Chronic folic acid administration confers no treatment effects in either a high or low dose following unilateral controlled cortical impact injury in the rat

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Abstract. Purpose: Traumatic brain injury (TBI) is a major health concern today and effective treatments must be developed in order to combat the numerous TBIs that occur each year. Multiple b-vitamins have been shown to have neuroprotective effects, however, folic acid (B9) has not been widely studied. The current study examined two different doses in a rodent model of controlled cortical impact (CCI) TBI.

Methods: Sham procedures or a unilateral parietal controlled cortical impact injury was induced. Rats were administered either vehicle or folic acid in an 80 μg/kg or 800 μg/kg dose. Rats were tested on the bilateral tactile adhesive removal task, rotarod task and the Morris water maze. Brains were examined to determine lesion size and neuronal loss.

Results: Neither of the folic acid-treated groups showed improvement on any behavioral task or anatomical measure post-CCI and the high dose group had increased neuronal loss compared to the vehicle. Administration of the high dose in sham animals resulted in some behavioral dysfunction and significant neuronal loss.

Conclusions: The results from this study suggest that folic acid may not represent an effective avenue for treatment and that higher doses may actually be detrimental following TBI.

Keywords: Vitamin-therapy, traumatic brain injury, neurotoxicity, therapeutic, recovery of function

1. Introduction

Despite the fact that traumatic brain injury (TBI) has been shown to be a major health concern in industrialized countries, no pharmaceutical treatment has been approved for use in humans. The Center for Disease Control estimates that 1.7 million TBIs occur in the United States each year, resulting in 52,000 deaths and 275,000 hospitalizations (Center for Disease Control, 2010). These numbers are even further compounded by the increase in TBIs due to military actions in the Middle East, resulting in significant increases in the incidence of blast-related TBI. A study of soldiers wounded in Iraq showed that over 50% of injuries were to the head and neck (Gondusky and Reiter, 2005). These numbers represent a significant burden on both the medical community and the families of victims and there is a strong need for adequate treatments for these TBI victims.

Treatments for TBI focus on preventing the secondary cascade of effects that occur as a result of TBI. The secondary cascade is complex and multimodal.
It consists of several factors such as excitotoxicity, increases in free radicals, apoptosis, edema, inflammation, and the glial response (Kochanek et al., 2000; Lenzinger et al., 2001; Raghupathi, 2004). Many of these factors are intricately tied together and treatments for TBI can affect several stages of the cascade by acting on these processes. In identifying potential therapeutic agents for TBI, many have argued that there is a need for extensive behavioral characterization, and that one of the reasons many drugs have not succeeded in human clinical trials is due to inadequate behavioral testing (Faden, 2002; Narayan et al., 2002; Saatman et al., 2008).

Recently, several of the B-group vitamins have been shown to be effective in the treatment of experimental traumatic brain injury. Riboflavin (B2), nicotinamide (B3), and pyridoxine (B6) have all been shown to directly reduce the detrimental effects of traumatic brain injury in both behavioral and physiological assays (Hoane et al., 2003; Hoane et al., 2006a; Hoane et al., 2008a; Hoane et al., 2005; Kuypers and Hoane, 2010). In addition, pantothenic acid (B5), folic acid (B9) and vitamin B12 have been identified as having a relation to reduced incidence of ischemic stroke in humans (He et al., 2004; Kelly et al., 2003). While stroke epidemiology studies suggest that folic acid and B12 may play a preventative role in brain damage, any mechanisms by which they are operating have not been fully investigated.

Folic acid in particular represents an interesting target because it plays such an important role in the nervous system, participating in the closing of the neural tube during fetal development, cellular division and maintenance of DNA methylation patterns. Despite these processes, relatively little research has been performed investigating its role in general neuroplasticity. It has been suggested by some researchers that it may have beneficial effects on cognition in older age, but the existence and extent of this effect is contentiously debated (Fioravanti et al., 1997; Sommer et al., 2003; Vogel et al., 2009).

