Decompressive craniectomy reduces white matter injury after controlled cortical impact in mice

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Decompressive Craniectomy Reduces White Matter Injury after Controlled Cortical Impact in Mice

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Abstract
Reduction and avoidance of increases in intracranial pressure (ICP) after severe traumatic brain injury (TBI) continue to be the mainstays of treatment. Traumatic axonal injury is a major contributor to morbidity after TBI, but it remains unclear whether elevations in ICP influence axonal injury. Here we tested the hypothesis that reduction in elevations in ICP after experimental TBI would result in decreased axonal injury and white matter atrophy in mice. Six-week-old male mice (C57BL/6J) underwent either moderate controlled cortical impact (CCI) (n = 48) or Sham surgery (Sham, n = 12). Immediately after CCI, injured animals were randomized to a loose fitting plastic cap (Open) or replacement of the previously removed bone flap (Closed). Elevated ICP was observed in Closed animals compared with Open and Sham at 15 min (21.4 ± 4.2 vs. 12.3 ± 2.9 and 8.8 ± 1.8 mm Hg, p < 0.0001) and 1 day (17.8 ± 3.7 vs. 10.6 ± 2.0 and 8.9 ± 1.9 mm Hg, p < 0.0001) after injury. Beta amyloid precursor protein staining in the corpus callosum and ipsilateral external capsule revealed reduced axonal swellings and bulbs in Open compared with Closed animals (32% decrease, p < 0.01 and 40% decrease, p < 0.001 at 1 and 7 days post-injury, respectively). Open animals were also found to have decreased neurofilament-200 stained axonal swellings at 7 days post-injury compared with Open animals (32% decrease, p < 0.001). At 4 weeks post-injury, Open animals had an 18% reduction in white matter volume compared with 34% in Closed animals (p < 0.01). Thus, our results indicate that CCI with decompressive craniectomy was associated with reductions in ICP and reduced pericontusional axonal injury and white matter atrophy. If similar in humans, therapeutic interventions that ameliorate intracranial hypertension may positively influence white matter injury severity.

Key words: axonal injury; controlled cortical impact; intracranial pressure; traumatic brain injury; white matter

Introduction
Traumatic axonal injury (TAI) is thought to be a major contributor to morbidity after severe traumatic brain injury (TBI).1–6 TAI is primarily a histopathological nomenclature, and our ability to diagnose axonal injury in vivo in the clinical setting is limited.7–9 The lack of easily accessible methods for in vivo detection of axonal injury after severe TBI has limited our understanding of the natural course of axonal injury during the acute phases of TBI. The primary goal of clinical care for severe TBI in the acute phase is the reduction and avoidance of secondary insults.10,11 It remains unclear whether TAI in white matter is entirely the result of primary injury or if commonly occurring secondary insults (such as increased intracranial pressure (ICP), hypoxia, or hypotension) after TBI can influence the extent and severity of axonal injury.12–15 Reduction and avoidance of elevations in ICP continue to be the mainstays of treatment patients with for severe TBI.10,11 Although there is evidence that sustained elevations in ICP > 20 mm Hg after severe TBI are associated with poor outcome, efficacy of threshold-targeted interventions has not been thoroughly established.10,16–21 Previous clinical investigations in pediatric TBI patients have demonstrated an association between raised ICP and white matter loss, as well as changes in diffusion tensor imaging of white matter in the corpus callosum at long-term follow-up.22,23 It remains unclear, however, whether sustained elevations in ICP play a causal role in secondary white matter injury or are simply associated because of the severity of underlying injury.

Several animal models have been developed to examine the role of elevated ICP after TBI.24–28 The primary histologic focus of many of these investigations, however, has been cortical lesion volumes and neuronal injury. Recently, Lafrenaye and associates26 investigated axonal injury in a central fluid percussion rat model of TBI with elevations in ICP. Using beta amyloid precursor protein (β-APP) immunohistochemistry, no difference in axonal swellings was observed in the cortex of animals that experienced persistent elevated ICP versus those that did not.26 These investigations did not explore the effect of elevated ICP on axonal injury of white matter. In the current study, we hypothesized that reduction in ICP elevations after controlled cortical impact (CCI) would result in...
decreased axonal injury in the ipsilateral corpus callosum and external capsule as well as sparing of white matter tract volumes.

