Administration of Riboflavin Improves Behavioral Outcome and Reduces Edema Formation and Glial Fibrillary Acidic Protein Expression after Traumatic Brain Injury

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ABSTRACT

Previous studies have shown that administration of riboflavin, vitamin B₂, significantly reduced edema formation following experimental stroke. The present study evaluated the ability of B₂ to improve behavioral function, reduce edema formation, and limit glial fibrillary acidic protein (GFAP) expression following frontal cortex contusion injury. Groups of rats were assigned to B₂ (7.5 mg/kg) or saline (1.0 ml/kg) treatment conditions and received contusion injuries or sham procedures. Drug treatment was administered 15 min and 24 h following injury. Rats were examined on a variety of tests to measure sensorimotor performance (bilateral tactile removal test), and cognitive ability (acquisition of reference and working memory) in the Morris water maze. Administration of B₂ following injury significantly reduced the behavioral impairments observed on the bilateral tactile removal test and improved the acquisition of both reference and working memory tests compared to saline-treated rats. The lesion analysis showed that B₂ reduced the size of the lesion. Examination of GFAP expression around the lesion revealed that B₂ significantly reduced the number of GFAP⁺ astrocytes. Edema formation following injury was also significantly reduced by B₂ administration. These findings are the first to show that B₂ administration significantly improved behavioral outcome and reduced lesion volume, edema formation, and the expression of GFAP following traumatic brain injury. These findings suggest that B₂ may have therapeutic potential for the treatment of TBI.

Key words: Vitamin B₂; Recovery of Function; GFAP; Rat; sensorimotor behavior; antioxidant

INTRODUCTION

Vita-nutrient therapies (such as magnesium, creatine, and vitamin E) have been very effective in attenuating the behavioral deficits and secondary effects following traumatic brain injury (TBI) (McIntosh et al., 1989; Smith et al., 1993; Inci et al., 1998; Heath and Vink, 1999a–c; Bareyre et al., 2000; Sullivan et al., 2000; Hausmann et al., 2002; Hoane and Barth, 2002; Vink et al., 2003; Fromm et al., 2004; Hoane, 2004, 2005; van den Heuvel and Vink, 2004). One particularly interesting family of vita-nutrients is the water soluble B group vitamins. Recent research has show that one such vitamin, nicotinamide (vitamin B₃), has had neuroprotective
effects in both cerebral ischemia and TBI. Administration of B3 following cortical contusion injury (CCI) has also been shown to improve functional recovery and reduce glial fibrillary acidic protein (GFAP) expression in injured rats (Hoane et al., 2003). In that study, we administered B3 (500 mg/kg) or vehicle 15 min following CCI and found that B3 significantly reduced the magnitude of the initial behavioral deficits on a test of sensorimotor dysfunction (bilateral tactile adhesive removal) and on the acquisition of working memory in the Morris water maze (MWM). Administration of B3 also significantly improved acquisition of reference memory in the MWM and reduced the size of the lesion cavity and GFAP expression around the site of the injury compared to vehicle treated controls. Thus, initially it appears that B3 showed substantial preclinical efficacy following TBI.

It was originally shown that B3 was very effective in animal models of ischemia. Administration of B3 up to 6 h after injury has proven to be very effective in reducing infarct size and improving behavioral deficits in various models of ischemia (Klaidman et al., 1996; Ayoub et al., 1999; Modudai et al., 2000; Sakakibara et al., 2000; Maynard et al., 2001; Ayoub and Maynard, 2002; Sakakibara et al., 2002). B3 has also proven effective in reducing depletion of nicotinamide adenine dinucleotide (NAD+; precursor for ATP) and curbing lactate proliferation following injury (Ayoub et al., 1999), as well as protecting against oxidative stress-induced apoptosis (Klaidman et al., 1996; Mukherjee and Adams, 1997).

