Int J Clin Exp Med 2014;7(11):4214-4219
www.ijcem.com /ISSN:1940-5901/IJCEM0001595

Original Article

Folic acid attenuates cognitive dysfunction in streptozotocin-induced diabetic rats

Rui Yang*, Rong-Ping Chen*, Hong Chen, Hua Zhang, De-Hong Cai

[1]*Department of Endocrinology, Zhujiang Hospital, Southern Medical University, Guangzhou 510282, China. *Equal contributors.*

Received July 30, 2014; Accepted September 20, 2014; Epub November 15, 2014; Published November 30, 2014

Abstract: Diabetic cognitive dysfunction is common in patients with diabetes but its pathogenesis is not clear. The aim of the present study is to investigate the role of 5', 10' methylene tetrahydrofolate reductase (MTHFR) in the development of diabetic cognitive impairment and test whether folic acid (FA) supplementation prevents cognitive dysfunction in diabetic rats. In the current study, three months after streptozotocin-induced diabetes onset, rats showed cognitive dysfunction including the prolonged escape latency, the decreased time spent in the target quadrant and the declined number of crossing the platform in Morris water maze test. Diabetic rats also presented elevated plasma homocysteine level and downregulation of MTHFR in hippocampus revealed by Western blotting. The diabetic cognitive dysfunction was attenuated by 30-day dietary FA treatment with a significantly decreased homocysteine level. In conclusion, these results suggest that MTHFR plays a crucial role in diabetic cognitive dysfunction and folate fortification might become a potent therapeutic strategy against diabetic cognitive impairment.

Keywords: Cognitive dysfunction, diabetes, tetrahydrofolate reductase, folic acid

Introduction

Cognitive impairment is common in patients with diabetes mellitus [1]. It is associated with elevated levels of anxiety, depression, and impaired social functioning and quality of life. Effective prevention strategies are needed to prevent or ameliorate diabetes-induced cognitive dysfunction. A first step in the development of such approaches is to identify the underlying mechanisms linking diabetes to cognitive impairment [2].

The mechanisms of diabetic cognitive dysfunction have not been clearly identified [3]. Earlier studies have shown that an increased plasma level of homocysteine is a risk factor for cognitive dysfunction and dementia [4]. Hyperhomocystinemia might be attributed to the decreased expression or activity of 5', 10' methylene tetrahydrofolate reductase (MTHFR) which utilizes folate to regenerate methionine from homocysteine [5]. Previous studies have demonstrated that diabetes is closely associated with MTHFR gene variation [6]. However, the role of MTHFR in the development of diabetes-related cognitive dysfunction has not been determined. Therefore, we hypothesized that decreased MTHFR and subsequently elevated plasma level of homocysteine could contribute to the genesis of diabetic cognitive impairment. Accordingly, folic acid (FA) supplementation might prevent diabetic cognitive dysfunction by lowering plasma level of homocysteine.

In the present study, we investigated the expression of MTHFR in hippocampus and the plasma level of homocysteine in streptozotocin (STZ)-induced diabetic rats and tested whether FA supplementation prevents cognitive dysfunction in diabetic rats.

Materials and methods

Animal care

Male healthy Sprague-Dawley rats (weight 220 to 250 g) were purchased from local animal center and were housed under room temperature (18°C to 25°C), controlled humidity (50%) and a 12/12-hour light-dark cycle with free access to food and water. All procedures regard-

ing animals were carried out in accordance with the National Institute of Health Guide for the Care and handling of animals. All experimental procedures were approved by the Hospital Animal Care and Use Committee. Animals were randomly divided into two groups: control (control group, n=10) and diabetic (n=20) group. Diabetes was induced by a single intraperitoneal injection of STZ (Sigma Chemical Co., St. Louis, MO, USA, 65 mg/kg of body weight) in 0.15 mol/L NaCl with 0.1 mol/L sodium citrate buffer (pH 4.2) [7]. Rats in control group were injected with similar citrate buffer [8]. Blood glucose concentrations were measured, with a commercial blood glucose test kit, in blood sample of the tail vein, before and after three days of STZ injection and were examined every two weeks during the experiments. The animals with blood glucose concentration above than 16.7 mmol/L were considered as diabetic and were used in the following study. After three months, the diabetic rats were randomly assigned to two groups: vehicle (DM group, n=10) and FA (FA group, n=10). FA (10 mg/kg/day) was administered by oral gavage and the vehicle was treated with saline for 30 days.

