The Effects of Chronic Administration Folic Acid on Memory Retrieval in Rats

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Summary

The effect of pre-treatment of folic acid on memory retrieval was evaluated using a step-through passive avoidance task. Folic acid (1 or 10 mg/kg), alone and with vitamin B6 (100 mg/kg), were administered intraperitoneally (i.p.) every other day for one month before training was initiated. Three retention tests were performed to assess memory in rats. The folate plasma level was also measured in the animals. Folic acid (1 or 10 mg/kg) alone significantly increased the step-through latency of the passive avoidance response compared to the control in the first retention test of the passive avoidance paradigm ($P<0.05$). Furthermore, vitamin B6 (100 mg/kg) with folic acid (1 mg/kg) significantly increased the cognitive ability compared to the control group ($P<0.01$). These results indicate that the administration of folic acid alone or in combination with vitamin B6 play a role in enhancing memory retrieval. In the combination therapy at specific doses, this effect was significant.

Keywords: Folic acid, Vitamin B6, Memory retrieval, Rat

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Introduction

The prevalence of Alzheimer's disease (AD) has increased progressively over the past 100 years, and Alzheimer's disease is the most common form of dementia. Memory impairment may precede impairment in other cognitive domains, which suggests that the cognitive profiles in these patients may be different [1,2]. Higher levels of circulating total homocysteine levels (tHcy) have been detected in people with neurodegenerative diseases. The higher baseline levels of tHcy in patients with AD show a more rapid progression of the disease over a three-year period [3]. Several cross-sectional and prospective investigative studies have shown a direct relationship between folate levels and cognition [4-7].

Serum folate levels are negatively correlated with the severity of post-mortem cerebral atrophy in AD patients [8]. It has been shown that higher folate intake could decrease the risk of AD, which was independent of other risk factors and the levels of vitamin B12 [9]. The total plasma homocysteine concentrations were higher in patients with dementia associated with Alzheimer's disease [10]. The simultaneous supplementation of folate and vitamin B12 attenuates the homocysteine-induced Aβ overproduction and memory deficits in rats [11]. Moreover, a significant correlation has been reported between the risk of Alzheimer's disease and the high plasma levels of homocysteine and the low levels of folic acid and vitamin B6 [12]. A B vitamin-deficient diet induced hyperhomocysteinemia in an inbred mouse strain, and this short-term dietary challenge significantly impaired cognition and caused hippocampal microvasculature deficits [13].

It has been shown that an increase in the intake of vitamin B6 and B12 in a community-dwelling, non-pathological aging population is associated with larger grey matter volume [14]. The association between vitamin consumption and cognitive function is of scientific interest. Because the results from several studies concerning the role of vitamin B6 and folic acid use different methods to examine cognition performance in humans are controversial, this can confound and limit the ability to study their effects in humans. Thus, this study demonstrates that pre-administering vitamin B6 and folic acid with the normal diet could accelerate cognition performance in rats.

Materials and methods

Animals

Male Wistar rats (200–250 g) were obtained from the Razi Institute (Karaj, Iran) and housed in groups of four per cage under standard laboratory conditions. They were kept at constant room temperature (21±2 °C) under a normal 12L:12D (light:dark) cycle with free access to food and water. All animal experiments were performed in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) to minimise the number of animals used and their suffering.

Drugs

Vitamin B6 was purchased in injectable form from Tamin Pharmaceutical Co., Iran. Folic acid was obtained from Merck and was diluted in saline before the experiment. Control animals received saline.

Experimental procedure

Animals were fed standard rodent chow (67.5% carbohydrate, 11.7% fat, 20.8% protein, and 1% supplement (0.15% folic acid and 0.15% vitamin B6); Khorak-Dame Pars, Tehran, Iran) Rats were divided into seven groups of 10 animals each. The first group received saline. In the remaining six groups, 50 or 100 mg/kg of vitamin B6, 1 or 10 mg/kg folic acid and 100 mg/kg vitamin B6 with 1 or 10 mg/kg folic acid were administered. All animals received the drugs intraperitoneally (i.p.) in a volume of 10 ml/kg every other day for one month prior to the experiments.

