Effect of Decompressive Craniectomy and Hypothermia on the Expression of c-Fos and Heat Shock Proteins Following Fluid Percussion Brain Injury in the Rat

Young-Min Han, M.D.¹, Do-Sung Yoo, M.D.², Pil-Woo Huh, M.D.², Kyoung-Suk Cho, M.D.², and Dal-Soo Kim, M.D.²

¹Department of Neurosurgery, Our Lady of Mercy Hospital College of Medicine, The Catholic University of Korea, Incheon, Korea
²Department of Neurosurgery, Uijeongbu St. Mary's Hospital, The Catholic University of Korea, Uijeongbu, Korea

Objective: In order to evaluate the cellular responses to different treatment modalities following traumatic brain injury, the expression of c-Fos protein and Heat shock protein (HSP70) in rat brain tissue subjected to moderate severity of fluid-percussion injury (FPI) was measured with Western blot analysis.

Methods: Male Sprague-Dawley (SD) rats (n=62, 200-250 g) were subjected to sham surgery alone (n=10) or to lateral fluid percussion brain injury of moderate severity (n=52, 2.1-2.5 atm). Parasagittal FPI induced the expression of c-Fos and HSP70 proteins in the hemisphere ipsilateral to the injury. Injured rats were treated with hypothermia (n=20) or decompressive craniectomy (n=20). Ten rats in each group were sacrificed 24h or 72h following FPI. The brain specimens were collected at 24, or 72 hours after injury and prepared for Western blot analysis.

Results: Expression of c-Fos protein and HSP70 following FPI showed a increased pattern at 24 hours post-injury and maintained till 72 hours post-injury. The expression of c-Fos and HSP70 was significantly reduced in rats treated with either moderate hypothermia or decompressive craniectomy at 24 and 72 hours post-injury, compared with those without treatments. The expression patterns of c-Fos and HSP70 were not same, however, these differences were not statistically significant.

Conclusion: These results suggest that treatment of traumatic brain injury with either decompressive craniectomy or hypothermia may similarly influence the cellular defense mechanism involving the expression of IEGs and HSP70.

Key Words: Traumatic brain injury, Immediate early gene, c-fos, Heat shock protein, Hypothermia, Craniectomy

INTRODUCTION

Induction of the family of immediate early genes (IEG) is associated with a variety of pathological insults to the central nervous system (CNS). IEGs such as Fos and Jun family proteins may mediate adaptive and defensive responses to acute brain injury. Jun and Fos proteins can interact to form activating protein-1 (AP-1), a class of transcription regulatory proteins controlling the expression of numerous late effector genes. Jun and Fos proteins are capable of forming a heterodimer responsible for the regulation of certain target genes, including nerve growth factor, amyloid precursor protein, and opioid precursor proteins, all of which produce protection against pathologic stresses. AP-1 binding activity increases in the ischemic brain injury model, and IEGs are associated with neuroprotective effects and apoptosis in the Fluid Percussion Injury (FPI) model. While a correlation exists between highly induced c-Fos expression and poor outcomes in human trauma, the exact role of the c-Fos protein remains unclear. Treatment of traumatic brain injury may utilize hypothermia or decompressive craniectomy. Hypothermia has been shown to produce behavioral neuroprotection, improve functional outcomes and reduce contusion volume in experimental brain injury models. While induction of Hsp70 and c-Fos proteins decreases in the FPI model treated with hypothermia, an association between...
reduced expression of these proteins and cell survival has not yet been established.

Decompressive craniectomy, a procedure whereby intracranial pressure (ICP) is relieved to improve cerebral blood flow, has been used in the treatment of severely injured head-trauma patients, producing relatively good outcomes. Decompressive craniectomy is also frequently carried out in patients with massive brain swelling. Although the procedure is believed to be effective in improving outcomes, cellular responses following decompression have rarely been studied. Thus, the objective of the present study was to compare the cellular effect of two different treatment modalities, hypothermia and decompression, by analyzing the relative induction of c-Fos protein and Hsp70.