Methods

Injury

All procedures were approved by the Washington University Animal Studies Committee and are consistent with the National Institutes of Health (NIH) guidelines for the care and use of animals. Six-week-old C57BL/6J male mice (Jackson Laboratory, Bar Harbor, ME) weighing 18–22 g were used in these experiments. Mice were sacrificed at three different time points: 24 h, 7 days, and 28 days (n = 20 for each time point). For each time point, four mice underwent Sham injury and 16 mice underwent CCI. The mice were anesthetized with 5% isoflurane at induction, followed by maintenance at 2% isoflurane for the duration of the procedure. The head was shaved, and head holders were used to stabilize the head within the stereotaxic frame (MyNeurlab, St. Louis, MO). Then, a single 5-mm craniotomy was performed by an electric drill on the left lateral side of the skull centered 2.7 mm lateral from the midline and 3 mm anterior to lambda. The 3-mm electromagnetic impactor tip was then aligned with the craniotomy site at 1.2 mm left of midline, 1.5 mm anterior to the lambda suture. The impact was then delivered at 2-mm depth. The head holders were released immediately after the injury. At the time of sacrifice, the mice were perfused with 0.3% heparin in phosphate buffered saline. Whole brains were sliced on a freezing microtome starting with the appearance of a complete corpus callosum and caudally to bregma ~3.08 mm. Sets of 12 sections were used as the ROI for white matter volume quantification.

The optical fractionator function was used to quantify target markers per cubic millimeter of tissue. A grid size of 250 μm × 250 μm, a counting frame of 40 μm × 40 μm, and a dissector height of 15 μm with a guard zone of 5 μm were used for all quantifications, resulting in 3% of the region of interest (ROI) being randomly sampled. All ROIs were traced at 4X magnification, and markers were counted at 60X magnification. The ipsilateral corpus callosum and external capsule spanning 12 sections were used as the ROI for the β-APP and neurofilament-200 (NF200) stains. This region was defined as the white matter area between midline and the lateral edge of the cingulum in rostral sections; in caudal sections, a horizontal line drawn laterally from the end of the fimbria served as the end boundary of the ROI. Injured axons were identified by β-APP-positive varicosities greater than 5 μm. Similarly, NF200-positive axonal varicosities greater than 5 μm in diameter were counted as injured axons during stereological assessment. Gunderson coefficients of error were <0.1 for both β-APP and NF200 quantifications. For quantification of NeuN positive cells, the CA3 region of the ipsilateral hippocampus was used as the ROI from the same 12 sections as described above. Intact neurons were identified as cell bodies with NeuN-positive nuclei. This ensured the Gunderson coefficient of error was <0.1.

White matter volume quantification

The ipsilateral corpus callosum and external capsule spanning 12 sections were used as the ROI for white matter volume quantification at 1 month after injury. This region was defined as the white matter area between midline and the lateral edge of the cingulum in rostral sections, and in caudal sections, a horizontal line drawn laterally from the end of the fimbria served as the end boundary of the ROI. White matter volume estimation was performed using the Cavalieri method.

Statistical analysis

All data were analyzed using Prism 6.0 software (GraphPad Software, San Diego, CA). The results are presented as mean ± standard deviation. For all data sets, there was no evidence for significant deviations from normal distribution (p > 0.05 by Shapiro-Wilk tests). ICP measurements were analyzed with a two-way analysis of variance (ANOVA). Significant main effects on animal group were subjected to post hoc analysis with Tukey tests with a significance level of p < 0.05. Quantitative histologic data were analyzed with one-way ANOVA, followed by Tukey tests for multiple comparisons with a significance level of p < 0.05. Spearman correlation was used to assess the correlation between peak
ICP measurements (15 min post-injury) and neuropathology at 1 day and 1 week post-injury time points.