Passive avoidance apparatus

A learning box consisting of 2 compartments, 1 light (white compartment; 20 cm × 20 cm × 30 cm) and 1 dark (black compartment; 20 cm × 20 cm × 30 cm), was used. A guillotine door opening (6 cm × 6 cm) was constructed on the floor of the box in the centre of the partition between the 2 compartments. Stainless steel grids (5 mm in diameter) were placed at 1-cm intervals on the floor of the dark compartment to produce the foot shock.

All animals were allowed to habituate in the experimental room prior to the experiments. The acquisition trial was performed 30 min after the habituation trial. In each trial, an animal was placed in the light compartment and, after 5 s, the guillotine door was opened. Once the animal crossed into the dark compartment, the door was closed and a foot shock (5 s, 0.2 mA intensity) was immediately delivered to the grid floor of the dark room by an insulated stimulator. Two minutes later, the procedure was repeated. The rat received a foot shock each time it re-entered the dark compartment and placed all 4 paws into it. The training was terminated when the rat remained in the light compartment for 120 consecutive s. The number of trials (entries into the dark chamber) was recorded. All animals were trained with a maximum of 3 trials [15-17].

One, two and seven days after training, the retention tests were performed to evaluate memory. Each animal was placed in the light compartment for 20 s, the door was opened, and the step-through latency was measured for entering into the dark compartment. The test session ended when the animal entered the dark compartment or remained in the light compartment for 300 s.

Plasma concentrations of folic acid

On the day after the behavioural study, animals were anaesthetized with ketamine/xylazine (60/6 mg/kg, i.p.), and the blood, which was rapidly removed by cardiac puncture and sampled into tubes with EDTA, was centrifuged (4 °C, 2500×g, 15 min) to retrieve the plasma fraction. Folate levels were determined using the Electrochemiluminescence Immunoassay (ECLIA) (folate III radioassay kit, Roche, Germany) method [18].

Data analysis

The data were analysed using a one-way ANOVA and Dunnett post-test. A level of $P < 0.05$ was considered to indicate statistical significance.

Results

The administration of folic acid at 1 and 10 mg/kg every other day for one month prior to training induced a significant increase in the memory retrieval compared to the control treated

group in the first retention test (one day after training) (P<0.05) (Fig. 1). In addition, folic acid at 1 and 10 mg/kg significantly increased the memory retrieval in the second and third retention test of the passive avoidance paradigm compared to the control (two days and one week after training, respectively) (P<0.05) (Fig. 1). The administration of vitamin B6 100 mg/kg with folic acid (1 mg/kg) every other day for one month prior to training induced a significant increase in memory retrieval compared to the control treated group in the first retention test (one day after training) (P<0.01) (Fig. 1). Further, the administration of vitamin B6 (100 mg/kg) with folic acid (10 mg/kg) every other day for one month prior to training induced a significant increase in memory retrieval compared to the control treated group in the first retention test (one day after training) (P<0.05) (Fig. 1).