A second member of this group of B vitamins that may have therapeutic potential is riboflavin (vitamin B2). Although not as extensively studied as B3, B2 has been shown to reduce cerebral edema following focal ischemia (Betz et al., 1994). In that study, a dose of 7.5 mg/kg of B2 was administered 1 h prior to middle cerebral artery occlusion (MCAO) and cerebral edema was assessed after 4 h of ischemia. It was found that administration of B2 reduced cerebral edema, measured by cerebral water content, significantly by 48% or 21% compared to vehicle controls; depending on the specific model of ischemia used (transcranial electrocautery or intracarotid thread occlusion MCAO, respectively). The proposed mechanism of action for B2 is fairly well established. B2 is broken down to dihydroriboflavin by NADPH-dependent flavin reductase. Dihydroriboflavin quickly reduces oxidized iron, thus protecting cells from oxidative injury. The link between reduction of oxidative injury and recovery of function following injury has been established (Hall and Braughler, 1993; Sen et al., 1994; Hoane et al., 1997; Fiskum, 2000; Atlante et al., 2001).

Given the recent evidence that B3 administration following TBI and stroke has substantial preclinical efficacy it is reasonable to assume that B2 may also be therapeutically following TBI, especially given B3’s ability to reduce oxidative damage. Thus, the purpose of the present experiment was to examine the potential preclinical efficacy of B2 in a model of TBI. The CCI model was utilized to deliver a bilateral frontal CCI in rats and to examine the ability of B2 to improve functional outcome on a range of behavioral measures. In addition, the ability of B2 to limit injury-induced GFAP expression and cerebral edema was also examined.

MATERIALS AND METHODS

Subjects

Forty-one male Sprague-Dawley rats, approximately 3 months old were used for this experiment. All experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee and the study was conducted in a facility certified by the American Association for the Accreditation of Laboratory Animal Care. Rats were maintained on a standard 12-h light/dark cycle with food and water available ad lib.

Surgery

The surgical procedure was performed using aseptic procedures and conditions. The CCI model utilized in the present study was based on previous studies (Lindner et al., 1998; Hoane et al., 2003; Hoane, 2005). Rats were anesthetized with Brevital (50 mg/kg, i.p.) and then prepared for surgery. The total time under anesthesia was approximately 20 min. When the rat was unresponsive (no ocular or pedal reflexes) the head was shaved and then scrubbed with 70% alcohol followed by Betadine and placed into a stereotaxic device. Ocular and pedal reflexes were continuously monitored during surgery. A midline incision was made in the skin and underlying fascia. A circular craniotomy (6.0 mm) was performed using a Dremel motor tool and a specially designed drill bit that prevented the bit from damaging the meninges and cortex. The craniotomy was bilateral and centered 3.0 mm anterior to bregma exposing the cortical region containing the medial prefrontal cortex. The contusion injury was created with a sterile, stainless steel impactor tip (5.0 mm diameter) that was attached to a piston and activated with compressed air (2.5 m/sec). The impactor tip was positioned above the cortex and upon activation of the piston the impactor tip made contact with the cortex for 0.5 sec, resulting in a 2.0 mm compression of the cortex. Following the contusion any bleeding was controlled with sterile sponges soaked in cold saline and the incision was closed with nylon suture material. The wound was then treated with a triple antibiotic ointment.
maintain normal body temperature during surgery and recovery the rats were maintained on isothermic (BrainTree Scientific Inc.) heating units (37°C). Rats receiving sham surgeries were anesthetized, received the surgical prep, and placed into the stereotaxic device, given a midline incision, sutured and allowed to recover.

**Drug Administration**

Following injury rats received B2 (7.5 mg/kg, i.p; n = 7) or saline (0.9%, 1.0 ml/kg, i.p; n = 8) 15 min following injury. A second dose was administered 24 h following injury. A group of vehicle shams was also included (n = 8). The dose of B2 was chosen based on a previous study (Betz et al., 1994). The half-life of B2 in the rat has been shown to be 6.53 days (Wase, 1956). All behavioral testing and histological analysis was conducted without knowledge of drug assignment.

**Bilateral Tactile Adhesive Removal Test**

This procedure tests somatosensory dysfunction following injury and has been described in detail (Hoane et al., 2003, 2004). Rats were tested on two trials per test day on post-operative days 2, 4, 6, 8, and 10. Small adhesive rectangular patches (105 mm²) (Avery, 05412) were applied to the radial aspect of each forelimb. The rat was returned to the home cage and the latency to remove the stimuli was recorded. A trial ended when the rat either removed both patches or two minutes elapsed. The dependent measure of interest was the total latency to remove both stimuli.