Morris water maze test

The cognitive function was evaluated by the Morris water maze test [9]. The spatial memory acquisition and retention of the rats were tested. The swimming pool filled with 25°C opaque water (made white with powdered milk) was divided into four quadrants with a small hidden platform (12 cm in diameter) fixed in a permanent position located 1-2 cm under the water's surface. The experiment was started by placing the rat on a start platform in the tank from 4 different randomly chosen start positions with the head facing the wall of the tank. The rat swims around until it finds the other platform to stand on. The researcher measures how long it takes for a rat to find hidden platform. Each experiment lasted until the rat finds the invisible platform at fixed positions in the water tank or for a maximum duration of one minute. The escape latency, the time to reach the platform, was recorded for each animal. If a rat could not find the platform within one minute, it was guided to the platform and also allowed to stay there for 10 seconds; the latency was scored as one minute. Acquisition trials were performed four times daily for five consecutive days. The second day after the last trial, the probe test was conducted as in the acquisition trial. However, the platform was removed in probe test. Animals were allowed to swim freely for one minute, the time spent in the target quadrant and the numbers of crossing the platform were recorded.

Biochemical analysis

Total plasma homocysteine was measured in blood samples obtained from the jugular vein by high-performance liquid chromatography.

Western blot analysis

The protein expression of MTHFR was detected by Western blotting as previously described [10]. At the end of the behavioral test, animals were immediately anesthetized deeply with pentobarbital sodium and decapitated. Immediately, the whole brains were excised and transferred to an ice-cold plate. The protein lysates were obtained by homogenizing the hippocampus with lysis buffer. The total protein concentration was determined by a simple colorimetric assay based on the Bradford dye-binding method with Bio-Rad protein assay reagent (Bio-Rad Laboratories, USA). Equal amounts of protein were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE, 12%). The samples were then transferred onto a polyvinylidene fluoride membrane (Boehringer Mannheim Corporation, USA). The membranes were then rinsed with 0.5 mol/L Tris-buffer saline (TBS) solution for three times and blocked with TBS containing 0.02% Tween and 5% non-fat milk for 1 hour at room temperature. The membranes were respectively probed with antibodies against β-actin and MTHFR (diluted in 1:500, Santa Cruz biotechnology, Inc., CA, USA) overnight at 4°C. The membranes were then incubated with a horseradish peroxidase-conjugated secondary antibody (diluted in 1:1000, Santa Cruz biotechnology, Inc., CA, USA) for 2 hour at room temperature. The membranes were then analyzed by enhanced chemiluminescence western blot substrate kit (Abcam, Cambridge, MA, USA). The densities of bands were scanned and analyzed by using the Multi-Analyst software package (Bio-Rad) and normalized to that of β-actin levels. Values were expressed as relative value units.

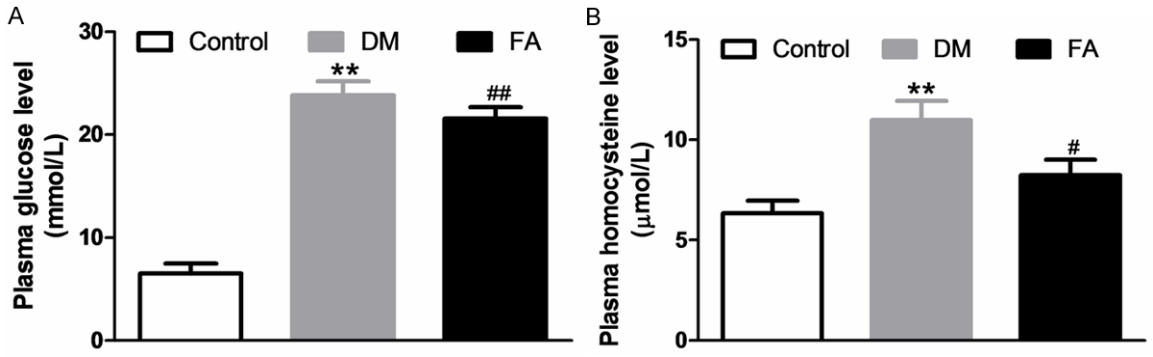

Figure 1. Plasma glucose and homocysteine level of the rats. A. The plasma glucose concentration of the rats from control, diabetes (DM) and folic acid (FA) group three months after streptozotocin injection. B. The plasma homocysteine level of the rats from control, DM and FA group one month after FA supplement. Values are means ± SEM; n=10 per group. **P<0.01 vs. control group, $^{\#}$P<0.05, $^{\#\#}$P<0.01 vs. DM group.

