Evaluation of combined fibroblast growth factor-2 and moderate hypothermia therapy in traumatically brain injured rats

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Abstract

Both the exogenous administration of fibroblast growth factor-2 (FGF-2) or the induction of moderate hypothermia have been shown to attenuate histopathology and improve functional outcome after traumatic brain injury (TBI). Since combined therapeutic strategies may be more beneficial than single therapies, we examined the potential synergistic effect of FGF-2 combined with moderate hypothermia treatment induced 10 min after TBI on functional and histological outcome following controlled cortical impact (CCI) injury. Fifty male Sprague-Dawley rats were randomized to one sham and four CCI treatment groups: Sham, VEH; FGF-2 (45 μg/kg/h for 3 h i.v.); Normothermia (37±0.5°C); FGF-2+Hypothermia (32±0.5°C for 3 h); VEH+Norm; VEH+Hypo. Vestibulomotor performance on the beam balance and beam-walk (BW) tasks on post-operative days 1–5 and spatial memory acquisition in the Morris water maze (MWM) on days 14–18 were assessed. After 4 weeks survival, histological evaluations (CA and CA cell counts and lesion volume) were performed. MWM performance improved in all treatment groups, but combined treatment was not more efficacious than either alone. The FGF-2+Hypo group performed significantly better than the other injured treatment groups in the BW task. Lastly, no significant group differences in beam balance or histological outcome were observed. These data suggest a suboptimal and incomplete synergy of combined FGF-2 and hypothermia treatment. These data may indicate that either our dose of FGF-2 or combination therapy was not optimized in our model.

Theme: Disorders of the nervous system
Topic: Trauma
Keywords: Controlled cortical impact; Neurotrophin; MWM

1. Introduction

The experimental evidence that moderate hypothermia is neuroprotective when given before or even after traumatic brain injury (TBI) or cerebral ischemia is overwhelming (for general review see [14,35,36]) and occurs across a variety of different models. Specifically, hypothermia has improved outcome following experimental TBI via central or lateral fluid percussion [5,9,34], weight drop-induced diffuse injury [29], and controlled cortical impact (CCI) [7,18,36]. While some TBI studies have shown that hypothermia after TBI reduces both neuronal death and axonal injury [6,13] while providing functional benefit [5], others have shown that moderate hypothermia initiated following CCI improves motor and spatial memory recovery despite no significant effect on structural pathology as reflected by cortical necrosis or hippocampal cell survival [18]. Thus, hypothermia may independently reduce both lethal and sublethal TBI damage. Despite positive preliminary reports using moderate hypothermia in clinical TBI [8,36], recent results from the multicenter National Brain Injury Study: Hypothermia (NABIS:H) clinical trial appear disappointing (personal communication). The results suggest that more refinement of the clinical application of hypothermia to severe TBI is needed and perhaps the beneficial actions of hypothermia need to

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be better clarified and enhanced while minimizing potential nonspecific effects, which can reduce therapeutic efficacy. Consequently, the best protocol and patient populations for the use of hypothermia in clinical TBI remain to be defined and optimized. One approach that may prove useful is to combine other treatments with hypothermia that are independently effective and have other beneficial specific mechanistic actions. The use of exogenous trophic factors is one class of candidates, especially since there is evidence that TBI alters neurotrophin receptors after injury [39] and hypothermia treatment can reduce the endogenous neurotrophic response of the brain after experimental CCI [11,23,24].

A number of studies in experimental TBI have shown that neurotrophins attenuate functional deficits or histopathology [16,44–46]. One of several neurotrophic factors, fibroblast growth factor-2 (FGF-2) has been shown protective in a number of in vitro injury models [33,37,52], and in vivo models such as fluid percussion TBI [12,25,38], stroke [27,28], and spinal cord injury [31,40]. Importantly, FGF-2 is effective when administered after injury, and retards functional recovery when its endogenous expression is inhibited [42].

Given the previous beneficial effects of FGF-2, we combined FGF-2 treatment with moderate hypothermia therapy to determine if synergistic effects occur by evaluating the effect of individual or combined FGF-2 and moderate hypothermia therapy on motor, cognitive, and histological outcome following a moderate CCI injury in rats.

