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BRAIN RESEARCH

Research Report

Temporal assessment of traumatic axonal injury in the rat corpus callosum and optic chiasm

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ARTICLE INFO

Article history:
Accepted 22 May 2012
Available online 28 May 2012

Keywords:
Traumatic axonal injury
Corpus callosum
Optic chiasm
Impaired axoplasmic transport
Neurofilament compaction
Impact acceleration model

ABSTRACT

Impaired axoplasmic transport (IAT) and neurofilament compaction (NFC), two common axonal pathology processes involved in traumatic axonal injury (TAI), have been well characterized. TAI is found clinically and in animal models in brainstem white matter (WM) tracts and in the corpus callosum (CC), optic chiasm (Och), and internal capsule. Previous published quantitative studies of the time course of TAI expression induced by the Marmarou impact acceleration model have been limited to the brainstem. Accordingly, this study assessed the extent of IAT and NFC in the CC and Och at 8 h, 28 h, 3 days and 7 days after traumatic brain injury (TBI) induction by the Marmarou impact acceleration model. IAT peak density was observed at 8 h in the CC and 28 h in the Och post-TBI. NFC peak density was observed at 28 h in both structures. The density of IAT and NFC decreased with increasing survival time in both structures. The NFC density time profile followed a similar trend in both the Och and CC, whereas the IAT density time profile was variable between the Och and CC. Furthermore, a strong linear relationship was observed between IAT and NFC in the CC but not in the Och. These findings highlight the heterogeneity of TAI as evidenced by variable IAT and NFC injury time profiles in each anatomical structure. This variability indicates the requirement of multiple markers for a comprehensive TAI evaluation and multiple targeted treatments for TAI polypathology within its therapeutic window time frame.

1. Introduction

Diffuse axonal injury (DAI), also referred to as traumatic axonal injury (TAI), is a well-recognized consequence of blunt head injury (Adams et al., 1982). TAI is considered a major contributor to morbidity and mortality after traumatic brain injury (TBI) (Adams et al., 1989; Bennett et al., 1995; Gentleman et al., 1995; Povlishock and Christman, 1995; Slazinski and Johnson, 1994).

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Abbreviations: β-APP, beta amyloid precursor protein; CC, corpus callosum; DAI, diffuse axonal injury; IAT, impaired axoplasmic transport; IR, immunoreactive; NFC, neurofilament compaction; NF-M, neurofilament medium; Och, optic chiasm; TAI, traumatic axonal injury; TBI, traumatic brain injury; WM, white matter

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doi:10.1016/j.brainres.2012.05.046

TAI is produced by rapid head acceleration/deceleration during a traumatic event (Kelley et al., 2006) with consequent tension or shear on axons.

Clinically, TAI has been reported to appear throughout the deep and subcortical white matter (WM) and has been noted to be predominantly common in the midline structures, including the corpus callosum (CC) and brainstem (Meythaler et al., 2001; Smith et al., 2003). In addition, vision impairment has also been reported to occur clinically following closed head injury (Perunovic et al., 2001). A post-mortem immunocytochemistry study of severely closed head injury cases revealed the presence of TAI at different sites within the optic pathways, such as the optic chiasm (Och), optic tract and optic radiations (Perunovic et al., 2001).