**Cognitive Assessment**

The Morris water maze (MWM) was used to assess cognitive function following injury and has been based on previous studies (Lindner et al., 1998; Hoane et al., 2003). A blue fiberglass tank 1.83 m in diameter and 76 cm deep was filled with room temperature water to a depth of 32 cm. A 10 cm² clear Plexiglas platform was submerged 1.0 cm below the surface of the water, resulting in an invisible escape platform. The San Diego Instruments video tracking system with SMART tracking software was used to track and record the movements of the white rat along the dark background.

Reference memory trials were conducted starting on post-operative day 12 and lasted for 5 days. For reference memory testing the platform was always located in the center of quadrant 1, halfway between the center of the tank and the outside wall. Rats were inserted into the water at one of four randomly chosen release points. Each day of testing consisted of four trials, one at each of the various starting points. Trials were terminated upon the rat reaching the platform or after 90 sec if the rat did not reach the platform. Rats that did not reach the platform were guided to the platform and allowed to escape and remain on the platform for ten seconds. The ITI was ~15 min. The swim distance and latency was determined for each trial.

Working memory trials, or repeated acquisition trials, were conducted following the reference memory trials starting on post-operative day 18. During working memory testing the escape platform was located in a new, randomly chosen location each day (quadrants 2, 3, or 4). Rats were given 4 trials per day (ITI 15 min) for 3 days. The swim latency and distance for trials 2, 3, and 4 were used for the analysis. Trial 1 was considered an information trial for the rat and was not used to determine working memory.

**Histology**

At 35 days post-lesion, rats were anesthetized with Nembutal (100 mg/kg, i.p.) and transcardially perfused with 0.9 % phosphate buffered saline followed by 10% phosphate buffered formalin. Brains were removed from the cranium and post-fixed in formalin. Brains were cryopreserved in 30% sucrose for 3 days prior to sectioning. The brains were sectioned frozen (40 μm) on a sliding microtome and serial sections were collected into a cryopreservative solution for storage and frozen.

**Lesion Analysis**

A series of sections were mounted on gelatin subbed microscope slides and stained with cresyl violet. The slides will then dehydrated and cover slipped. The extent of the injury was examined by analyzing the amount of remaining cortical tissue surrounding the lesion cavity using a video-assisted Olympus microscope (BX-51). Brain sections at the level of the lesion were captured using a digital capturing system (DP-70 digital camera) and the area of the remaining cortex was measured using ImageTool Software. Total remaining cortical volume was calculated based on the Cavalieri method (Coggeshall, 1992). The mean area of cortex at each of four bregma coordinates surrounding the lesion (4.2, 3.2, 2.2, and 1.2) was multiplied by the section thickness (40 μm) and by the number of total sections.

**GFAP Immunohistochemistry**

A second series of sections were used to label reactive astrocytes (Hoane et al., 2003). Sections were removed from cryoprotectant and washed with 0.1 phosphate buffer (PB) and processed for GFAP immunoreactivity using a free floating protocol. Tissue sections were incubated with a GFAP rabbit anti-cow primary antibody (DAKO, Z334, diluted 1:3000) for 48 h, rinsed in PBS +
The number of GFAP stained with hematoxylin, dehydrated, and cover slipped were mounted on subbed microscope slides, counter-then increased to 40/H11003 the tissue surrounding the lesion were viewed at 4
determined using cell counts. Eight sites (Fig. 7) from the total number of GFAP for the analysis.

sulfate, 0.05% DAB, and 0.01% H2O2. Tissue sections containing acetate-imidizole buffer, 2.5% nickel ammonium error rate stable, thus not inflating á due to multiple comparisons (Levin et al., 1994). Anatomical data were analyzed with one-way ANOVA’s and Fischer’s LSD. Eighteen additional animals were used for the edema analysis, resulting in n = 6 per group. For all statistical tests, a p value of <0.05 was considered significant. All data are shown as mean ± SEM.

Edema Analysis

Edema formation was assessed by measuring the tissue water content in the brains at the site of injury. At 48 h after injury, the animals were sacrificed with CO2 and their brains were rapidly harvested. The frontal pole of the brain containing the injured cortex was cut into a 4.0-mm coronal slab using a brain mold and was placed on a cold plate. Tissue punches (4.0 mm) were made with a steel biopsy punch (Roboz Instruments) through the injury core in the cortex or equivalent tissue in uninjured rats and placed into preweighed 1.5-mL tubes. The tubes were then re-weighed, and then uncapped and placed in an oven at 65°C for 48 h. Tubes were then removed from the oven, allowed to return to room temperature, and then weighed. Water content was determined by (wetdry)/wet.