Results

ICP elevations following bone flap replacement after CCI

As previously described by others, we used a rodent CCI model with bone flap replacement to generate elevations in ICP.24,30,31 We used two separate cohorts of mice to evaluate ICP over time. In the first cohort, ICP measurements were obtained after craniectomy but before injury, 15 min after injury, and just before sacrifice at 24 h after injury (Fig. 1A). In a two-way repeated measures ANOVA of before injury, 15 min after injury, and just before sacrifice at 24 h, there were significant main effects of group (F = 56.6, p < 0.0001) and time of measurement after injury (F = 30.8, p < 0.0001), as well as a significant group × time interaction (F = 16.34, p < 0.0001). In post hoc analysis with Tukey tests, ICP measurements in the Closed group were significantly elevated compared with Sham and Open 15 min and 24 h after injury (Fig. 1A). In the 1 week survival cohort, significant elevations in ICP at 15 min in the Closed group were again observed that were moderated by 1 week but still statistically elevated compared with Sham and Open (Fig. 1B).

ICP elevations are associated with an increase TAI in pericontusional white matter

After CCI in mice, the pericontusional corpus callosum and external capsule have been observed to be ROI with large amounts of TAI.32,33 To determine the effects of elevations in ICP on TAI, we assessed TAI in the ipsilateral corpus callosum and external capsule with two different markers—β-APP and NF200.

Consistent with previous reports, we observed β-APP accumulations in varicosities in the pericontusional corpus callosum and external capsule at both 1 day and 7 days post-injury, with a reduction in immunohistochemical staining over time (Fig. 2A–I).12 Injured mice with elevations in ICP (Closed) appeared to have increased β-APP staining in the pericontusional white matter compared with mice in the Open group (Fig. 2). We did not observe any immunohistochemical staining in the contralateral hemisphere (Fig. 3).

Stereological quantification of β-APP in the pericontusional white matter confirmed our qualitative observations (Fig. 2J, K). At 1 day post-injury, one-way ANOVA revealed a significant main effect of group (F = 48.1, p < 0.0001). Post hoc Tukey tests demonstrated a higher number of β-APP positive varicosities and swellings in Closed compared with Open (p < 0.01). Similarly, at 1 week post-injury, one-way ANOVA revealed a significant group effect (F = 30.4, p < 0.0001), and post hoc Tukey analysis confirmed a higher number of B-APP stained swellings and varicosities in Closed compared with Open (p < 0.001). Immunohistochemistry with NF200 demonstrated increased background staining compared with B-APP immunohistochemistry, but increased amounts of NF200 swellings in the pericontusional corpus callosum and external capsule were still observed (Fig. 4A–I). We did not observe any immunohistochemical staining in the contralateral hemisphere. Stereological quantification of NF200 in pericontusional white matter revealed increased NF200 positive swellings in injured mice compared with Sham at 1 day and 7 days after injury. One-way ANOVA revealed a significant main effect of group (F = 26.6, p < 0.0001); however, post hoc Tukey tests did not demonstrate a significant difference between Closed and Open at 1 day post-injury. At 7 days post-injury, one-way ANOVA confirmed a significant group effect (F = 39.0, p < 0.0001). Post hoc Tukey test also showed a significantly higher number of NF200 swellings in Closed compared with Open (p < 0.001) (Fig. 4J, K). Thus, elevated ICP in the Closed group was associated with increased white matter axonal injury at two time points using B-APP and using two different immunohistochemical markers at 1 week post-injury.