2. Material and methods

2.1. Subjects

Fifty adult male Sprague–Dawley rats weighing 370–400 g on the day of surgery were used. Animals were group-housed (two per cage) in standard steel/wire mesh cages with ad libitum access to food and water. All procedures carefully conformed to the guidelines outlined in the Guide for the Care and Use of Laboratory Animals from the U.S. Department of Health and Human Services and were approved by the University of Pittsburgh Institutional Animal Care and Use Committee. The animals were randomized to one sham or four CCI groups: Sham+ vehicle (VEH); FGF-2 (45 μg/kg/h for 3 h i.v.) + Normothermia (37±0.5°C); FGF-2 (same as above) + hypothermia (32±0.5°C for 3 h); VEH+Norm; VEH+ Hypo.

2.2. Surgical and injury protocols

Prior to surgery, previously trained animals were tested on both the beam balance and beam walking tasks to establish a baseline measure. Anesthesia was induced with 4% isoflurane (Anaquest, Memphis, TN) in oxygen and the rats intubated with a 14-gauge angiocatheter and mechanically ventilated with 2.0% isoflurane/66% N₂O/balance O₂ for surgery. Venous and arterial catheters were placed in the tail for FGF-2 infusion and the continuous monitoring of blood pressure and arterial glucose and blood gas sampling. A rectal probe was inserted for continuous monitoring of body temperature, and a microprobe thermistor (Type MT-29/1, Physiotemp Instruments Inc., Clifton, NJ) was inserted into a temporalis muscle for indirect monitoring of brain temperature. A midline incision was made, the soft tissues reflected, and a 7 mm craniotomy was made between lambda and bregma and centered 5 mm laterally on the right side of the central suture. The rats received a CCI through the craniotomy at a velocity of 4 m/s with a tissue deformation depth of 2.6 mm. Shams underwent identical surgical procedures, but did not receive the impact. After injury, the scalp incision was closed with interrupted nylon sutures and the rat was removed from the injury device and returned to the heating pad. The rat was stabilized on anesthesia for 30 min after which arterial blood gas (ABG), glucose, and hematocrit were checked just before injury. Mean arterial pressure was kept above 90 mm Hg, brain and body temperature were kept at 37±0.5°C, and pCO₂ at 40±5 mm Hg.

2.3. Injury device

The CCI injury device [15] consisted of a small (1.975 cm) bore, double-acting, stroke-constrained, pneumatic cylinder with a 5.0 cm stroke. The cylinder was rigidly mounted in an angled vertical position on a crossbar. The lower rod end had an impactor tip attached (i.e., that part of the shaft that comes into contact with the exposed dura) and the upper rod end was attached to the transducer core of a linear velocity displacement transducer (LVDT). The velocity of the impactor shaft was controlled by gas pressure and measured directly by the LVDT (Shaevitz Model 500 HR; Macro Sensors, Pennsauken, NJ), which produced an analog signal that was recorded by a PC-based data acquisition system (Axoscope; Axon Instruments, Inc., Foster City, CA) for analysis of time/displacement parameters of the impact.

2.4. FGF-2 infusion and hypothermia induction

Ten minutes after injury, rats randomized to the hypothermic groups were cooled to a brain temperature of 32±0.5°C with ice packs; those randomized to normothermic groups were maintained at 37±0.5°C with a heating pad. Hypothermic rats were maintained at 32±0.5°C for 3 h, and then gradually (over a 1 h period) rewarmed to 37±0.5°C. Normothermic rats were maintained at 37±0.5°C for 4 h, beginning 10 min post injury, as were the sham controls. Infusion of FGF-2 began 30 min after injury for 3 h at a dose of 45 μg/kg/h for a total dose of
135 μg/kg of FGF-2. The sham rats were infused with vehicle at the same rate and for the same time as the CCI groups. All lines were subsequently removed at 4 h, anesthesia was discontinued, and the animals were allowed to regain consciousness before being returned to their home cages.