Recently, studies have begun to investigate the role of folic acid following neurological damage. Iskandar and colleagues (2004) looked at this question by examining the dose-response curve of folic acid following a spinal cord injury (SCI) in rats. They identified 80 μg/kg as the optimal dose for nerve regeneration and also noted that the highest dose used (800 μg/kg) impaired nerve regeneration. Subsequently they tested the 80 μg/kg dose on measures of functional recovery and found that it significantly improved scores on an overall motor test evaluating recovery from SCI. They concluded that the effect of folic acid was primarily exerted on axonal regrowth and thus led to functional recovery in this model. Examinations into the efficacy of folic acid following brain damage have also begun in the area of TBI. Naim and coworkers (2011) utilized the same 80 g/kg dose in an acceleration model of closed head injury in the piglet and evaluated many different behavioral measures. They found moderate improvements on day 1 of testing for exploratory open field performance, for the balance beam task and for the T-maze, but did not see improvement on their other three tasks or any sparing on histological measures. No improvements were found on post-injury day 4 in any task. In both of these studies, the behavioral testing has been relatively limited. In the case of Iskandar and colleagues (2004), there was only a single outcome measure and in the case of Naim and colleagues (2011), while multiple behaviors were assessed, testing only occurred at time points of one day and four days after injury. In addition, the effects found in the piglet study appear to be marginal as they were only seen on the first day after injury and only on select tasks.

In the current study, we investigated the effects of the previously established dose of folic acid (80 μg/kg) on recovery of function following a unilateral controlled cortical impact (CCI) with a widely established behavioral battery. Behavioral assessments included tasks designed to measure somatosensory, motor and cognitive functioning. In addition, we evaluated the effects of a high dose (800 μg/kg) in the same model because previous work did not look at multiple doses in vivo. Based upon the previous research discussed above, we expected folic acid administration to improve recovery of function following TBI.

2. Methods

Experiment 1

2.1. Animals

Thirty-six male Sprague-Dawley rats, approximately 3.5 months of age with an average weight of 403.0 g at the time of injury were used in this study. All procedures described in this study were approved in advance by the Institutional Animal Care and Use Committee and the study was conducted in laboratory facilities certified by the American Association for the
Accreditation of Laboratory Animal Care. Animals were housed singly in standard cages with ad lib access to food and water on a 12 h light : dark cycle. Testing was conducted during the light cycle.

2.2. Surgery

Surgical procedures were performed according to previous studies and under aseptic conditions (Hoane et al., 2003; Hoane et al., 2008a; Vonder Haar et al., 2011). Animals were anesthetized under combined isoflurane (2–4%) and oxygen (0.8 L/min) and placed into a stereotaxic device. Body temperature was monitored and adjusted using a heated surgical stage (37°C). Animals received either a unilateral parietal CCI injury or sham procedures. A 5.0 mm unilateral craniotomy was made over the sensorimotor cortex centered at AP = −2.4, ML = 2.4. Care was taken to avoid damage to the meninges and dura. The cortical area containing the sensorimotor representation of the forelimb and hindlimb was exposed. A 4.0 mm diameter stainless steel impactor tip attached to an electromagnetic impactor (myneurolab.com) was used to induce the injury. The cortex was impacted at 2.75 m/s to a depth of 2.5 mm with a contact time of 0.5 s. After injury, bleeding was stopped, the incision was sutured and the animal was placed in a heated recovery chamber. Sham procedures included all of the above, with the exception of the impact.

2.3. Drug administration

Rats were randomly assigned to four groups. Thirty minutes following injury, rats were given an injection of either folic acid (80/1000 g/kg or 800/1000 g/kg, i.p.) or vehicle (0.9% saline, 1 ml/kg, i.p.) following methods adapted from previous studies (Iskandar et al., 2004). Group one received CCI and was given 80/1000 g/kg folic acid injections (B9-low, n = 9). Group two received CCI and was given 800/1000 g/kg folic acid injections (B9-high, n = 9). Group three received sham procedures and was given vehicle injections (Vehicle, n = 9). Group four received sham procedures and was given vehicle injections (Sham, n = 9).