White matter atrophy increases after CCI without decompressive craniectomy

At 4 weeks post-injury, little to no immunohistochemical staining with β-APP or NF200 was observed in the pericontusional white matter. To assess the effects of ICP elevations on white matter at longer time points, we assessed white matter atrophy at 4 weeks after injury. White matter volume of the ipsilateral corpus callosum and external capsule was estimated by the Cavalieri method (Fig. 5) after immunohistochemical staining with β-APP. One-way ANOVA analysis demonstrated a strong effect of group (F = 23.9, p < 0.0001) and post hoc Tukey test revealed Closed animals had increased white matter atrophy compared with Open, resulting in smaller corpus callosum and external capsule volumes (p < 0.01).

Decompressive craniectomy after CCI reduces hippocampal CA3 neuronal loss

Neurons in the CA3 region of the hippocampus have been reported to be highly susceptible to injury after TBI.34,35 To explore the effects of ICP elevations on neuronal injury, we performed stereological analysis of the CA3 region of the hippocampus with

![FIG. 1](image-url) Controlled cortical impact with immediate bone flap replacement (Closed) resulted in elevations in intracranial pressure (ICP). (A) ICP measurements in mice survived for 24 h after injury or Sham surgery. (** p < 0.001, Tukey test). (B) ICP measurements in mice survived for 7 days after injury or Sham surgery. (* p < 0.01, ** p < 0.001, Tukey tests).
FIG. 2. Controlled cortical impact with immediate bone flap replacement (Closed) resulted in increased beta amyloid precursor protein (β-APP) stained axonal swellings. (A–C) β-APP staining in the pericontusional white matter of Sham, Open, and Closed mice respectively at 1 day post-injury; scale bar 250 μm. (D–I) Higher magnification of the pericontusional white matter at 1 day and 1 week post-injury; scale bars 25 μm. (J, K) Stereological quantification of numbers of β-APP positive axonal swellings per cubic millimeter of the ipsilateral corpus callosum and external capsule: J at 1 day post-injury and K at 1 week post-injury (*p<0.01, **p<0.001, #p<0.0001 compared with Sham, Tukey test).

FIG. 3. Controlled cortical impact with or without immediate bone flap replacement did not result in increased beta amyloid precursor protein (β-APP) stained axonal swellings in the contralateral white matter. (A–C) β-APP staining in the contralateral white matter of Sham, Open, and Closed mice respectively at 1 day post-injury; scale bar 250 μm. (D–I) Higher magnification of the contralateral white matter at 1 day and 1 week post-injury; scale bars 25 μm.
NeuN immunohistochemistry at 1 day, 1 week, and 4 weeks after injury (Fig. 6, 7). At 1 day post-injury, one-way ANOVA revealed no significant differences between groups in number of NeuN stained cells in the CA3 region ($F = 0.5, p = 0.62$). At 1 and 4 weeks post-injury, however, a significant group effect was observed ($F = 22.34, p < 0.0001$ at 1 week and $F = 30.31, p < 0.0001$ at 4 weeks). Post hoc Tukey analysis demonstrated a significantly greater reduction in NeuN positive cells in Closed compared with Open at 1 and 4 weeks post-injury ($p < 0.05$ and $p < 0.01$, respectively).

Correlation of ICP measurements with neuropathology

The association between ICP measurements in Closed animals 15 min after injury and neuropathology ($\beta$-APP, NF200, and NeuN) at 1 day and 1 week post-injury was assessed. There were no significant correlations between ICP measurements at 15 min after injury and any of the three immunohistochemical markers at 1 day post-injury. There was a significant positive correlation, however, between ICP measurements and the extent of axonal injury determined by stereological quantification of $\beta$-APP immunohistochemistry at 1 week after injury ($r = 0.833, p < 0.05$) but not with NF200 or NeuN (Fig. 8).