2.5. Functional assessments

2.5.1. Motor performance

Gross vestibulomotor function was assessed on a beam-balance task that consisted of placing the animal on a suspended, narrow wooden beam (1.5 cm wide) and recording the duration it remained on the beam for a maximum of 60 s. Training prior to injury consisted of three trials, during which baseline measurements were taken. More complex vestibulomotor function and coordination were assessed using a modified beam-walking task. Briefly, this task consisted of training rats to escape a bright light and high decibel white noise (Lafayette gelatinized glass slides. One set of sections was stained

2.5.2. Cognitive performance

A Morris water maze task variant was used to compare acquisition rates between groups. The maze consisted of a plastic pool (180 cm in diameter and 60 cm in depth) filled with water to a depth of 28 cm with a clear Plexiglas stand (10 cm in diameter and 26 cm high, i.e., 2 cm below the water’s surface) used as the hidden goal platform. The pool was located in a 2.5×2.5 m room with numerous extra-maze cues (e.g., posters) that remained constant throughout the experiment. Water maze testing began on day 14 post-injury in order to avoid possible confounds with the motor deficits observed in the first few days following injury. The rats were given four trials per day for five consecutive days to assess performance. For each daily block of four trials, the subjects were placed in the pool facing the wall. Trials were initiated from each of the four possible start locations (north, east, south, west) in a randomized manner. The goal platform was positioned 45 cm from the outside wall and was placed in the north east, south east, south west, or north west quadrant of the maze. The location of the platform was held constant for each animal. A maximum of 120 s was allowed to each rat to find the hidden platform. If the rat failed to find the platform within the allotted time, it was placed on the platform by the experimenter where it remained for 30 s before being placed in a heated incubator between trials (4 min intertrial interval).

2.6. Tissue preparation

Four weeks after injury, animals were deeply anesthetized with pentobarbital (Nembutal, 80–100 mg/kg; Abbott Laboratories, North Chicago, IL) and transcardially perfused with 100 ml of 0.1 M phosphate buffered saline with 5 U/ml heparin pH 7.4, followed by 500 ml 4% paraformaldehyde. After perfusion, the brain was removed and immersed in 4% paraformaldehyde in 0.1 M phosphate buffer pH 7.4 at 4°C overnight. The brain was next transferred to 15% sucrose in 0.1 M phosphate buffer pH 7.4 at 4°C for 24 h then to 30% sucrose in 0.1 M phosphate buffer pH 7.4 at 4°C until sunken. The cryoprotected rat brain was frozen and two series of 7 μm thick coronal sections were cut on a cryostat (Jung CM 1800; Brodersen Instruments, Valencia, PA) and mounted on gelatinized glass slides. One set of sections was stained with Cresyl violet for morphological analysis and the other set was prepared for immunohistochemistry.

2.6.1. Morphology

Treatment efficacy on the survival of vulnerable neurons in the hippocampal CA1 and CA3 regions was evaluated from coronal sections underlying the area of contusion, approximately 3.5 mm posterior to bregma, using a double blind analysis for each of the following groups (Sham+VEH=6; FGF-2+Norm=4; VEH+Norm=4; FGF-2+Hypo=4, and VEH+Hypo=5). To reduce counting errors associated with false positive identification of dying neurons, the total number of CA1 and CA3 surviving neurons were counted using a Nikon Eclipse E600 microscope (Nikon Corporation, Tokyo, Japan). All data are reported as the percent of total percent neurons in the ipsilateral (injured) CA1 and CA3 regions relative to the contralateral side. Cortical volume was determined by outlining both the contralateral and ipsilateral (injured) hemispheres (MCID, Imaging Research, Ontario, Canada) then subtracting the area of the ipsilateral hemisphere from the contralateral and summing the differences to calculate a mean volume.