2.4. Bilateral tactile adhesive removal task

The bilateral tactile adhesive removal task has been established to be an effective method of testing somatosensory dysfunction. It has been applied across a variety of injury models and shows considerable deficits following unilateral injuries (Goffus et al., 2010; Hoane et al., 2008b; Schallert et al., 1982). To test somatosensory function, this test was administered on days 2, 4, 6, 8, 10 and 14 post-CCI following methods outlined in previous studies (Hoane et al., 2003; Hoane et al., 2008a; Hoane et al., 2006b). There were two days of pre-testing prior to injury to establish baseline performance. Small circular adhesive patches (1.3 cm diameter, Avery product #05062) were applied to the lateral aspect of each forelimb and the rat was returned to its home cage. The order and latency of contact and removal for each patch was recorded with a stopwatch. A trial ended when both patches were removed or 120 s had elapsed. There were two trials per test day with an intertrial interval (ITI) of 10 mins. The measure of interest was the latency to remove the patches. The latencies from the two trials were averaged together to form a score for the day for each rat.

2.5. Rotarod testing

The rotarod task has been shown to be a sensitive measure of motor impairment following TBI in both constant-speed and accelerating speed paradigms (Hamm et al., 1994). It consists of a 7 cm diameter cylinder situated at a height of between 0.5 m and 1.3 m above a foam pad. The cylinder is mechanically controlled via computer to rotate at set speeds and/or accelerate (San Diego Instruments, San Diego, CA). To evaluate motor function, the constant acceleration paradigm of this test was administered on days 7–11 post-CCI following methods from a previous study (Buitrago et al., 2004). Rats were given no pre-training prior to testing on this task. Starting on day 7 post-CCI, rats were given an adaptation trial on the cylinder in order to familiarize them with the consequences of falling. They were required to stand on a stationary cylinder for 1 min without falling. If a rat fell, it was placed back on the cylinder and the timer started over. Once rats met this criterion, testing began. During testing, rats were never put back on the cylinder. The cylinder started at a steady rate of 5 rpm for 20 s. Rats were placed on the rotating cylinder facing inward. After 20 s had elapsed, the cylinder began accelerating from 5 to 50 rpm over a period of 300 s (acceleration rate of 0.055 cm/s² or 0.15 rpm/s). Rats were given four trials a day with an ITI of 10 min
for five days in a row. The measure of interest was the latency to fall for the rat for each trial. The four latencies were averaged to form a score for the day for each rat.

### 2.6 Morris water maze

To examine cognitive-spatial function, this test was administered on days 12–18 post-CCI as described in previous studies (Hoane et al., 2003; Hoane et al., 2008a; Vonder Haar et al., 2011). On days 12–15, the reference memory task took place with a clear Plexiglas platform (10 cm × 10 cm) submerged 2 cm below the surface in 32 cm of room temperature water (22 °C) in the center of the northwest quadrant of a 1.5 m diameter circular tank. Rats were lowered in at randomized start points facing the wall of the tank. A trial ended after rats reached the platform or 90 s had elapsed. Rats unable to reach the platform after 90 s were guided by hand to the platform, given 10 s to locate spatial cues and then removed from the platform. Rats were placed under a heat lamp to dry after each trial. There were four trials per day with an ITI of 15 min. Path data, including latencies and distances, were recorded by computer movement tracking software (SMART, San Diego Instruments). Latencies from the four trials were averaged to form a score for the day.

The working memory task followed on days 16–18 following methods outlined in previous studies (Hoane et al., 2003; Hoane et al., 2008a; Vonder Haar et al., 2011). The procedure was the same, except on each day, the platform was placed in the center of a randomized new quadrant of the tank (Northeast, Southwest or Southeast). The first trial of the day was considered an acquisition trial and was not included; the latencies from the last three trials were averaged to form a score for the day for each rat.

### 2.7 Histology

On day 23 post-CCI, rats were anesthetized with a lethal dose of sodium pentobarbital (Euthasol, Virbac Animal Health; 0.3 mL i.p.). Once eye-blink and pedal responses disappeared, rats were transcardially perfused with ice cold 0.9% phosphate buffered saline (PBS), followed by 10% phosphate buffered formalin (PBF). After the brain was removed from the skull, it was post-fixed for three days in PBF. Brains were then placed in a 30% sucrose solution for three days, slabbéd and sliced frozen on a sliding microtome at 40 μm.

### 2.8 Cresyl violet

A series of slices transversing the lesion cavity (0.0 to −6.0 mm from bregma) were mounted on gelatin-subbed slides for staining. These were rehydrated, stained with cresyl-violet, dehydrated, cover-slipped and prepared for light microscopy to examine the extent of the lesion and perform cell counts.

