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Research paper

Single severe traumatic brain injury produces progressive pathology with ongoing contralateral white matter damage one year after injury

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ABSTRACT

There is increasing recognition that traumatic brain injury (TBI) may initiate long-term neurodegenerative processes, particularly chronic traumatic encephalopathy. However, insight into the mechanisms transforming an initial biomechanical injury into a neurodegenerative process remain elusive, partly as a consequence of the paucity of informative pre-clinical models. This study shows the functional, whole brain imaging and neuropathological consequences at up to one year survival from single severe TBI by controlled cortical impact in mice. TBI mice displayed persistent sensorimotor and cognitive deficits. Longitudinal T2 weighted magnetic resonance imaging (MRI) showed progressive ipsilateral (il) cortical, hippocampal and striatal volume loss, with diffusion tensor imaging demonstrating decreased fractional anisotropy (FA) at up to one year in the il-corpus callosum (CC: − 30%) and external capsule (EC: − 21%). Parallel neuropathological studies indicated reduction in neuronal density, with evidence of microgliosis and astrogliosis in the il-cortex, with further evidence of microgliosis and astrogliosis in the il-thalamus. One year after TBI there was also a decrease in FA in the contralateral (cl) CC (− 17%) and EC (− 13%), corresponding to histopathological evidence of white matter loss (cl-CC: − 68%; cl-EC: − 30%) associated with ongoing microgliosis and astrogliosis.

These findings indicate that a single severe TBI induces bilateral, long-term and progressive neuropathology at up to one year after injury. These observations support this model as a suitable platform for exploring the mechanistic link between acute brain injury and late and persistent neurodegeneration.

1. Introduction

There is increasing evidence that, rather than a single, acute, self-limiting event, for many individuals traumatic brain injury (TBI) can trigger a chronic, sometimes life-long disease process (Masel and DeWitt, 2010). Particular attention has focused on the relation between TBI and increased risk of late neurodegenerative disease, such as chronic traumatic encephalopathy (CTE) (Washington et al., 2016). Originally described in clinical studies of boxers as the 'punch-drunk' syndrome (Martland, 1928), the associated neuropathology later became recognized as dementia pugilistica, more recently CTE (Corsellis et al., 1973). However, in the past decade increasing descriptions of this pathology in non-boxer athletes exposed to repetitive brain injury (McKee et al., 2014; Stewart et al., 2016) and in individuals surviving a year or more from single moderate or severe TBI (Johnson et al., 2012) suggest it is exposure to TBI, independent of the circumstance, that predisposes to neurodegenerative pathology (Smith et al., 2013; McKee et al., 2016).

The neuropathology of late survival from TBI is complex and multifaceted and includes abnormalities in tau, amyloid β, TDP-43,

Abbreviations: AD, axial diffusivity; CC, corpus callosum; CCI, controlled cortical impact; cl, contralateral; CTE, chronic traumatic encephalopathy; CV, Cresyl violet; DTI, diffusion tensor imaging; EC, external capsule; EPI, echo-planar imaging; FA, fractional anisotropy; FPI, fluid percussion injury; il, ipsilateral; LFB, luxol fast blue; MD, mean diffusivity; MRI, magnetic resonance imaging; MWM, Morris water maze; r, remaining; RD, radial diffusivity; ROI, regions of interest; T2w, T2 weighted; TBI, traumatic brain injury

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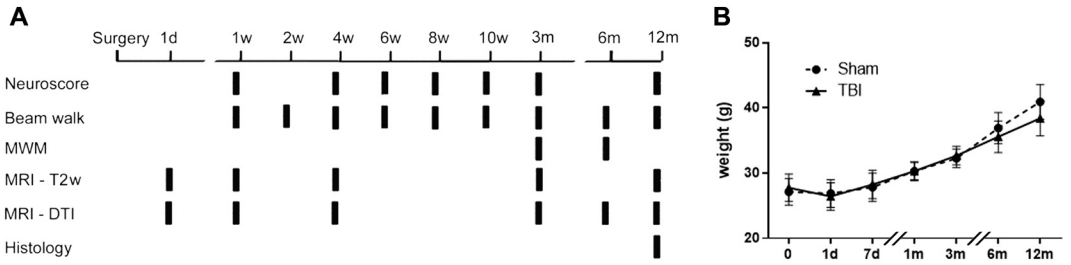

Fig. 1. Experimental design and weights. Mice were subjected to controlled cortical impact (CCI) or sham injury. Functional deficits and MRI analysis were assessed at multiple intervals up to one year post injury/sham at the time points indicated (A). Both sham and TBI mice gained body weight over time ($p < 0.0001$), with no significant difference between sham and TBI weight at any time. (B). Data are mean ± SD, $n = 7–8$.

neuroinflammation, axonal degeneration, neuronal loss and white matter degradation (Hay et al., 2016). Although there have been advances in describing these pathologies, our understanding of the processes linking acute phase TBI to late neurodegenerative pathology is still incomplete. As such, in the absence of candidate pathways driving late poor outcome, progress in identifying strategies for intervention has been limited partly because of the paucity of relevant pre-clinical models for late TBI survival, with the overwhelming majority of models defining 'late' follow-up as two months post-injury (Gold et al., 2013; Osier et al., 2015).