2.6.2. Immunohistochemistry

Glial fibrillary acidic protein (GFAP) immunohistochemistry was conducted on paraffin-embedded tissue. Sections were pre-blocked with 10% normal rabbit serum (NRS) and 0.1% Triton X-100 in 0.1 M PBS (TX-PBS). Sections were incubated with primary antibody, mouse anti-GFAP monoclonal antibody (1:3000; Chemicon International, Temecula, CA) with 5% NRS and TX-PBS at 4°C for 16–24 h. Affinity-purified rabbit anti-mouse IgG (1:50; Jackson ImmunoResearch Laboratories, West Grove, PA) was incubated as secondary antibodies with 5% NRS and TX-PBS at 4°C for 2 h on a shaker. Mouse peroxidase– anti-peroxidase (PAP) soluble immune complexes (20 g/
ml; Jackson ImmunoResearch Laboratories, West Grove, PA) were used to visualize immunoreactivity with 2% NRS and TX-PBS at 4°C for 3 h. Tissue slides were rinsed between all steps with TX-PBS three times for at least 10 min each time. The peroxidase reaction was developed with DAB Substrate Kit (Vector; Burlingame, CA) until a dark brown reaction product was evident. Sections were rinsed in water, dehydrated in alcohols, defatted in xylenes, and coverslipped for light microscopic analysis. Some sections were counterstained with hematoxylin (Vector; Burlingame, CA). At least three sections through the hippocampus of each animal were processed for immunostaining. Control experiments were run in parallel to confirm specificity. Primary antibody was omitted and polyclonal antibody to GFAP (DAKO, Denmark) was used for comparison.

2.7. Statistical analysis

Analyses were performed using Statview software on a Macintosh computer. Behavioral data were analyzed using repeated-measures analysis of variance (ANOVA). If a significant effect was found with the ANOVA, individual group comparisons were made with Fisher’s PLSD post-hoc tests. Histological and physiological data were analyzed with unpaired t-tests. Data were expressed as mean±S.E.M. and a significance level of P<0.05 was used.

3. Results

3.1. Physiology

Arterial blood gases were measured at 10, 30, 60, 120, 180 and 240 min post injury (Table 1). Mean arterial blood pressure (MABP) was never below 90 mmHg in any rat although some slight group differences were seen (Fig. 1). As seen in Table 1, slight elevations in blood glucose levels were observed during hypothermia before returning near baseline after rewarming (240 min after TBI) in both hypothermic groups. Hypothermia is known to increase circulating catecholamines [26] which is likely responsible for the slight increase in glucose seen in our hypothermic rats.

3.2. Motor performance

A repeated-measures ANOVA on beam balancing ability revealed a significant group difference ($F_{4,45}=4.066$, $P=0.0067$), a significant time (days) difference ($F_{3,225}=28.397$, $P<0.0001$), and a significant group×day interaction ($F_{20,225}=3.614$, $P<0.0001$). Post-hoc analysis revealed that all CCI rats had motor deficits compared to the Sham+VEH group ($P<0.001$). However, there were no significant differences among the injured groups (Fig. 2). In contrast, a repeated measures ANOVA of the beam walking task also showed a significant group ($F_{4,45}=17.181$, $P<0.0001$), time ($F_{5,225}=86.633$, $P<0.0001$), and

<table>
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30 min
| pH | 7.44±0.01 | 7.44±0.01 | 7.45±0.01 | 7.46±0.01 | 7.46±0.00 |
| pCO₂ | 38.8±0.5 | 38.9±0.7 | 36.4±0.7 | 37.3±0.6 | 36.1±0.3 |
| pO₂ | 174.2±5.1 | 178.7±4.3 | 181.6±4.5 | 201.1±3.5 | 198.2±4.1 |
| pH | 160.4±5.9 | 163.4±6.6 | 158.3±5.2 | 214.2±12.2* | 191.9±10.3* |

120 min
| pH | 7.44±0.01 | 7.42±0.01 | 7.42±0.01 | 7.44±0.01 | 7.44±0.01 |
| pCO₂ | 38.7±0.5 | 38.4±1.1 | 38.9±0.8 | 37.9±0.5 | 37.3±0.8 |
| pO₂ | 179.5±3.5 | 175.0±3.5 | 176.4±5.3 | 198.0±4.5 | 197.5±3.0 |
| pH | 159.7±6.2 | 151.6±7.1 | 153.4±4.6 | 209.3±16.5* | 193.1±15.1* |

240 min
| pH | 7.42±0.01 | 7.41±0.01 | 7.42±0.01 | 7.40±0.02 | 7.39±0.01 |
| pCO₂ | 39.7±0.7 | 39.0±0.8 | 38.0±0.9 | 40.2±0.8 | 40.5±1.0 |
| pO₂ | 176.7±4.6 | 175.0±4.2 | 175.6±2.5 | 175.7±4.4 | 170.8±5.5 |
| pH | 160.2±4.2 | 148.8±7.4 | 154.7±3.8 | 200.1±21.3* | 163.5±10.3* |