### 2.9 Lesion analysis

In order to examine the extent of the lesion cavity, four sections were selected (−1.3, −2.3, −3.3, −4.3 mm from bregma) and examined under a light microscope. An Olympus microscope (BX-51) with an Olympus 13.5 megapixel camera (DP-70) was used to image sections. The area of the hemispheres from each section were measured using imaging software (ImageTool, UTHSCSA). The area of each hemisphere was measured and the volume calculated using the Cavalieri method, as described in previous studies (Coggeshall, 1992; Goffus et al., 2010; Hoane et al., 2003). The mean area from the four coordinates was multiplied by the thickness of the sections (40 μm) and total number of sections (4). The extent of cortical injury was then calculated by examining the percent reduction from the contralateral hemisphere using the formula [100– (ipsilateral hemisphere volume/contralateral hemisphere volume) * 100].

### 2.10 Neuronal cell counts

To quantify neuronal loss, a series of cresyl-violet stained sections from the center of the lesion (−2.4, −2.8 and −3.2 mm from bregma) were selected and cell counts of neuronal bodies were performed in the hippocampus and thalamus following procedures similar to previous studies (Hoane et al., 2003; Holland et al., 2008). For each section, two sites were selected for counts from the CA3 field of the hippocampus both ipsilateral and contralateral to the lesion and imaged at 40× using an Olympus camera (DP-70) mounted to an Olympus microscope (BX-51) (see Fig. 6). The counts from the ipsilateral sites were averaged together as were the contralateral. The ipsilateral average was then divided by the contralateral average to obtain a percent reduction score. The
percent reduction scores from each section were averaged together for an overall measure of cell loss. For each section, two sites were also selected for counts from the ventral posterior nucleus (VPN) of the thalamus. The hypothalamus was used as a landmark and, at a 10× view, the frame was moved 2.5 mm lateral and 1 mm ventral, then zoomed to 40×. The sites ipsilateral and contralateral to the lesion were then captured at 40× (see Fig. 6). The counts from the ipsilateral were averaged together, as were the contralateral. The ipsilateral average was then divided by the contralateral average to obtain a percent reduction score. The percent reduction scores from each section were averaged together for an overall measure of cell loss.

2.11. Statistical analysis

All data were analyzed without knowledge of group assignment. The mean and standard error of the mean (SEM) were calculated for all the data. The General Linear Model (GLM) ANOVA with repeated measures was used to evaluate the effects of folic acid treatment across days. The Huynh-Feldt correction was used to correct for repeated measures. Tukey’s Honestly Significant Difference (HSD) was used for pairwise post-hoc comparisons between the B9-low group and vehicle group, B9-high group and vehicle group, and B9-high group and sham group. One-way ANOVAs were used to evaluate histological data and were followed up with HSD post-hoc analyses. The statistical level of significance was a p-value of less than 0.05.

Experiment 2

2.12. High-dose sham assessment

Due to concerns over potential toxicity from the high dose of folic acid, a second experiment was conducted. A group of animals (n=6) was given sham procedures and run through all the behavior tests exactly as described above in experiment 1 except that they were given the high dose of folic acid (800 µg/kg) daily for 14 days. This group was then compared against the original sham group using a repeated measures ANOVA for the behavioral measures. For anatomical measures, they were compared regarding the average number of cells in the CA3 field of the hippocampus and VPN of the thalamus using an independent samples t-test. For a sham comparison, the absolute number of cells is a more viable measure than the percent reduction used for injured animals.

3. Results

Experiment 1

3.1. Bilateral tactile adhesive removal task

The pre-injury scores were averaged to give a baseline measurement and compared in a one-way ANOVA. There were no differences between the groups, F(3, 32) = 2.08, p = 0.123. The post-injury data was analyzed in a 4 × 6 repeated measures ANOVA (Group [Sham, Vehicle, B9-low, B9-high] × Day [2, 4, 6, 8, 10, 14]). There was no significant interaction between group and day, F(10.63, 113.34) = 0.93, p = 0.517, but there was a significant main effect of day, showing that rats improved on this task over time, F(3.54, 113.34) = 9.85, p < 0.001. There was a significant overall difference between the groups, F(3, 32) = 5.03, p = 0.006. The B9-high group was not significantly different than the vehicle group, HSD(16) = 15.15, p = 0.098, but performed significantly worse than the sham group, HSD(16) = 23.94, p = 0.003. There was no difference between the B9-low group compared to the vehicle group, HSD(16) = 1.06, p = 0.998 or compared to the sham group, HSD(16) = 7.73, p = 0.617 (see Fig. 1).