Statistical Analysis

Analysis of variance (ANOVA) tests were performed using the procedures for general linear models (SPSS 8.0 for Windows) with options for repeated measures where appropriate. The between groups factors were treatment (B2 or saline) and injury (CCI or sham) and the within factor was day of testing. Huyn-Feldt (HFP) probabilities were used for the assessment of the repeated measures factor. The HFP is a more conservative estimate of probability, which reduces the problems associated with repeated measures factors. The statistical analysis revealed no significant differences between the two groups of shams (saline [n = 4] and B2 [n = 4]) on any of the behavioral tests (p > 0.05). Therefore, the sham-saline and sham-B2 groups were combined to form one sham group. One animal from the injured-B2 group died following injury. This resulted in a two-factor ANOVA consisting of group (B2 [n = 7], saline [n = 8], or sham [n = 8]) and day of testing which was used for the analyses. Post-hoc analyses were conducted using Fischer’s least significant difference Test (LSD) for comparison of means. Given that k (number of groups) = 3 in the present experiment, it has been shown that Fischer’s LSD is the best procedure for holding the family-wise (Type I) error rate stable, thus not inflating á due to multiple comparisons (Levin et al., 1994). Anatomical data were analyzed with one-way ANOVA’s and Fischer’s LSD. Eighteen additional animals were used for the edema analysis, resulting in n = 6 per group. For all statistical tests, a p value of <0.05 was considered significant. All data are shown as mean ± SEM.

RESULTS

Bilateral Tactile Adhesive Removal Test

The latency to remove the tactile stimuli was analyzed in a 3 × 5 ANOVA including groups (B2, saline, sham) and test day (1–5) as factors in the analysis, treating day as a repeated measure. Rats became more efficient in removing the tactile stimuli on successive trials; the main effect for day was statistically significant [F (2,20) = 26.55, p < 0.001] (Fig. 1). Bilateral contusions produced significant effects in the latency to remove tactile stimuli; the main effect of group was statistically significant [F (2,20) = 26.55, p < 0.001]. There was a significant difference in the rate of recovery; the group × day interaction was significant [F (6.97,69.67) = 3.812, p < 0.002]. In order to examine the effect of B2 treatment the following post-hoc comparison was conducted with Fischer’s LSD test (B2 + saline) for each day. This analysis revealed that treatment with B2 significantly reduced the lesion deficit on test days 2, 4, and 6 compared to saline treatment ([LSD (14) significantly reduced the lesion deficit on test days 2, 4, and 6 compared to saline treatment ([LSD (14) = 35.21, p < 0.001]; [LSD (14) = 19.74, p < 0.001]; [LSD (14) = 24.36, p < 0.001, respectively).

Cognitive Assessment: Reference Memory

The latency to reach the hidden platform was analyzed in a 3 × 5 ANOVA, including groups (B2, saline, sham) and test days (1–5) as factors in the analysis, treating day as a repeated measure. Rats became more efficient at finding the platform on successive trials; the main effect for latency was statistically significant [F (3.52,70.40) = 47.018, p < 0.001] (Fig. 2). Bilateral contusions produced a significant difference in the severity of the reference memory deficit; the main effect of group was statistically significant [F (2,20) = 4.67, p < 0.03]. There
were no differences in the rate of acquisition; the group ×
day interaction was not significant [F (7.04,70.40) =
1.53, \( p > 0.17 \)]. In order to examine the effect of B$_2$
treatment the following post-hoc analysis was performed on
the significant main effect for group utilizing Fischer's
LSD. This analysis revealed that B$_2$ significantly in-
creased the acquisition of reference memory compared
to treatment with saline on test days 3 and 5 ([LSD (14) =

**FIG. 1.** The effect of a regimen of vitamin B$_2$ or saline administered following bilateral frontal CCI or sham surgery on the bi-
lateral tactile adhesive removal test. Treatment with vitamin B$_2$ significantly reduced the removal deficit compared to saline treatment (**\( p < 0.001 \)).