Discussion

Even moderate elevations in ICP after CCI in mice without decompressive craniectomy were associated with increased axonal injury and white matter atrophy. We observed increased axonal injury in mice with elevations in ICP using two different markers of axonal injury—$\beta$-APP and NF200—at 1 week post-injury in the ipsilateral corpus callosum and external capsule. Our findings were further supported by increased white matter atrophy at 1 month after injury in the mice with elevated ICP. Together, these data lend support to our hypothesis that elevations in ICP after CCI in mice worsen axonal injury in white matter. CCI without decompressive craniectomy in the mouse has been shown to increase ICP, contusional lesion volume, brain edema, and blood–brain barrier disruption.24,30,36,37 Its influence on axonal injury, however, has not been previously reported, to our knowledge. In our experiments, the difference in the extent of axonal injury between groups was more pronounced at 1 week post-injury compared with 1 day post-injury. Previous investigations in mice using a CCI model of TBI have demonstrated peak contusional volume at 24 h post-injury.30

We postulate that at our 24 h post-injury assessment of axonal injury, the complete effects of secondary insults (elevations in ICP, decreased cerebral perfusion pressure, and brain edema resulting in vascular compromise) on susceptible axons were not yet manifest.
At 1 week post-injury evaluation, elevations in ICP were still present at a reduced level, but the effects of the ICP elevations on white matter were more pronounced. Neuropathological investigations of nonsurvivors of TBI and non-TBI have attempted to correlate patterns of axonal injury with injury mechanism based on β-APP immunoreactivity as well as the role secondary insults such as hypoxia or vascular compromise related to elevations in ICP.14,15,38 Neuropathology from disabled human survivors of head injury have demonstrated strong associations of diffuse axonal injury and raised ICP with poor outcomes months after injury.14 Further, the number of lesions detected by T2-weighted magnetic resonance imaging 4 weeks after closed head injury in adults correlated with intracranial hypertension detected in the first few days after injury.39 These previous clinical investigations along with our own investigations support our hypothesis that elevations in ICP after CCI exacerbate axonal injury in a delayed fashion. Despite a small sample size, we did observe a strong correlation between peak ICP measurements and extent of axonal injury determined by stereological analysis of β-APP immunohistochemistry at 1 week but not 1 day post-injury. We postulate that intracranial hypertension produces vascular compromise and decreased cerebral blood flow to susceptible axons resulting in exacerbation in the amount of detectable axonal injury. An alternative hypothesis, however, is that increased expansion of contusional volume in mice receiving bone flap replacement compromises blood flow to the pericontusional white matter. Previous investigations have observed reductions in ICP and contusional volume when early decompression is performed in mice undergoing CCI.24,30 Additional experiments involving independent manipulations of ICP will be required, however, to further assess the causal role of ICP per se, independent of contusion evolution to exacerbate pericontusional axonal injury.

White matter volume assessment at 1 month post-injury was used as a longer term pathological assessment. Animals in the Closed group had significantly increased white matter atrophy in the ipsilateral corpus callosum and external capsule compared with Open and Sham animals. Increased contusional expansion in the Closed group animals is a possible explanation for the reduced white matter volumes. Nonetheless, taken together, neuropathological assessments of axonal injury at various post-injury time points provide strong evidence to support the hypothesis that ICP elevations are associated with exacerbated pericontusional axonal injury. A separate question is whether clinically relevant delayed decompression or other approaches to reduce ICP improve pericontusional axonal injury. Addressing this question will require a different study design.
Clinical studies on the effects of elevated ICP on white matter after TBI are limited. Tasker and associates investigated changes in the corpus callosum of adolescent patients with TBI at long-term follow-up (mean 4.9 years). Using diffusion tensor imaging, they observed volume thinning of the corpus callosum; reduced fractional anisotropy; and increased mean, radial, and axial diffusivity in patients who had experienced elevations in ICP during the acute phase of treatment. Our findings of increased white matter atrophy in mice with elevations in ICP after injury are consistent with these clinical observations. Neurons in the CA3 region of the hippocampus have been reported to be highly susceptible to injury after TBI. We performed stereological analysis of the CA3 region of the hippocampus to assess the association of elevations of ICP with neuronal loss. At 24 h, we did not observe significant neuronal loss in either injury group compared with Sham, but stereological analysis at 7 days and 4 weeks post-injury revealed significant neuronal loss in both the Open and Closed groups. Further, animals that had experienced elevations in ICP (Closed) had an even greater increase in neuronal loss, demonstrating that the secondary insult of ICP elevation in our CCI model worsened both axonal and neuronal injury. This model provides an opportunity to evaluate therapeutics that may have the potential to ameliorate or prevent the effects of ICP elevation on neuronal injury.