3.2. Rotarod testing

The rotarod scores were analyzed in a 4 × 5 repeated measures ANOVA (Group [Sham, Vehicle, B9-low, B9-high] × Day [7–11]). There was no significant day by group interaction, F(12, 107.59) = 1.59, p = 0.119, but rats improved on the task over the testing period as shown by the main effect of day, F(4, 107.59) = 44.97, p < 0.001. There was a significant overall difference between the groups, F(3, 32) = 10.36, p < 0.001. Despite a large separation in latency between the B9-high group and vehicle group, HSD(16) = 80.07, p < 0.001. There was no significant difference between the B9-low group and the vehicle group, HSD(16) = 9.08, p = 0.929, but the
3.3. Morris water maze

The latencies from the reference memory paradigm of the Morris water maze (MWM) were analyzed in a 4 × 4 repeated measures ANOVA (Group [Sham, Vehicle, B9-low, B9-high] × Day [12–15]). Latencies decreased across the testing period, as shown by the main effect of day, $F(3, 96) = 57.72, p < 0.001$. There was no significant interaction between group and day, $F(9, 96) = 1.72, p = 0.095$. There was a significant overall difference between groups, $F(3, 32) = 3.30, p = 0.033$. However, there was no significant difference between the B9-high group and vehicle group, $HSD(16) = 4.38, p = 0.862$, or the B9-low group and sham group, $HSD(16) = 11.91, p = 0.167$. There was no significant difference between the B9-low group and vehicle group, $HSD(16) = 2.67, p = 0.964$ or between the B9-low group and sham group, $HSD(16) = 13.61, p = 0.092$ (see Fig. 3).

The latencies from the working memory paradigm of the MWM were analyzed in a 4 × 3 repeated measures ANOVA (Group [Sham, Vehicle, B9-low, B9-high] × Day [16–18]). There was no significant group by day interaction, $F(6, 64) = 1.78, p = 0.0117$, or main effect of day, $F(2, 64) = 1.35, p = 0.286$. There was no significant difference between the groups, $F(3, 32) = 2.73, p = 0.060$ (see Fig. 4).

3.4. Lesion analysis

The percent reduction score for the ipsilateral hemisphere was analyzed in a one-way ANOVA. There was a significant difference between the groups, $F(3, 32) = 98.27, p < 0.001$. There was no
3.5. Neuronal cell counts

The average percent reduction score from the neuronal cell counts in the CA3 region of the hippocampus were analyzed in a one-way ANOVA. There was no significant overall difference between the groups, $F(3, 32) = 2.88, p = 0.051$ (see Fig. 6).

The average percent reduction score from the neuronal cell counts in the VPN of the thalamus were analyzed in a one-way ANOVA. There was a significant overall difference between the groups, $F(3, 32) = 14.88, p < 0.001$. The B9-high group had significantly reduced VPN neurons compared to the vehicle group, $HSD(16) = 27.68, p = 0.002$ as well as compared to the sham group, $HSD(16) = 45.75, p < 0.001$. However, there was no significant difference between the B9-low group and vehicle group, $HSD(16) = 4.18, p = 0.933$ or between the B9-low group and the sham group, $HSD(16) = 13.89, p = 0.218$ (see Fig. 6).
Fig. 6. Panel A shows the locations selected for neuronal cell counts: the CA3 field of the hippocampus and the VPN of the thalamus. Panel B shows a graph of the neuronal reduction in the CA3 field of the hippocampus. Each injured group had substantial neuronal loss, but they were not different from each other. Panel C shows a 40× zoom of the VPN of the thalamus of an exemplar animal from each group. Panel D shows a graph of neuronal reduction in the VPN of the thalamus. The high dose folic acid group had a significant reduction in neurons compared to the vehicle by a 2.5-fold margin ($p = 0.002$).