1. Materials and Methods

1. Fluid Percussion Injury

Male Sprague-Dawley rats (n=62) weighing 200–250 g each were anesthetized with ketamine (70 mg/kg) and xylazine (6 mg/kg). A 3-mm-diameter craniectomy was centered over the right parietal cortex 4 mm from the sagittal suture and midway between bregma and lambda; the dura was left intact. A plastic Luer adapter was positioned over the exposed dura using dental cement, and a male Luer-Lok was introduced and attached to the injury device (HPD-1700, Dragonfly R&D, Silver Spring, MD). Animals were secured in a stereotaxic frame and received lateral FPI of moderate (2.1-2.5 atm, n=52) intensity by allowing a pendulum to strike a piston filled with sterile saline. A sham-injured group (n=10) underwent the same procedure without receiving FPI. The temporal expression patterns of c-Fos protein and HSP70 were examined in the injured groups. Expression patterns according to the different treatment modalities in the injured rats were also examined.

2. Decompressive Craniectomy

Moderately injured rats (n=20) were secured in a stereotaxic frame for 5 min post-injury. The temporalis muscle was then stripped off the parietal bone and a craniectomy was performed from the coronal suture to the lambdoid suture and near the base of temporal bone laterally. The scalp was approximated with nylon sutures. Expression of c-Fos protein and HSP70 were examined at 24 hours and 72 hours post-injury.

3. Moderate Hypothermia

The temporalis muscle of the moderately injured rats (n=20) was cooled to 32°C beginning 5 min post-injury by the external application of ice packs and fanning, maintained for 60 min, and then re-warmed slowly for 1 hour. The expression of c-Fos protein and HSP70 were examined at 24 hours and 72 hours post-injury.

4. Tissue Collection

Rats receiving no treatment were decapitated at 24 and 72 hours (n=5, respectively) post-injury. Rats treated with hypothermia or decompression were decapitated at 24 and 72 hours (n=10, respectively) post-injury. Following decapitation, the injured hemispheres were dissected and frozen in liquid nitrogen.

5. Tissue Preparation and Western Blot Analysis

Whole cell lysates were prepared by homogenizing 1 g of brain tissue in 4 mL of lysis buffer (0.5% Triton X-100, 20 mM HEPES (pH 7.4), 150 mM NaCl, 12.5 mM glycerophosphate, 1.5 mM MgCl₂, 2 mM EGTA, 10 mM NaF, 2 mM dithiothreitol, 1 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride, and 20 M aprotinin. Lysis reactions were centrifuged at 2,900× g for 20 min at 4°C. The supernatant was re-centrifuged at 15,000× g for 20 min at 4°C. Whole cell lysates collected from the final supernatant were resolved by SDS-polyacrylamide gel electrophoresis and then transferred to Hybond-P membranes (Amer-sham, Aylesbury, U.K.). The membranes were immunoblotted with various antibodies and visualized with horseradish peroxidase-conjugated antibodies and rabbit, mouse, and goat IgG using the enhanced chemiluminescence Western blotting system (ECL, Amer-sham, Aylesbury, U.K.). The density of each band was analyzed with densitometry, and relative optical band densities were calculated and normalized using β-actin signal according to the formula: (density of sample protein band/ density of β-actin).

6. Statistical Analysis

Comparisons within groups were made using 2-way repeated measurement ANOVA. All tests of significance were for p values less than 0.05.
**RESULTS**

1. Expression of c-Fos and HSP70

In the injured rats, immunoreactivity to c-Fos protein and HSP70 was detected at 24 hours, maintained until 72 hours post-injury (Fig. 1A and 2A). The increased expression of c-Fos and HSP70 in the injured group compared to the sham injured group was statistically significant respectively (p<0.05).