There are limitations to our experimental design that must be considered when translating our findings to the clinical setting. TBIs in humans can be quite heterogeneous. In this investigation, we used a focal contusion model that is highly reliable and consistent in its pathologic response, but does not encompass the full spectrum of TBIs. It remains unclear whether elevations in ICP in other models of TBI would produce the same association with axonal injury and white matter injury. A fluid percussion injury (FPI) rat model of TBI failed to demonstrate increased axonal injury after elevations in ICP; however, there may be several reasons for the difference in findings. Axonal injury in the FPI rat model was only assessed at 6 h post-injury and the ROI included only the neocortex. We observed the greatest significant difference in axonal pathology using two different immunohistochemical markers at 7 days post-injury suggesting that a 6 h post-injury end-point may be too early to fully assess the entire effects of elevations of ICP on axonal pathology. Further, differences in the characteristics of the injury model such as variences in the intracranial pulse pressure waves generated and typical pathology observed in each model may also be responsible for the differences in axonal pathology. In addition, we did not perform invasive blood pressure monitoring to determine whether there were differences in mean arterial pressure and cerebral perfusion pressure across groups. In children with severe TBI, hypotension and cerebral perfusion pressures below 40 mm Hg have been associated with poor outcomes. We also did not investigate the effects of elevated ICP in female mice, nor in mice of different ages.

In our model, ICP elevations occurred immediately after injury whereas in humans, intracranial hypertension can be delayed after TBI. It is not known whether or not the difference in timing of peak ICP influences axonal or neuronal injury. Modulating the timing of decompression in this model may provide some insight into the window of vulnerability of pericontusional white matter to elevations in ICP and the length of the therapeutic window for rescue. Although unlikely, it has not been fully established whether the effects of immediate bone flap replacement apart from elevations in ICP influence injury severity. An orthogonal method of ICP manipulation would be needed to establish whether there are effects of bone flap replacement unrelated to ICP elevations, such as artificially increasing cerebrospinal fluid pressure.

FIG. 6. Controlled cortical impact with immediate bone flap replacement (Closed) resulted in decreased NeuN positive cells in the hippocampus. (A–L) NeuN staining of the hippocampus of Sham, Open, and Closed mice, respectively; scale bar 200 \( \mu \)m. (M–N) Stereological quantification of numbers of NeuN positive cells per cubic millimeter of the ipsilateral CA3 region of the hippocampus: M at 1 day post-injury and N at 1 week post-injury (*\( p < 0.05 \), #\( p < 0.01 \) compared with Sham, Tukey test).
volume. ICP measurements in our studies were performed under anesthesia with isoflurane, an inhaled anesthetic, which is known to influence cerebrovascular hemodynamics. Direct translation of the actual ICP values observed in these experiments to the clinical environment is premature.

No apparent histological abnormalities were observed in the contralateral white matter or hippocampus using the immunohistochemical markers described above, but these regions were not included in our detailed stereological analysis. Prominent silver staining contralateral to the impact site after CCI in mice has been observed by others. Future investigations evaluating the influence of ICP elevations on brain tissue remote to the impact site are planned.

Conclusion

CCI in mice, without decompressive craniectomy, resulted in significant elevations in ICP. Reductions in ICP after decompressive craniectomy were found to be associated with decreased white matter axonal injury as determined by two different markers up to 7 days after injury, as well as reduced white matter atrophy 1 month after injury. In the future, it will be important to test whether therapeutic interventions that prevent or reduce intracranial hypertension influence white matter injury severity and associated long-term outcomes.

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