Experiment 2

3.6. High-dose sham assessment

3.6.1. Bilateral tactile adhesive removal task

The averaged pre-injury scores for the bilateral tactile adhesive removal task were analyzed with an independent samples $t$-test. There was no significant difference between the B9-treated sham group and sham group, $t = -0.92, p = 0.377$. Post-injury performance was analyzed with a repeated measures ANOVA. There was no significant difference between the B9-treated sham group and sham group, $F(1, 13) = 3.83, p = 0.072$ (see Fig. 7).

3.6.2. Rotarod task

Performance on the rotarod task was analyzed with a repeated measures ANOVA. There was no significant difference between the B9-treated sham group and sham group, $F(1, 13) = 3.75, p = 0.075$ (see Fig. 7).

3.6.3. Morris water maze

Performance on the reference memory paradigm of the MWM was analyzed with a repeated measures ANOVA. There was no significant difference between the B9-treated sham group and sham group, $F(1, 13) = 2.90, p = 0.114$ (see Fig. 7). Performance on the working memory paradigm of the MWM was
Fig. 7. Panel A shows the performance of the two sham groups on the bilateral tactile adhesive removal task. There was no significant difference between the two groups ($p = 0.072$). Panel B shows the performance of the two sham groups on the rotarod task. There was no significant difference between the two groups ($p = 0.075$). Panel C shows a graph of the average number of cells in the VPN of the thalamus. The sham group that received high-dose B9 treatment had significantly less neurons than the normal sham group ($p = 0.002$). Panel D shows the performance of the two sham groups on the reference memory paradigm of the MWM. There was no significant difference between the groups ($p = 0.114$). Panel E shows the performance of the two sham groups on the working memory paradigm of the MWM. There was no significant difference between the groups ($p = 0.314$). Panel F shows a graph of the average number of cells in the CA3 region of the hippocampus. The sham group that received high-dose B9 treatment had significantly fewer neurons than the normal sham group ($p = 0.001$).

3.6.4. Neuronal cell counts

Cell counts from the CA3 fields of the hippocampi were averaged across hemispheres and analyzed with an independent samples $t$-test. The B9-treated sham group had a significantly reduced number of neurons compared to the sham group, $t = 4.41, p < 0.001$ (see Fig. 7). Cell counts from the VPN of the thalamus were averaged across hemispheres and analyzed with an independent samples $t$-test. The B9-treated sham group had a significantly reduced number of neurons compared to the sham group, $t = 3.76, p < 0.002$ (see Fig. 7).

4. Discussion

The results from this study suggest that administration of folic acid does not improve recovery of function in either an 80 μg/kg or 800 μg/kg dose. This finding stands in contrast to previously published work that showed benefits from folic acid in two different injury models (Iskandar et al., 2004; Naim et al., 2011). Additionally, the results from experiment 2 suggest that folic acid may even be toxic at high doses. In experiment 1, there was no beneficial effect of folic acid administration on the tactile adhesive removal task, rotarod task, the reference memory or the working memory paradigm of the MWM. Additionally, there were no reductions in lesion size and no neuronal sparing in the treated animals. Interestingly, rats treated with the high dose had a trend toward greater motor impairments on the rotarod task, though the difference was not significant ($p = 0.063$) as well as a trend towards greater somatosensory deficits on the bilateral...
tactile adhesive removal task, though the difference was not significant (0.098). In injured animals, the high dose of folic acid increased neuronal loss in the VPN of the thalamus and a trend towards increased lesion size was seen (p = 0.079). The increase in cell loss in the VPN is particularly interesting, because this area may mediate performance on sensorimotor behavioral tasks due to its role as a somatosensory relay station in the thalamus. It is also interesting to note that there were no differences between the treated groups and vehicle on either of the MWM tasks and no differences in cell loss in the CA3 field of the hippocampus, an area that has been shown to mediate spatial learning (Moser et al., 1993). In experiment 2, high-doses of folic acid administered to sham animals did not cause behavioral deficits, although there were trends towards impairment on the bilateral tactile adhesive removal task (p = 0.072) and the rotarod task (p = 0.075). However, when the brains of sham animals given the high dose were examined, they had fewer neuronal bodies in both the hippocampus and the VPN of the thalamus. Taken in conjunction with the trends towards poorer behavioral performance, this may indicate potential toxic effects of high-dose folic acid even in uninjured rats. The current study attempted to replicate effects seen in previous work utilizing folic acid after injury. Our results stand in contrast with the currently published literature. Previously, Naim and colleagues (2011) had shown that an 80 μg/kg dose of folic acid improved recovery on the first day after injury on three tasks. No improvement was seen at day four, when testing concluded. The testing window for treatment effects of folic acid suggested by that study falls somewhere between one and four days after injury. In the current study, testing began on day two post-CCI, with the bilateral adhesive removal task and no benefit of folic acid administration was seen on any day. This suggests that if there is a treatment effect for folic acid, it may be limited to the immediate, initial period following TBI.