2. Differences according to treatment modality

In injured rats treated by moderate hypothermia, immunoreactivity to c-Fos protein and HSP70 was significantly reduced at 24 hours relative to rats without treatment. A similar pattern was observed at 72 hours (Fig. 1B and 2B). In the rats treated with decompressive craniectomy, the immunoreactivity to c-Fos and HSP70 was similar to rats treated by hypothermia. The decreased

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**Fig. 1.** A: Western blot analysis of c-Fos and HSP70 at 24 hours post-injury. Expression of c-Fos and HSP70 showing a decreased pattern in both groups treated with hypothermia or decompression relative to the injured group without treatment. Actin is shown as a loading control. B: Graph showing relative band densities (Ratio). The increased expression of c-Fos and HSP in the injured group compared to the sham injured group was statistically significant respectively (p<0.05). The decreased expression of c-Fos and HSP70 in the injured groups receiving treatments relative to the injured group receiving no treatment was statistically significant (p<0.05). Differences between treatment groups were not statistically significant. Data is shown as mean ± standard deviation. C = sham injured rat; I = injured rat; H = injured rat treated by hypothermia; D = injured rat treated by decompressive craniectomy.

**Fig. 2.** A: Western blot analysis of c-Fos and HSP70 at 72 hours post-injury. Actin is shown as a loading control. B: Graph showing relative band densities (Ratio). The decreased expression of c-Fos and HSP70 in the treatment groups relative to the injured group receiving no treatment was statistically significant (p<0.05); however, no significant differences were observed between treatment groups. Data is shown as mean ± standard deviation. C = sham injured rat; I = injured rat; H = injured rat treated by hypothermia; D = injured rat treated by decompressive craniectomy.
expression of c-Fos and HSP70 in the injured groups receiving treatments relative to the injured group receiving no treatment was statistically significant (p<0.05). Differences between treatment groups were not statistically significant.