**FIG. 2.** The effect of a regimen of vitamin B$_2$ or saline administered following bilateral frontal CCI or sham surgery on ac-
quisition of a reference memory task in the MWM (A), data collapsed across all trials (B). Treatment with vitamin B$_2$ signifi-
cantly improved the acquisition of reference memory compared to saline treatment (**\( p < 0.05 \)).
The latency to reach the hidden platform was analyzed in a $3 \times 3$ ANOVA including groups (B2, saline, sham) and test days (1–3) as factors in the analysis, treating day as a repeated measure. Rats did become more efficient at finding the platform on successive trials; the main effect for day was statistically significant [$F(2.00,40.00) = 6.176, p < 0.006$] (Fig. 3). Bilateral contusions produced significant deficits in the acquisition of working memory; the main effect of group was statistically significant [$F(2,20) = 4.330, p < 0.03$]. There was a significant difference in the rate of acquisition; the group x day interaction was significant [$F(4.00,40.00) = 2.693, p < 0.05$]. In order to examine the effect of B2 treatment the following post-hoc analysis was performed on the significant main effect for group utilizing Fischer’s LSD. This analysis revealed that B2 significantly increased the acquisition of working memory compared to treatment with saline on test days 1 and 2 ($[\text{LSD}(14) = 19.36, p < 0.01]; [\text{LSD}(14) = 24.71, p < 0.05]$, respectively). The distance to reach the hidden platform was also analyzed in a $3 \times 5$ ANOVA and resulted in similar effects (data not shown).

**Lesion Analysis**

The volume of the remaining cortex surrounding the lesion cavity was analyzed in a one-factor ANOVA including group (saline and B2) as the factor in the analysis. There was a significant difference in the size of the lesion; the main effect of group was statistically signifi-

**Cognitive Assessment: Working Memory**

The effect of a regimen of vitamin B2 or saline administered following bilateral frontal CCI or sham surgery on acquisition of a working memory task in the MWM. Treatment with vitamin B2 significantly prevented the occurrence of working memory deficit compared to saline treatment ($^* p < 0.05$; $^{**} p < 0.01$).

FIG. 3. The effect of a regimen of vitamin B2 or saline administered following bilateral frontal CCI or sham surgery on acquisition of a working memory task in the MWM. Treatment with vitamin B2 significantly prevented the occurrence of working memory deficit compared to saline treatment ($^* p < 0.05$; $^{**} p < 0.01$).

FIG. 4. The effect of a regimen of vitamin B2 or saline administered following bilateral frontal CCI or sham surgery on lesion volume. Treatment with vitamin B2 significantly reduced the volume of the lesion compared to saline treatment ($^* p < 0.05$).
The reduction in lesion size can be seen in Figure 5.

**GFAP Analysis**

The number of GFAP$^+$ cells were analyzed in a one-factor ANOVA, including group (B$_2$, saline, sham) as the factor in the analysis. There was a significant difference in the number of GFAP$^+$ cells around the lesion; the main effect of group was statistically significant [$F(2,22) = 40.460, p < 0.001$] (Fig. 6). Post-hoc analysis with Fischer’s LSD was performed to determine if there was a difference between the saline-treated rats and the B$_2$-treated rats. This analysis revealed that treatment with B$_2$ significantly reduced the expression of GFAP around the lesion [LSD (16) = 59.38, $p < 0.016$]. This effect is observable in the photomicrograph shown in Figure 7.

**Edema Analysis**

The effect of B$_2$ on edema formation was analyzed in a one-factor ANOVA including group (B$_2$, saline, sham) as the factor in the analysis. There was a significant difference in the formation of edema; the main effect of group was statistically significant [$F(2,17) = 44.927, p < 0.001$] (Fig. 8). Post-hoc analysis with Fisher’s LSD was performed to determine if there was a difference between the saline-treated rats and the B$_2$-treated rats. This analysis revealed that treatment with B$_2$ significantly reduced the formation of edema [LSD (11) = 2.44, $p < 0.001$].

**DISCUSSION**

The results from this study have shown that administration of B$_2$ following CCI of the frontal cortex significantly improves recovery of function following injury.
The bilateral tactile adhesive removal test showed that administration of B2 following injury significantly reduced the magnitude of the behavioral deficit and improved the subsequent recovery on this test. The acquisition of a reference memory task in the MWM was also significantly facilitated compared to saline-treated rats following injury. In a similar manner, B2 administration also improved the working memory deficit in the MWM. The improvements in behavioral function following injury in the present study are consistent with, and very similar to, the results seen with the administration of B3 in the CCI model (Hoane et al., 2003). This appears to be the first study to show that administration of B2 improves behavioral performance following either TBI. These results suggest that B2 has substantial preclinical efficacy following injury.