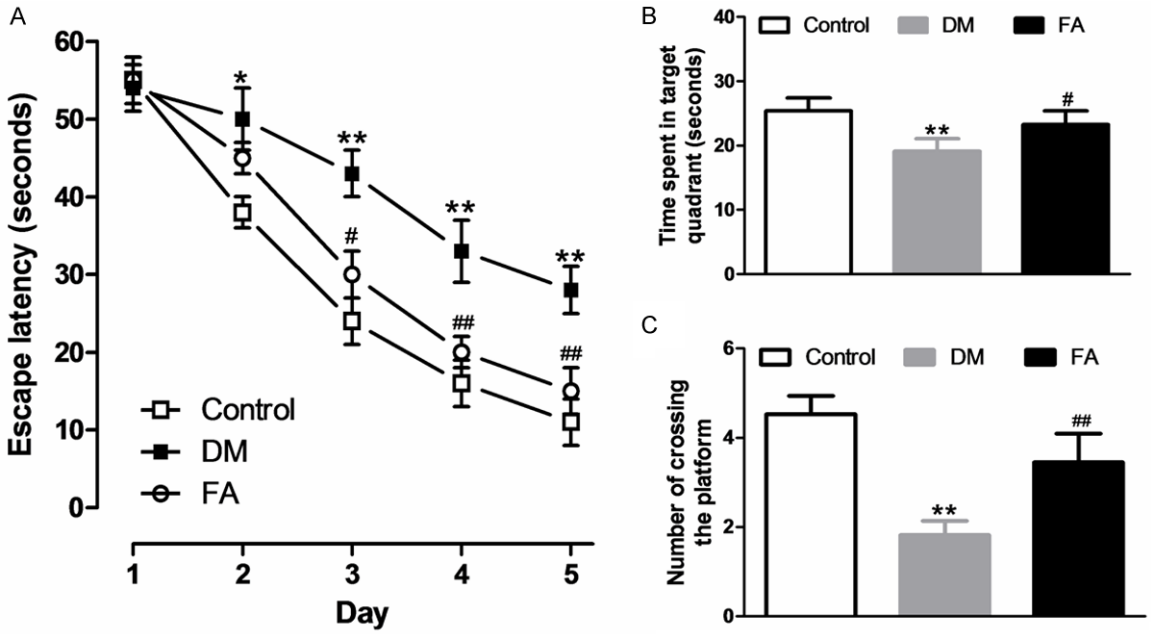

Figure 2. The cognitive function obtained in Morris water maze test. The escape latency (A), the time spent in target quadrant (B), and the number of crossing the platform (C) of the rats from control, diabetes (DM) and folic acid (FA) group one month after FA supplement. Values are means ± SEM; n=10 per group. *P<0.05, **P<0.01 vs. control group, $^{\#}$P<0.05, $^{\#\#}$P<0.01 vs. DM group.

Statistical analysis

Data are presented as means ± SEM. Indicators of the Morris water maze tests were analyzed by ANOVA, followed by student's t test comparison between groups. The immunostaining results were statistically analyzed by one-way ANOVA, followed by Student's t test statistics. All of the analysis was performed by using SPSS 13.0 software (SPSS Inc., Chicago, IL). Results were considered significant when P<0.05.

Results

Plasma glucose and homocysteine

No difference in basal fasting plasma glucose concentration was observed among these groups at onset of the study (data not shown). Three months after STZ injection, rats treated with STZ in the DM group and FA group showed significant hyperglycemia comparing to control group (23.82 ± 1.36 and 21.56 ± 1.12 mmol/L

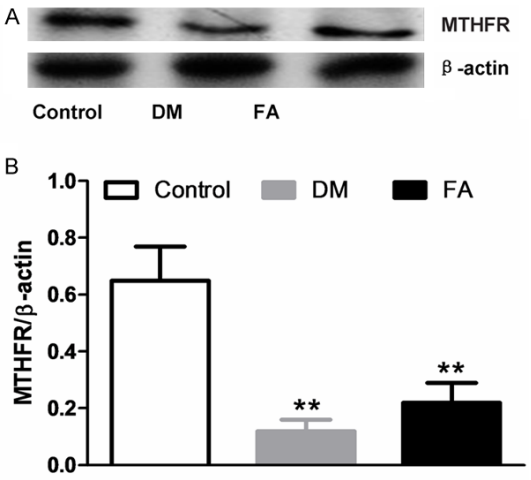

Figure 3. The protein expression of tetrahydrofolate reductase (MTHFR) detected by Western blotting. A. The proteins extracted from the rats in control, diabetes (DM) and folic acid (FA) group were analyzed by Western blotting. B. MTHFR/β-actin is shown in the bar graph. Values are means ± SEM; n=3 per group. **P<0.01 vs. control group.

vs. 6.52 ± 0.96 mmol/L, both *P*<0.01) (**Figure 1A**). After treatment with FA or vehicle for 30 days, plasma homocysteine level in DM group was significantly higher than control group (10.98 ± 0.97 μmol/L vs. 6.33 ± 0.64 μmol/L, *P*<0.01), while the homocysteine level in FA group was significantly lower than DM group (8.24 ± 0.77 μmol/L vs. 10.98 ± 0.97 μmol/L, *P*<0.01) (**Figure 1B**).