*Both hypothermia groups are significant over time, as well as over all other groups (P<0.05). Data at 10, 60, and 180 min not shown, but are consistent with other intra group findings. p. Glu=plasma glucose.
group×day interaction ($F_{20.225}=6.488$, $P<0.001$). Post-hoc analysis showed that the combined FGF-2+hypo group displayed significantly improved beam walking function compared to the other groups ($P<0.0042$ vs. FGF-2+Norm, $P<0.0021$ vs. VEH+Norm, and $P<0.0153$ vs. VEH+Hypo).

### 3.3. Cognitive performance

A repeated measures ANOVA revealed significant differences in the latency to find a hidden platform among groups ($F_{4.45}=9.42$, $P<0.0001$) and time ($F_{4.180}=37.869$, $P<0.0001$), but there was no significant group×day interaction ($F_{5.648}=6.488$, $P<0.001$). Post-hoc analysis showed that the combined FGF-2+hypo group displayed significantly improved beam walking function compared to the other groups ($P<0.0042$ vs. FGF-2+Norm, $P<0.0021$ vs. VEH+Norm, and $P<0.0153$ vs. VEH+Hypo).

![Graph showing mean arterial blood pressure (MABP) data before and 4 h after the injury in all the animal groups. Values are mean (±S.E.M.).](image1)

**Fig. 1.** Mean arterial blood pressure (MABP) data before and 4 h after the injury in all the animal groups. Values are mean (±S.E.M.).

![Graph showing mean (±S.E.M.) beam walk latency scores before, and Days 1–5 following, CCI injury. Injured animals treated with 135 μg/kg of FGF-2 or 3 h hypothermia after injury did not differ significantly from the control injury group. The FGF-2 combined with hypothermia treatment had a significantly better outcome over time than either treatment alone.](image2)

**Fig. 2.** Mean (±S.E.M.) beam walk latency scores prior to, and Days 1–5 following, CCI injury. Injured animals treated with 135 μg/kg of FGF-2 or 3 h hypothermia after injury did not differ significantly from the control injury group. The FGF-2 combined with hypothermia treatment had a significantly better outcome over time than either treatment alone.
interaction ($F_{16,180}=0.609, P=0.874$). Post-hoc analyses showed that all injured groups had deficits ($P<0.001$). Additionally, all treated but injured groups showed reduced deficits compared with untreated injured animals ($P<0.05$) however, there were no differences seen between treatment groups (Fig. 3). Post-hoc analysis showed significant differences at days 14, 15, and 18 with both the FGF-2+Norm and VEH+Hypo groups performing better than the VEH+Norm group ($P<0.05$). The FGF-2+Hypo group exhibited a significantly reduced latency to find the hidden platform compared to the VEH+Norm group only at post-operative day 18 ($P<0.029$, Fisher). In addition, all injured groups with or without treatment demonstrated significantly shorter swim latencies ($P<0.05$) during the visible platform trials suggesting that their performance deficits during the hidden platform trials were not confounded by non-specific deficits such as visual processing and motivation.

3.4. Histology

3.4.1. Neuronal density

Hippocampal CA1 and CA3 neuronal density underlying the contusion was examined from each group. TBI produced significant reductions in CA1 and CA3 surviving neurons in the hippocampus ipsilateral to impact compared to the contralateral hippocampus in all the CCI-injured groups. The percentage of CA1 neuronal survival was 98.49±5.68 (Sham+VEH), 35.43±4.13 (FGF-2+Norm), 34.94±7.69 (VEH+Norm), 35.43±4.13 (FGF-2+Hypo), and 43.62±9.71 (VEH+Hypo). All injured groups were significantly different from the Sham+VEH group ($P<0.05$), but no significant differences were observed among injured ($P>0.05$, unpaired $t$-test). The mean percentage of CA3 neuronal survival was 104.88±6.22 (Sham+VEH), 54.29±7.45 (FGF-2+Norm), 53.51±6.75 (VEH+Norm), 73.32±8.92 (FGF-2+Hypo), and 76.75±9.67 (VEH+Hypo). An unpaired $t$-test on cortical tissue loss did not reveal a significant difference among injured groups ($P>0.05$, n.s.). The mean (±S.E.M.) cortical volume was 22.34±3.07 mm$^3$ (FGF-2+Norm), 23.41±3.30 mm$^3$ (VEH+Norm), 21.10±3.23 mm$^3$ (FGF-2+Hypo), and 20.47±1.62 mm$^3$ (VEH+Hypo).