The beneficial effects of B2 were also supported by the anatomical analyses conducted in this experiment. Treatment with B2 significantly reduced the extent of the lesion compared to saline treatment. The analysis of reactive astrocytosis in the tissue surrounding the lesion cavity also revealed a significant effect. Administration
of B2 following injury significantly reduced the number and size of GFAP+ cells in the tissue around the lesion cavity, suggesting a reduction in tissue damage at the site of injury. These findings are consistent with previous research that has shown that administration of B3 reduced lesion size and reactive gliosis following CCI and ischemia (Ayoub et al., 1999; Modudai et al., 2000; Sakakibara et al., 2000, 2002; Maynard et al., 2001; Ayoub and Maynard, 2002; Hoane et al., 2003). Furthermore, the analysis of cortical edema also revealed that B2 significantly reduced the amount of post-injury water content in the tissue. This effect is consistent with the ability of B2 to reduce tissue water content “edema” following ischemia (Betz et al., 1994).

The present study has shown that the ability of B2 to improve functional outcome following TBI is associated with its ability to limit injury-induced pathophysiological changes. Improvements in behavioral function and in the reduction of cortical tissue loss, GFAP expression and edema formation were all observed in the present study. The links between TBI and free radical generation have been well established (Hall and Braughler, 1993; Sen et al., 1994; Hoane et al., 1997; Fiskum, 2000; Atlante et al., 2001), including the link between iron, free radicals, and oxidative injury (McCord, 2004). There is strong evidence in the literature that the mechanism of action for B2 is its ability to reduce oxidative damage, especially mediated through oxidized iron. NADPH-dependent flavin reductase is the enzyme that reduces riboflavin into dihydroriboflavin. Dihydroriboflavin then reduces heme-proteins that contain higher oxidative states of iron, such as Fe(IV)O and Fe(V)O, which have been shown to be associated with oxidative tissue damage (Hultquist et al., 1993; Betz et al., 1994). These iron containing heme-proteins contribute to cellular injury and administration of B2 protects the heart, lung, and brain from ischemic and reperfusion injury (McCord, 2004). Thus, in the present study the administration of B2 following injury may have had its beneficial effects by scavenging oxygen free radicals (Christensen, 1993). Recent research has demonstrated the reactivity of B2 with organic radicals (Ksendzova et al., 2004) and the ability to protect murine cerebellar granule cells from glutamate toxicity (Lin et al., 2004).

As mentioned, the beneficial effects observed in the present study appear to be very similar to the effects we have shown with B3 using the same model of TBI (Hoane et al., 2003). However, B3 provided a much stronger reduction in the initial magnitude of injury deficit on the bilateral tactile removal and working memory tests than B2 did. Although the behavioral effects appear similar these B vitamins have different mechanisms of action. The mechanism of action most attributed to B2 is based on free radical scavenging (see above); whereas, with B3 the mechanisms that may limit brain injury include the prevention of depletion of nicotinamide adenine dinucleotide (NAD+) and prevention of ATP depletion (Ayoub et al., 1999), poly-ADP-ribose polymerase (PARP) inhibition (Ayoub et al., 1999; Sakakibara et al., 2002), and inhibition of lipid peroxidation (Klaidman et al., 1996; Mukherjee and Adams, 1997). Changes in blood pressure, heart rate, or hemodilution have not been shown to be the cause of B3’s beneficial effects in ischemia models (Modudai et al., 2000). In a similar manner it is unlikely that peripheral effects of B2 accounted for the reduction of edema in the present study. Administration of flavin mononucleotide (FMN), the phosphorylated form of B2, which does not cross the blood–brain barrier had no effect on cerebral edema following ischemia (Betz et al., 1994) This also suggests that the beneficial effects on recovery of function and tissue loss observed following CCI may not be attributed to changes in peripheral actions (e.g., blood pressure or heart rate).

The results of this study have shown that administration of B2 following TBI produces significant improvements in behavioral performance and reduces lesion size, GFAP expression, and cerebral edema. A single study by itself does not establish pre-clinical efficacy; however, our findings clearly show that B2 as a treatment for TBI is intriguing and needs further examination. Given the present data and the recent B3 data (Hoane et al., 2003), it appears that the B group vitamins may be a promising treatment avenue for brain injured patients.

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