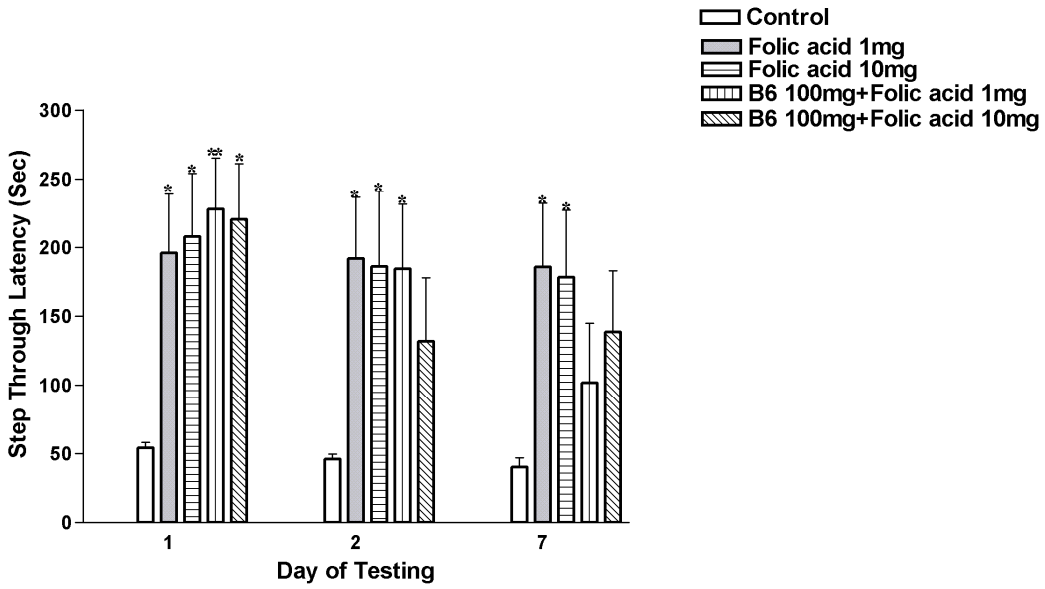

Fig 1. The effects of folic acid (1 or 10 mg/kg) and vitamin B6 (100 mg/kg) with folic acid (1 or 10 mg/kg) on the step-through latencies in rats. Values are expressed as means ± SEM. Rats (10 per group) were i.p. injected with drugs every other day for one month prior to the experiments. The retention tests were performed one, two and seven days after training, *P < 0.05, **P< 0.01, compared to the same day control, Dunnett test.

Folic acid at 1 or 10 mg/kg with vitamin B6 (100 mg/kg) also increased the memory retrieval compared to the control treated group in second and third retention test, but this effect was

significant for folic acid at 1 mg/kg with vitamin B6 (100 mg/kg) in the second retention test ($P<0.05$) (Fig. 1). Moreover, there was no significant difference in the plasma concentration of folate in the folic acid treated group compared to control (Fig 2).

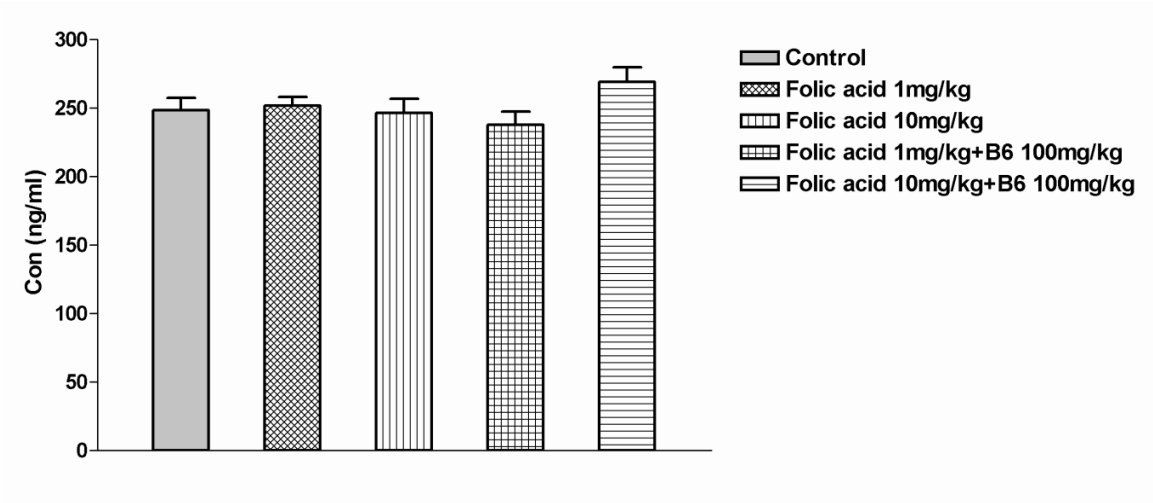

Fig 2. The plasma concentration of folic acid (1 or 10 mg/kg) and vitamin B6 (100 mg/kg) with folic acid (1 or 10 mg/kg) on the step-through latencies in rats.