3.4.2. Immunohistochemistry

GFAP staining showed marked astrogliosis in all injured brains (Fig. 4). An increase in GFAP-immunoreactivity was observed in the ipsilateral hippocampus cortex and thalamus in all injured groups (Fig. 4). GFAP staining in the ipsilateral CA3 corresponded directly to the area of maximal hippocampal cell loss caused by CCI injury. However, FGF-2 and/or hypothermia-treated animals showed an attenuation of GFAP expression compared to

![Water Maze of FGF-2 and Hypothermia Study](image)

Fig. 3. The effect of post-CCI FGF-2 and hypothermia treatment on Morris water maze place learning after TBI. Graph plotting the latencies to find a hidden platform beginning 2 weeks after TBI. Injured animals treated with 135 μg/kg of FGF-2 and/or 3 h hypothermia after injury had significantly shorter latencies to find the hidden platform than the VEH+Norm group. However, the combination was not better than FGF-2 or hypothermia alone.
Fig. 4. Photomicrograph of GFAP-immunoreactivity counterstained with hematoxylin at 4 weeks after CCI injury revealing marked astrogliosis in all injured brains (D–O) compared to the sham controls (A–C). An attenuation of GFAP over-expression is observed in the VEH+Hypo (G–I), FGF-2+Norm (J–L), and FGF-2+Hypo (M–O) groups compared to the VEH+Norm (D–F) group. Scale bar 2 mm (A,D,G,J,M), 500 \( \mu \text{m} \) (B, E, H, K, N), 100 \( \mu \text{m} \) (C, F, I, L, O).
the injured VEH+Norm-treated control by immunohistochemistry (Fig. 4).

4. Discussion

In the present study, we demonstrate that behavioral morbidity after CCI in the rat can be reduced by either moderate hypothermia or FGF-2 treatment in an injury model that results in enduring motor and MWM dysfunction and histopathological damage. However, additional benefit by the combination of moderate hypothermia with FGF-2 treatment was only observed in one of several functional assessments, suggesting that optimal combined treatment may not have been obtained. The exogenous administration of FGF-2 [12,38] or the induction of moderate hypothermia [4,5,9,12,13,29,34,47] have previously been shown to reduce injury when given alone following experimental TBI. However, to our knowledge, our study is the first to investigate the combined effects of FGF-2 and hypothermia after TBI. We found improvements in beam walking and MWM performance in animals receiving FGF-2, hypothermia or combined optimal individual therapy. Additional benefit was found in combined treatment, but only in the beam walking task, suggesting the possibility that optimal combined treatment was not obtained, or ineffective on higher-order functioning. Importantly, while there was minimal synergy seen with the one trial of combined therapy used in the present study, a therapeutic benefit was still seen that was comparable or better to either treatment alone. These data suggest that the two treatments were at least compatible with each other and further study may be required to determine if optimal conditions for combined therapy exist.

FGF-2 is an 18 kD, 154-amino acid protein with potent trophic actions on neurons, and glia. Neurprotection has been reported with FGF-2 in vitro [30,41] and in vivo following focal [3,28] or global ischemia [10], spinal cord injury [1,31,40,48] traumatic brain injury [12,51], and seizures [32].

In the first of several previous studies in TBI, Dietrich et al. [12] found that exogenous FGF-2-treatment significantly decreased neuronal death and contusion volume following mild-to-moderate parasagittal fluid-percussion brain injury. In another study, McDermott and colleagues [38] did not observe a FGF-2-mediated decrease in hippocampal cells loss or contusion size in a lateral fluid percussion model, but did show attenuation of post-traumatic memory dysfunction. The results from our current study are more similar to lateral [38] than parasagittal fluid percussion [12] in that we found no histological protection, but some functional improvement. However, the histopathological consequence of injury in our CCI model is more analogous to lateral fluid percussion than parasagittal fluid-percussion which typically produces less severe histological damage. These data showed that the histopathological consequences of these different types of injury are quite different. It may be that FGF-2 histological protection is limited by injury intensity, but that functional benefit can still be obtained by modulatory FGF-2 actions with less overt pathology.