Cognitive function

The escape latency in the DM group was significantly longer than the control group on day 2 to 5 (*P*<0.05 or *P*<0.01), the time spent in the target quadrant was shorter than control group (19.13 ± 1.94 vs. 25.42 ± 2.01, *P*<0.01), and the number of crossing the platform was less than the control group (1.82 ± 0.32 vs. 4.53 ± 0.41, *P*<0.01) (**Figure 2**). The escape latency in the FA treatment group was significantly shorter than the DM group on day 3 to 5 (*P*<0.05 or *P*<0.01), the time spent in the target quadrant was longer than DM group (23.24 ± 2.14 vs. 19.13 ± 1.94, *P*<0.05), and the number of crossing the platform was more than the DM group (3.45 ± 0.64 vs. 1.82 ± 0.32, *P*<0.01) (**Figure 2**).

Expression of MTHFR

The expression of MTHFR in hippocampus from control group was significantly higher than both DM group and FA group (both *P*<0.01) (**Figure 3**).

Discussion

There are two novel findings in the present study. First, diabetic cognitive dysfunction is associated with decreased expression of MTHFR and increased plasma level of homocysteine. Second, FA supplement significantly attenuates diabetes-induced cognitive impairment associated with decreased plasma homocysteine level.

There is evidence that increased serum homocysteine levels are associated with declining cognitive function and dementia [11]. Increased plasma homocysteine level has consistently been shown to be an independent risk factor for cognitive dysfunction in general population [12]. The present study demonstrated that increased plasma homocysteine level also mediates diabetic cognitive impairment. There may be a number of biological pathways by which hyperhomocysteinemia causes cognitive impairment [4]. However, the distinct pathway has not been determined.

The metabolism of homocysteine plays an important role in the development of cognitive dysfunction. MTHFR catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which serves as a methyl donor in the reaction converting homocysteine to methionine [13, 14]. A common MTHFR gene mutation C677T results in decreased activity of the MTHFR enzyme [15]. People with homozygous C677T variant exhibit significantly reduced MTHFR activity and higher levels of homocysteine [16]. It has been reported that MTHFR C677T mutation results in the increase of serum homocysteine level which is bad for cognitive function [5]. The resent study indicated that decreased MTHFR might at least partially contribute to diabetic cognitive dysfunction.

Low folate status is associated with poor cognitive function and dementia in the elderly [17]. The lowering effect of FA supplement on homocysteine level has been well established [18]. There are studies reported that supplementation with FA for a long term appears to reduce the rate of cognitive decline in older adults [19]. However, some other studies declared that supplementation of vitamins B12 and FA alone or in combination do not appear to improve cogni-

tive function in individuals with or without existing cognitive impairment [20, 21]. This controversial may be attributed to the variant basal level of folate and the duration of FA supplement. The effect of FA supplement in diabetic patients has not been addressed. The present study showed that folate fortification significantly attenuates diabetes-related cognitive damage.

In summary, the present data provides a novel insight for the prevention or treatment strategy against diabetic cognitive dysfunction.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Hong Chen, Department of Endocrinology, Zhujiang Hospital, Southern Medical University, 253# industry Road, Guangzhou 510282, China. Tel: +86-020-62782330; Fax: +86-020-61643196; E-mail: rubychq@yeah.net

