ± 0.31	1.07 ± 0.20	
Reaction time post	1.23 ± 0.27	1.06 ± 0.19	
Difference	−0.01 ± 0.14	−0.01 ± 0.15	0.57

4. Discussion

Short-term supplementation with folic acid reduced serum homocysteine concentrations but did not enhance the psychomotor performance of healthy elderly subjects. Similarly, no significant effect on hemorheology was observed. This is the first study to evaluate the effects of folic acid on psychomotor performance of subjects with normal folic acid levels and normal cognitive function. Previous studies showing beneficial effects of

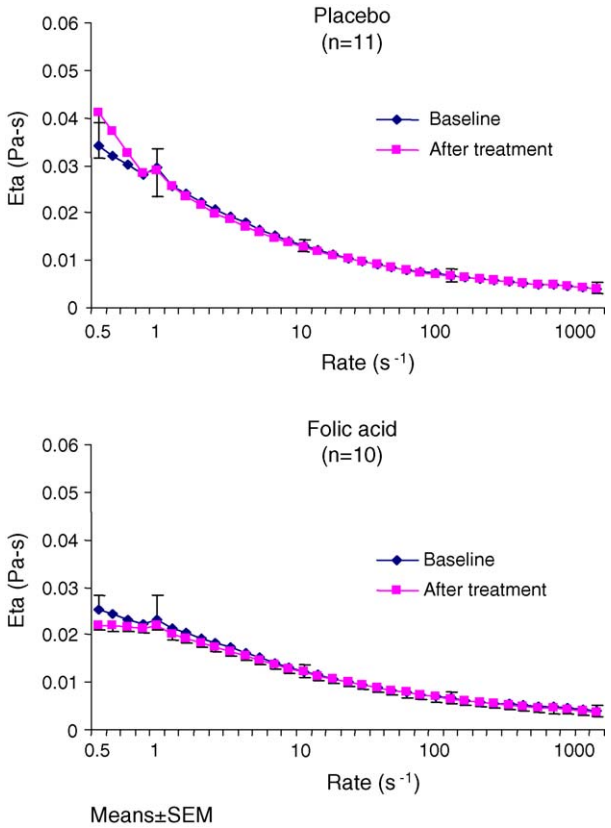

Fig. 1. Blood viscosity curves for different shear rates (logarithmic scale) in the group receiving placebo (upper panel) and the group receiving folic acid (lower panel) at baseline and after treatment.

folic acid on psychomotor performance have studied subjects with folic acid deficiency and cognitive impairment (Nilsson et al., 2001; Sommer et al., 2003).

Folic acid has been used in subjects without folic acid deficiency or cognitive impairment as an agent to reduce homocysteine levels in studies of vascular function. In this context it has been shown to improve endothelial function, thrombotic activity, and in animal studies, oxidative damage to neurones (Undas et al., 1999; Chambers et al., 2000; Mangoni et al., 2002; Mattson, 2003). There have been no studies on the effects of folic acid on cerebral blood flow or reactivity in elderly subjects. In young healthy subjects the single administration of folic acid did not improve cerebral blood flow acutely (within 24 h), but neither did it change serum homocysteine levels (Rosengarten et al., 2003).

There may possibly be two main reasons why no change in psychomotor performance was demonstrated in this study. The first is that folic acid supplementation and homocysteine lowering may not have any additional benefit to confer on this group of healthy subjects. Some studies have shown that in healthy elderly subjects blood homocysteine and vitamin levels are not associated with cognitive skills (Ravaglia et al.,

2000). In this report, homocysteine plasma levels were relatively low. The percentage of subjects with homocysteine levels >14 μmol/l was 4% compared to 29% in an unselected population (Ravaglia et al., 2000). Moreover, in some tests of cognitive function, subjects with mild to moderate dementia have not demonstrated improvement with folic acid supplementation in the presence of ''normal'' homocysteine levels (Nilsson et al., 2001).

Another explanation is that the period of supplementation was too short to demonstrate a change. Previous psychomotor studies that showed the benefits of folic acid supplementation in subjects with folic acid deficiency or prior cognitive impairment were at least of 2 months duration (Nilsson et al., 2001; Sommer et al., 2003). This is in contrast with the folic acid-induced endothelial and coagulation changes, which have been shown to occur within 4 weeks (Mangoni et al., 2002).

These results suggest that in healthy elderly subjects with normal serum folic acid short-term supplementation with folic acid does not enhance psychomotor performance. These findings do not support the use of folic acid fortification to prevent cognitive decline in this group. However, further studies of longer duration in individuals with higher vascular load should be considered.

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