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Research Report

A semicircular controlled cortical impact produces long-term motor and cognitive dysfunction that correlates well with damage to both the sensorimotor cortex and hippocampus

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ARTICLE INFO

Article history:
Accepted 28 May 2014
Available online 4 June 2014

Keywords:
Traumatic brain injury
Controlled cortical impact
Behavior
Histology
Mice

ABSTRACT

Animal models of traumatic brain injury (TBI) are essential for testing novel hypotheses and therapeutic interventions. Unfortunately, due to the broad heterogeneity of TBI in humans, no single model has been able to reproduce the entire spectrum of these injuries. The controlled cortical impact (CCI) model is one of the most commonly used models of contusion TBI. However, behavioral evaluations have revealed transient impairment in motor function after CCI in rats and mice. Here we report a new semicircular CCI (S-CCI) model by increasing the impact tip area to cover both the motor cortex and hippocampal regions in adult mice. Mice were subjected to S-CCI or CCI using an electromagnetic impactor (Impactor One™, MyNeuroLab; semicircular tip: 3 mm radius; CCI tip diameter: 3 mm). We showed that S-CCI, at two injury severities, significantly decreased the neuroscore and produced deficits in performance on a rotarod device for the entire duration of the study. In contrast, the CCI induced motor deficits only at early stages after the injury, suggesting that the S-CCI model produces long-lasting motor deficits. Morris water maze test showed that both CCI and S-CCI produced persisting memory deficits. Furthermore, adhesive removal test showed significant somatosensory and motor deficits only in the S-CCI groups. Histological analysis showed a large extent of cortical contusion

Abbreviations: TBI, traumatic brain injury; CCI, controlled cortical impact; S-CCI, semicircular controlled cortical impact; PBS, phosphate-buffered saline

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http://dx.doi.org/10.1016/j.brainres.2014.05.042

lesions, including both the sensory and motor cortex, and hippocampal damage in the S-CCI. These findings collectively suggest that the current model may offer sensitive, reliable, and clinically relevant outcomes for assessments of therapeutic strategies for TBI.

1. Introduction

Traumatic brain injury (TBI) is a common cause of death and disability worldwide, especially in children and young adults. In the United States, more than 1.7 million people suffer a TBI annually (Loane and Faden, 2010). Death and disability with TBI stem from 2 major mechanisms: a primary mechanical injury, and a secondary injury driven by a complex interplay of multiple injury factors such as inflammatory mediators, free radicals, and glutamate excitotoxicity initiated by the primary injury (Loane and Faden, 2010; Marklund and Hillered, 2011). This cascade of secondary damage has been the focus of much research, but to date there are limited pharmaceutical interventions with proven efficacy, and the complex relationships between various mediators are still poorly understood (Morales et al., 2005; Loane and Faden, 2010; Marklund and Hillered, 2011).

Animal models of TBI are essential for testing novel hypotheses and therapeutic interventions. Unfortunately, due to the broad heterogeneity of TBI in humans, no single model has been able to reproduce the entire spectrum of these injuries (Morales et al., 2005; Marklund and Hillered, 2011). The controlled cortical impact (CCI) model is one of the most commonly used models of contusion TBI. The CCI model utilizes a metal impactor rod to transiently and rapidly deform the cortex exposed via a craniotomy. CCI was initially developed for use in ferrets as bilateral injury of the brain (Lighthall, 1988; Lighthall et al., 1990). It was subsequently adapted for use in rats (Dixon et al., 1991) and mice (Smith et al., 1995) as lateral CCI paradigms. One advantage of this model is that the injury is primarily focal and one can easily delineate what regions of the brain are damaged and should be targeted for therapeutic intervention.

Experimental CCI produces graded, reproducible brain injury. However, behavioral evaluations have revealed transient impairment in various aspects of gross motor function and fine motor coordination after CCI in rats and mice (Dixon et al., 1991; Fox et al., 1998; Raghupathi et al., 1998; Hannay et al., 1999; Nakamura et al., 1999; Shear et al., 2004). For example, in the mouse CCI model, the motor deficits in forelimb reflex and hindlimb reflex were seen up to 15 d (Nakamura et al., 1999), rotarod deficits up to 7 d after CCI (Fox et al., 1998), and strength up to 7 d post-injury (Raghupathi et al., 1998; Morales et al., 2005). Additionally, neuroscores were significantly decreased at 15 d after CCI (Nakamura et al., 1999), which may be the result of brain lesion only covering a small area of motor cortex (Fig. 1).

Mouse models are being increasingly used because transgenic and knockout mice are available for the study of genetic and molecular mechanisms. In addition, the low cost and small body weights of mice are also attractive for testing therapeutic interventions. In humans, besides cognitive deficits that result from TBI, motor deficits are a major problem in both adult and young patients (Gagnon et al., 1998; Katz et al., 1998), even after mild concussive trauma (Tremblay et al., 2011). Prolonged motor deficits are an important clinical feature (Hillier et al., 1997; Heitger et al., 2006; Kozlowski et al., 2013). Thus, animal models with long-lasting motor deficits will be beneficial for pharmaceutical and rehabilitation evaluations. To produce long-lasting motor function impairment, we developed a new semicircular CCI (S-CCI) model in adult mice to increase the impact area to cover both sensorimotor cortex and hippocampus (Fig. 1D–F). Our new model produced not only brain tissue lesion in both regions, but also induced long-lasting sensorimotor and cognitive deficits associated with damage to these regions for the duration of investigation (up to 4 wk).

2. Results

A total of 38 female C57/BL6 mice were used in this study. Six mice were excluded from the study before the injury because their average latency to locate the platform on day 5 during training was greater than 45 s. Thirty-two mice were randomly divided into 4 groups: sham ($n=8$), 0.5 mm S-CCI ($n=8$), 1.0 mm S-CCI ($n=8$) and 1.0 mm CCI ($n=8$). Over the course of the 4-wk study period, 2 of the 32 mice died. One mouse from the CCI group died within 1 d, and the other from the 1.0 mm S-CCI group died within 1 wk.

2.1. *Behavioral characteristics of TBI*

A series of tests were performed on consecutive days following TBI to evaluate motor and sensorimotor function in all groups. All behavioral tests were blindly performed.

Neuroscore: There were statistically significant effects of injury group ($F_{(3,\ 26)}=128.9$, $p<0.0001$) and test day ($F_{(4,\ 104)}=5.141$, $p<0.001$; repeated-measures ANOVA) for neuroscore. No statistical significance was found for the interaction of injury group and test day ($F_{(12,\ 104)}=0.6191$, $p=0.8218$). Neurological function scores were significantly decreased in 0.5 and 1.0 mm S-CCI groups (Fig. 2, *post hoc* test, $p<0.01$). This decrease began 2 d post-injury and persisted for the entire duration of the study when compared to sham mice. However, in the CCI group, neuroscores were significantly decreased only at 2 d, 1 and 2 wk after injury (*post hoc* test, $p<0.01$). There was no significant difference between the 0.5 and 1.0 mm S-CCI groups at any of the time points studied (*post hoc* test, $p>0.05$). The scores from the CCI group were significantly higher than those in the both 0.5 and 1.0 mm S-CCI groups throughout almost the entire period investigated (*post hoc* test, $p<0.05$–0.01).