In addition to the traditional role of trophic factors in the CNS as survival, growth, and differentiation factors, recent studies have shown acute modulatory effects of trophic factors on axonal branching and arborization, ion channel function, and synaptic efficacy [20]. Furthermore, the expression and secretion of trophic factors are regulated by electrical activity, suggesting that many activity-dependent interactions at the synapse may be mediated by trophic signals [20]. Trophic signal cascades interact with excitatory and inhibitory neurotransmitter systems producing both transient and persistent changes in neuronal signaling. There are many examples of relatively fast modulation of pre- and postsynaptic inhibitory and excitatory transmission by neurotrophins [43]. Additionally, important roles for trophic factors in synaptic plasticity such as long term potentiation (LTP) and memory and learning are also well established [43], which suggests one possible explanation for why FGF-2 provides behavioral protection in the absence of structural protection after TBI in our CCI model.

The trophic actions of exogenous trophic factor treatment may reduce excitotoxic damage by buffering increases in free radicals and intracellular calcium [37]. However, exogenous trophic factor administration may acutely contribute to excitotoxicity by further activating phospholipases D (PLD) and A2 (PLA2) via protein tyrosine kinase (PTK) pathways [22,49], which in turn, can transiently increase neuronal free radicals and cellular calcium by the excessive production of diacylglycerol and arachidonic acid cascades. Evidence in support of detrimental diacylglycerol and arachidonic acid cascades after TBI is provided by studies showing CDP-choline treatment reduces brain edema and blood–brain barrier breakdown [2] as well as attenuate water maze performance deficits [17]. CDP-choline treatment has been shown to prevent the release of free fatty acids after cerebral ischemia by stimulating the choline phosphotransferase reaction towards phosphatidylcholine formation [19,50]. Thus, exogenous trophic factors given during or immediately after an excitotoxic neurotransmitter surge could have detrimental as well as protective actions. In addition, NGF may mediate damaging signaling cascades via p75 receptors but beneficial actions via trk receptors [21]. Thus, exogenous FGF-2 given early could be less effective than if given later. In the past, this may be one reason there have been contradicting reports of beneficial action of exogenous trophic factors in excitotoxic conditions and suggests that one should not conclude on the basis of the limited data of the present study that there is no therapeutic potential for the combined use of hypothermia and FGF-2.
It has been shown in various ischemia and trauma studies that the duration of hypothermia is critical in whether or not neuroprotection is demonstrated in a particular model. In the present study, no histopathologic protection was seen with either FGF-2 treatment alone or in combination with 3 h of moderate hypothermia. This result is consistent with our previously reported study showing that moderate hypothermia for 3 h exhibits protective effects on behavioral deficits but not histopathological improvement following CCI injury in the rat [18]. It is possible that contusion volume, especially following CCI, is the ultimate morphological expression of an irreversible pathological process leading to cell death within regions of focal necrosis. These pathological processes may be insensitive to a variety of therapeutic interventions including moderate hypothermia [18]. It is also possible that a longer duration of hypothermia may be more protective and thereby attenuate histopathology. Finally, studies of hypothermia or FGF-2 have employed a variety of hypothermic protocols, injury models, and magnitudes as well as histopathological endpoints that could contribute to different results. These observations highlight the need for rigorously controlled studies that systematically examine the effects of moderate hypothermia or FGF-2 on different magnitudes of TBI and associated histopathological damage.

In summary, our data indicate that FGF-2 and hypothermia treatments, while producing some benefit on their own, were only slightly more effective when combined. The lack of a synergistic effect in all behaviors may be related to the dose and timing of combined treatments, but at least the combined approach is compatible in that therapeutic benefit was still obtained. Further work is necessary to determine if different timing and dose regimens exhibit greater synergistic effects.

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