**DISCUSSION**

Acute brain trauma can induce the expression of various genes, including the family of IEGs. c-Fos is an IEG associated with traumatic brain injury, and its induction has been frequently reported in experimental traumatic brain injury. In a human study of head injury, expression of c-Fos and c-Jun mRNA was observed in 50% and 64% of patients, respectively, and expression of c-Fos and c-Jun was associated with the final outcomes of head trauma patients: patients with poorer outcomes had higher levels of gene expression. Different sites of induction have been reported in various injury models, and the sites of induction varied according to severity of the injury. In the focal ischemia model, small cortical infarcts can induce c-Fos throughout the cranial hemisphere, with the immunoreactivity to IEGs detected in various areas. In the model of penetrating brain injury, induction of IEGs is restricted to the injured hemispheres. Expression of c-Fos may be induced by calcium entry into the cell according to the theory of cortical spreading depression. Specifically, the administration of calcium channel blockers such as N-methyl-D-aspartate (NMDA) prevents the induction of c-fos. In the FPI model, cortical impact can induce depolarization of neurons, which spreads into the adjacent cortical surface through the cortico-cortical connection. Similarly, c-Fos induction may be extended via other tracts. In several studies of traumatic brain injury, the induction of IEGs is observed not only in the impact site periphery, but also in remote sites such as the hippocampus, striatum, thalamus and even the cerebellum. The hippocampus is known to be vulnerable to pathologic insults, and the induction of c-Fos and HSP70 in this area has been repeatedly demonstrated. In these studies, the expression of c-Fos was widely observed over the brain surface ipsilateral to the injury and the expression in the contralateral hemisphere was limited to vulnerable areas. Thus, we evaluated c-Fos and HSP70 protein expression in the hemisphere ipsilateral to the injured site. In some previous studies, the increase in c-Fos induction did not appear to be directly related with injury intensity, and also suggested the expression of c-Fos protein might be a multifactorial response. The induction of c-Fos may exert a neuroprotective effect through gene transregulation by AP-1, and a number of gene products that bear neuroprotective properties such as HSP70 might be regulated by AP-1. Additionally, induction of IEGs like c-Fos is associated with neurotrophic factor expression. Brain derived neurotrophic factor (BDNF) and nerve growth factor (NGF) are well known neuroprotective factors, and the infusion of NGF into a cortical lesion site 24 hours after trauma resulted in improved behavioral outcome, whereas neuronal damage was not attenuated. Our data showed that FPI increased induction of c-Fos and HSP70, and that treatment with either hypothermia or decompression decreased induction. Our data suggested that IEG and HSP70 may be associated with a cellular mechanism involved in neuronal and/or glial injury. Previous investigations about head trauma have shown different patterns of expression regionally and temporally, indicating that different inducing factors, neurochemical alterations in a cell, and injury-spreading pathways may be involved. A variety of pathological insults such as ischemia, seizure, and trauma are likely to share a final common signal transduction pathway that influences the induction of IEGs. The induction of IEGs is also associated with apoptotic reactions inducing programmed cell death. Zhang et al reported that reduced Fos protein induction is correlated with an increase in cerebral infarct size in the focal ischemia model. Other studies suggest the involvement of c-Fos in an apoptotic cascade following traumatic brain injury. The induction of IEGs may influence pathways of both neuroprotection and apoptosis; however, the identity of the dominant pathway remains unclear. Previous studies have shown that this role may be dependent on injury mechanism, injury severity, or treatment modality. Thus, the effect of c-Fos induction remains unclear and requires further analysis. Moderate hypothermia is a proven and beneficial method for the treatment of traumatic brain injury, often improving behavioral outcomes. Hypothermia reduces cerebral blood flow and the inflammatory reaction, a significant cause of brain damage. In FPI, the blood brain barrier (BBB) is disrupted, thus aggravating inflammation by allowing leukocyte and lymphocyte entry into...
damaged brain tissue from circulation. Uncoupling between metabolic needs and cerebral blood flow (CBF) may cause anaerobic glycolysis and decreased pH (acidosis). Such acidosis may cause depolarization of neuronal cells and calcium entry, the cause of the cortical spreading depression. Hypothermia has been reported to reduce the opening of the BBB, inflammation, and increased intracranial pressure. Our data showed decreased induction of c-Fos and HSP70 protein in hypothermia group. Significant reduction suggests that hypothermia may be associated with the cellular defense mechanism against FPI. Heat shock proteins have been reported to be cellular stress responders and are likely to be related to the intensity of ischemic or traumatic stress. Other evidence suggests that the stress response is an important component of the cellular defense mechanism, and the induction of HSP is critical to survival following pathologic insults. Decompressive craniectomy has been reported to be effective in the control of ICP. Clinically, decompressive craniectomy with intact dura shows nearly 50% reduction of ICP. In our study, FPI induced-expression of HSP70 was reduced in the hypothermia and decompression treated groups relative to the group receiving no treatment, as was IEG in the groups treated with decompression. Decompressive craniectomy showed no definitive differences in the induction of IEG or HSP70. There are few experimental studies examining the effect of decompression on neurobehavioral and functional outcome. This may be, in part, attributable to the differences in skull anatomy of humans and rats. Based on our data, we suggest that in rat models, the mechanism for neuroprotection may be similar to part of the signal transduction pathway on a cellular level. The exact mechanism of cellular defense and response following decompression treatment is unclear and requires further study. Our data also suggested that treatment of head trauma with a combination therapy consisting of both hypothermia and decompression may enhance the overall therapeutic effect.

CONCLUSION

Although the exact role of IEG and HSP expression in the FPI model is unknown, our studies showed that the expression pattern of c-Fos and HSP70 is similar between FPI rats treated with hypothermia and those treated with decompressive craniectomy. Our data suggest that these different treatment modalities may induce similar molecular changes.

REFERENCES