Discussion

The present study shows that the administration of folic acid every other day prior to training enhanced memory retrieval in rats. Folic acid (1 or 10 mg/kg) significantly increased the retrieval of memory in the first retention tests of a passive avoidance task compared to the control group. Furthermore, vitamin B6 with folic acid (1 or 10 mg/kg) significantly increased the retrieval of memory in the first retention tests of a passive avoidance task compared to the control group. However, the cognitive-enhancing effect of vitamin B6 (100 mg/kg) with folic acid (1 mg/kg) was higher than the other groups.

In addition, there was no difference between the plasma concentrations of folate in the folic acid treated group compared to the control group. However, the memory enhancing effect of folic acid was different from the plasma concentration and was independent of the dose response.

Therefore, in our study, with increasing doses of folic acid, the memory performance did not improve compared to that observed at the lower dose.

Recently, several studies have examined the role of folic acid in human cognition [19,20]. One study has shown that higher folate intake was associated with the rapid cognitive decline in older people whose vitamin B status was unknown [21]. Similarly, high serum folate was directly associated with both anaemia and cognitive impairment in people with low vitamin B levels [22]. Thus, this could explain why, with increasing doses of folic acid, we found that the cognition performance did not increase. Furthermore, as we have shown in our study on normal animals, the combination therapy increased the cognition enhancing effects more than the monotherapy. Folate is thought to be necessary to preserve cognition function because it acts as a carrier of 1-carbon groups for the methylation cycle [23].

The administration of nutraceutical supplements containing phosphatidylserine, *Ginkgo biloba*, vitamin E and pyridoxine improved short-term memory performance in aged dogs [24]. Vitamin B6 inhibits glutamate release from rat cortical synaptosomes via the suppression of presynaptic voltage-dependent Ca^{+2} entry and protein kinase C (PKC) activity [25]. In this study, the protective effect of vitamin B6 against excitotoxicity has been shown. The role of pyridoxal phosphate (PLP) in neurological disorders, like seizures, has been examined previously. In epilepsy, neurotransmitter metabolism can be regulated by modulating the synthesis of PLP. PLP has been used for the treatment of epilepsies from infantile spasms to status epilepticus in adults in Japan [26]. It has been reported that pyridoxine and pyridoxal are as effective antioxidants as vitamin C and vitamin E and protect against reactive oxygen free radicals [27]. Treatment of diabetic rats with pyridoxine increased 5-HT synthesis, which may be a result of the desensitisation of receptors and thereby, modify the synthesis and release of 5-HT. Pyridoxal phosphate levels in the hippocampus regulates the decarboxylation of the 5-HTP, which is the precursor of 5-HT [28]. It has been suggested that vitamin B6 has modulatory effects on the metabolism of neurotransmitters in the brain, which explains the cognition enhancing effects of vitamin B6 and the ability to act as a free radical scavenger.

In this study, we observed that the plasma concentration of folate in the group treated with vitamin B6 (100 mg/kg) and folic acid (10 mg/kg) was higher than the other groups, but it was not significant compared to the controls. This high serum folate could be attributed to the high dose of folic acid (10 mg/kg) administered to the animals, which resulted in the appearance of unmetabolised folic acid in the plasma. These results were similar to a previous study [29].

Conclusion

These results indicate that folic acid and vitamin B6 and play a potential role in enhancing memory retrieval. Also, folate levels in the last retention test were not significant between the treated groups compared to the controls. However, the specific dose of vitamin B6 and folic acid used in the combination therapy on memory performance is necessary. Further studies are necessary to determine whether administration of both vitamin B6 and folic acid will also reduce the risk of cognitive diseases and/or improve cognitive functioning.

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