References

[1] Kodl CT, Seaquist ER. Cognitive dysfunction and diabetes mellitus. Endocr Rev 2008; 29: 494-511.
[2] Luchsinger JA. Type 2 diabetes and cognitive impairment: linking mechanisms. J Alzheimers Dis 2012; 30: S185-198.
[3] Luchsinger JA. Diabetes, related conditions, and dementia. J Neurol Sci 2010; 299: 35-38.
[4] Garcia A, Zanibbi K. Homocysteine and cognitive function in elderly people. CMAJ 2004; 171: 897-904.
[5] Cheng DM, Jiang YG, Huang CY, Kong HY, Pang W, Yang HP. Polymorphism of MTHFR C677T, serum vitamin levels and cognition in subjects with hyperhomocysteinemia in China. Nutr Neurosci 2010; 13: 175-182.
[6] Boger CA, Stubanus M, Haak T, Gotz AK, Christ J, Hoffmann U, Riegger GA, Kramer BK. Effect of MTHFR C677T genotype on survival in type 2 diabetes patients with end-stage diabetic nephropathy. Nephrol Dial Transplant 2007; 22: 154-162.
[7] Yang C, Zhu B, Ding J, Wang ZG. Isoflurane anesthesia aggravates cognitive impairment in streptozotocin-induced diabetic rats. Int J Clin Exp Med 2014; 7: 903-910.
[8] Ma LY, Zhang DM, Tang Y, Lu Y, Zhang Y, Gao Y, Xia L, Zhao KX, Chai LY, Xiao Q. Ghrelin-attenuated cognitive dysfunction in streptozotocin-induced diabetic rats. Alzheimer Dis Assoc Disord 2011; 25: 352-363.
[9] Zuo ZF, Wang W, Niu L, Kou ZZ, Zhu C, Zhao XH, Luo DS, Zhang T, Zhang FX, Liu XZ. RU486 (mifepristone) ameliorates cognitive dysfunction and reverses the down-regulation of astrocytic N-myc downstream-regulated gene 2 in streptozotocin-induced type-1 diabetic rats. Neuroscience 2011; 190: 156-165.
[10] Yang D, Ma S, Tan Y, Li D, Tang B, Zhang X, Sun M, Yang Y. Increased expression of calpain and elevated activity of calcineurin in the myocardium of patients with congestive heart failure. Int J Mol Med 2010; 26: 159-164.
[11] Eussen SJ, Ueland PM, Clarke R, Blom HJ, Hoefnagels WH, van Staveren WA, de Groot LC. The association of betaine, homocysteine and related metabolites with cognitive function in Dutch elderly people. Br J Nutr 2007; 98: 960-968.
[12] Kalmijn S, Launer LJ, Lindemans J, Bots ML, Hofman A, Breteler MM. Total homocysteine and cognitive decline in a community-based sample of elderly subjects: the Rotterdam Study. Am J Epidemiol 1999; 150: 283-289.
[13] Almeida OP, Flicker L, Lautenschlager NT, Leedman P, Vasikaran S, van Bockxmeer FM. Contribution of the MTHFR gene to the causal pathway for depression, anxiety and cognitive impairment in later life. Neurobiol Aging 2005; 26: 251-257.
[14] Karabacak E, Aydin E, Ozcan O, Dogan B, Gultepe M, Cosar A, Muftuoglu T. Methylenetetrahydrofolate reductase (MTHFR) 677C>T gene polymorphism as a possible factor for reducing clinical severity of psoriasis. Int J Clin Exp Med 2014; 7: 697-702.
[15] Ford AH, Flicker L, Hankey GJ, Norman P, van Bockxmeer FM, Almeida OP. Homocysteine, methylenetetrahydrofolate reductase C677T polymorphism and cognitive impairment: the health in men study. Mol Psychiatry 2012; 17: 559-566.
[16] Tsai SJ, Hong CJ, Yeh HL, Liou YJ, Yang AC, Liu ME, Hwang JP. Heterozygote advantage of the MTHFR C677T polymorphism on specific cognitive performance in elderly Chinese males without dementia. Dement Geriatr Cogn Disord 2011; 32: 159-163.
[17] Ramos MI, Allen LH, Mungas DM, Jagust WJ, Haan MN, Green R, Miller JW. Low folate status is associated with impaired cognitive function and dementia in the Sacramento Area Latino Study on Aging. Am J Clin Nutr 2005; 82: 1346-1352.
[18] Chen F, Cui CL, Wu P, Xie NZ. Relationship of serum homocysteine level with nutritional status and HbA1 level in elderly inpatients. Int J Clin Exp Med 2013; 6: 779-784.
[19] Bogaisky M, Leipzig RM. Daily folic acid supplementation for 3 years improved cognitive func-

tion in older persons. Evid Based Med 2007; 12: 83.

[20] Balk EM, Raman G, Tatsioni A, Chung M, Lau J, Rosenberg IH. Vitamin B6, B12, and folic acid supplementation and cognitive function: a systematic review of randomized trials. Arch Intern Med 2007; 167: 21-30.

[21] Celik G, Annagur BB, Yilmaz M, Kara F. Findings of multidimensional instruments for determining psychopathology in diabetic and non-diabetic hemodialysis patients. Int J Clin Exp Med 2012; 5: 346-354.