Stronger beneficial results were found in a model of SCI (Iskandar et al., 2004). This original study, which established the dosing regimen for folic acid found improved axonal regeneration as well as locomotor recovery after SCI. In addition to the differences in mechanism between SCI and TBI, the Iskandar study also used a pretreatment regimen. In the current study, we wanted to assess the effectiveness of folic acid after TBI. Since a pretreatment was not evaluated, there is potential for increased benefit based on a pretreatment regimen. One of the large problems of comparing the current study to the Iskandar and colleagues study (2004) besides the large differences in injury models, is the disparity in behaviors tested. In the current study, we evaluated animals on tasks that assess sensory, motor and cognitive behaviors compared to the single locomotion score rated in the Iskandar study. Regardless of the differences, the mild effects seen in either of the previous studies and the lack of effect seen in the current study should call into question how effective folic acid may be as a treatment for TBI. Even given mild treatment effects, folic acid does not appear to show the large improvements that have been shown with other TBI treatments such as nicotinamide or progesterone (Hoane et al., 2008a; Shear et al., 2002). The low dose does not appear to provide substantial recovery of function, and high enough doses may even impair and contribute to neurotoxicity following brain injury.

Although it is by no means clear from this study how folic acid caused the impairments and cell loss from the high dose, one potential mechanism for folic acid-induced toxicity can be hypothesized from examining its role in the homocysteine-methionine cycle. Both folic acid and vitamin B12 participate in the homocysteine-methionine cycle. Increases in folic acid assist in demethylation of homocysteine into methionine via the 5-methyltetrahydrofolate pathway (Fench, 2001). Normally, reducing homocysteine levels is beneficial, however, increases in methionine may lead to increases in DNA methylation through the S-adenosyl methionine pathway (Achón et al., 2000). Preservation of methylation patterns is an important function of this pathway, but either hypo- or hyper-methylation can be detrimental for organisms (Murgatroyd et al., 2009). DNA methylation effectively silences genes by binding to and blocking sections of coding DNA. These large increases in DNA methylation may be detrimental following TBI as massive gene shifts are occurring in response to the injury and heavy methylation may prevent rapid changes in genes necessary to counteract detrimental processes (Hayes et al., 1995).

Though we see some potentially detrimental effects of high-dose folic acid following TBI, particularly in neuronal loss in the thalamus, it is important not to over-interpret the results. First, the high dose that was given to the rats in the current study is approximately double what the daily intake for a human is. Second, other studies have shown remarkably little effects of
very high dose folic acid administration in animals, even under high stress situations such as pregnancy (Achón et al., 2000; Sunder-Plassmann et al., 2000). It is also important to note that the administration in this study was a chronic dosing regimen. It is possible that folic acid may be beneficial in the short term, and that the daily dosing caused some of the deficits seen in the treated animals. It is also possible that there may be a more ideal dose of folic acid following TBI. The current study evaluated two ends of a continuum and did not test any dose-response relations between 80 and 800 μg/kg/day. However, the results do suggest that the upper end of that continuum should likely be avoided when considering treatments. While the current results are not very favorable for eventual translation of folic acid into a treatment for TBI, there is still some work to be done to examine its role following TBI. Particularly of interest is identifying a dose-response curve in terms of toxicity from folic acid following TBI. If a toxicity mechanism is identified, there is the potential to identify detrimental processes which could be targeted for inhibition by future drugs as well as increase our current knowledge of the mechanisms of TBI.

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Conflict of interest

The authors have no financial relationship or commercial interests based on the outcome of this study.

References