Limited evidence assessing outcomes one year after experimental TBI across models and injury severities indicates that sensorimotor and cognitive deficits persist up to one year after severe fluid percussion injury (FPI) (Pierce et al., 1998; Immonen et al., 2009) with progressive tissue loss, white matter damage and associated ongoing axonal pathology in the ipsilateral hemisphere (Pierce et al., 1998; Immonen et al., 2009; Smith et al., 1997; Bramlett and Dietrich, 2002). Persistence of functional deficits (Shelton et al., 2008; Shear et al., 2004; Dixon et al., 1999) has also been reported after focal brain injury by controlled cortical impact (CCI) in mice, with progressive tissue loss, reduction in cerebral blood flow (Dixon et al., 1999; Kochanek et al., 2002) and persistent neuroinflammatory processes, again in the ipsilateral (il) cortex, corpus callosum (cc) and thalamus (Loane et al., 2014). Similarly, models of repetitive mild TBI report long-term cognitive deficits associated with late pathologies (Mouzon et al., 2014; Winston et al., 2016), with variable results depending on the inter-injury interval (Meehan et al., 2012; Mannix et al., 2013). However, while there have been notable descriptions of persisting and evolving pathologies adjacent to the site of injury and in the ipsilateral hemisphere, there has been little insight into remote and contralateral pathologies so far.

In this work, we have longitudinally analysed the behavioral outcomes of a single severe TBI. Using *in vivo* quantitative magnetic resonance imaging (MRI) techniques and parallel neuropathological studies we describe the long-term and progressive, bilateral hemispheric consequences of TBI. We show a progression of pathology to regions remote from the original injury and into contralateral structures at one year after injury, with ongoing white matter pathology, including active neuroinflammation.

2. Materials and methods

2.1. Animals

C57BL/6 mice (Harlan Laboratories, Italy) were housed in a specific pathogen free vivarium at a constant temperature (21 ± 1 °C) with a 12 h light–dark cycle and free access to food and water. The IRCCS-Istituto di Ricerche Farmacologiche Mario Negri (IRFMN) adheres to the principles set out in the following laws, regulations, and policies governing the care and use of laboratory animals: Italian Governing Law (D.lgs 26/2014; Authorization n.19/2008-A issued March 6, 2008 by Ministry of Health); Mario Negri Institutional Regulations and Policies providing internal authorization for persons conducting animal experiments (Quality Management System Certificate – UNI EN ISO 9001:2008 – Reg. No. 6121); the NIH Guide for the Care and Use of Laboratory Animals (2011 edition) and EU directives and guidelines (EEC Council Directive 2010/63/UE). The Statement of Compliance (Assurance) with the Public Health Service (PHS) Policy on Human Care and Use of Laboratory Animals has been recently reviewed (9/9/2014) and will expire on September 30, 2019 (Animal Welfare Assurance #A5023–01). All efforts were made to minimize animal suffering and reduce the number of animals used.

2.2. Experimental design

Sham and TBI mice ($n = 8$/group) were longitudinally analysed following the experimental plan shown in Fig. 1A. Sensorimotor deficits were evaluated by neuroscore (1, 4, 6, 8 and 10 weeks, 3 and 12 months) and beam walk tests (1, 2, 4, 6, 8 and 10 weeks, 3, 6 and 12 months) by the same operator for the entire duration of the study. Cognitive deficits were evaluated by Morris water maze test at 3 and 6 months. Imaging studies were done as T2 weighted (T2w) magnetic resonance imaging (MRI) (1, 7 days, 1, 3, 12 months) and diffusion tensor imaging (DTI) MRI (1, 7 days, 1, 3, 6, 12 months). All mice were sacrificed at 12 months for histopathological analysis. Body weight at all time points was recorded in all mice (Fig. 1B). TBI did not result in any acute mortality; one mouse died three months after injury.

2.3. Experimental traumatic brain injury

Eight-week-old male mice were anesthetized with isoflurane inhalation (induction 3%; maintenance 1.5%) in an N_2O/O_2 (70%/30%) mixture and placed in a stereotaxic frame. Rectal temperature was maintained at 37 °C. Mice were then subjected to craniectomy followed by induction of CCI brain injury as previously described (Pischiutta et al., 2014; Zanier et al., 2011; Zanier et al., 2014; Zanier et al., 2016). Briefly, the injury was induced using a 3 mm diameter rigid impactor driven by a pneumatic piston rigidly mounted at an angle of 20° from the vertical plane and applied vertically to the exposed dura mater, between bregma and lambda, over the left parieto-temporal cortex (antero-posteriority: − 2.5 mm, laterality: − 2.5 mm), at impactor velocity of 5 m/s and deformation depth 1 mm, resulting in a severe level of injury (Brody et al., 2007; De Blasio et al., 2017). The craniotomy was then covered with a cranioplasty and the scalp sutured. Sham-operated mice received identical anesthesia and surgery without brain injury.

2.4. Behavioral tests

Sensorimotor deficits were evaluated by neuroscore and beam walk tests, and cognitive deficits were evaluated by the Morris water maze test as previously described (Pischiutta et al., 2014; Zanier et al., 2011; Zanier et al., 2014; Zanier et al., 2016; Zanier et al., 2013).