The Marmarou impact acceleration weight-drop model has been widely used since its origination in 1994 to induce closed head TBI in rats in order to study TAI and other pathological changes. This model has been reported to induce DAI, particularly in the CC, internal capsule, optic tracts, cerebral and cerebellar peduncles and in the long tracts in the brainstem (Foda and Marmarou, 1994). Despite clinical findings of TAI in the subcortical WM and optic pathways, laboratory studies of TAI in these anatomical structures are limited, especially those using impact acceleration injury models. The majority of TAI studies using the Marmarou model have focused on the brainstem (Buki et al., 2000; Marmarou and Povlishock, 2006; Marmarou et al., 2005; Povlishock et al., 1997; Stone et al., 2000, 2001, 2004; Suehiro et al., 2001). The original histological study of the impact acceleration model by Foda and Marmarou (1994) reported that TAI does occur in the brainstem and to a lesser extent in the cortical WM. To the best of our knowledge, only five histological studies utilizing the Marmarou model have reported TAI in the cortical WM in a quantitative manner (Ding et al., 2001; Kallakuri et al., 2003; Kallakuri et al., 2012; Li et al., 2011a; Li et al., 2011b). These studies showed that it is possible to induce TAI in the CC (Kallakuri et al., 2003; Kallakuri et al., 2012; Li et al., 2011a, 2011b) and optic chiasm (Och) (Ding et al., 2001) using the Marmarou model. Because clinical studies have indicated that the CC and Och pathways are prominent areas of TAI after TBI, further laboratory studies are needed to assess the extent of TAI in these regions following closed-head TBI. The few laboratory studies that have quantified TAI in the CC (Kallakuri et al., 2003; Kallakuri et al., 2012; Li et al., 2011a, 2011b) and the Och (Ding et al., 2001) using this model are limited to studying TAI expression at 24 h post-TBI. However, a temporal assessment is lacking of TAI density in these structures following TBI induced by impact acceleration.

Beta amyloid precursor protein (β-APP) immunostaining has been found valuable in the detection of TAI as early as 30–35 min after the traumatic event in rats and humans (Gorrie et al., 2002; Hortobagyi et al., 2007; Marmarou et al., 2005; Stone et al., 2001) and was used extensively as a marker of impaired axoplasmic transport (IAT) (DiLeonardi et al., 2009; Geddes et al., 2003; Gorrie et al., 2002; Hortobagyi et al., 2007; Marmarou et al., 2005; McKenzie et al., 1996; Pal et al., 2006; Stone et al., 2001; Suehiro et al., 2001). In addition to IAT, the intra-axonal cytoskeletal alteration characterized as neurofilament compaction (NFC) has been increasingly investigated by utilizing specific neurofilament medium chain (NF-M) antibodies (Czeiter et al., 2008; Stone et al., 2001; Suehiro et al., 2001). NFC was shown to be associated with either dephosphorylation or calpain-mediated proteolysis of neurofilament sidearms (Buki et al., 1999a; Giza and Hovda, 2001; Marmarou et al., 2005). The monoclonal RMO14 antibodies specifically recognize epitopes on the NF-M rod domain that become available after dephosphorylation, enabling the detection of NFC (Trojanowski et al., 1989). IAT and NFC are two distinct processes occurring in distinct axonal populations (Stone et al., 2001). Compared to other histological methods such as silver staining, immunohistochemistry has the advantage of detecting subcellular aspects of TAI such as IAT and NFC (DiLeonardi et al., 2009; Marmarou et al., 2005; Pal et al., 2006; Stone et al., 2001; Suehiro et al., 2001). The purpose of this study was to assess the subcellular aspects of TAI density over time in two clinically relevant WM tracts. Specifically, the temporal subcellular TAI density changes in the CC and Och were quantified at 8 h, 28 h, 3 days and 7 days after TBI by β-APP and RMO14 immunocytochemistry.

2. Results

2.1. *Qualitative analysis*

In the immunocytochemistry controls, in which primary antibodies were deleted, no immunoreactivity was observed. The sham group showed no β-APP-immunoreactive (IR) axons and RMO14-IR axons. In the injured rats' sections, β-APP-IR and RMO14-IR axonal morphological changes were present in the Och and CC (Figs. 1–3). In the Och, β-APP-IR swollen axons were detected 8 h post-TBI, with some forming retraction balls (Fig. 1A). At 28 h post-TBI, β-APP-IR axons with their still-swollen profiles were observed, but they appeared thinner compared to their previous time point. Additionally, other axon profiles at 28 h were punctuated dense or wavy axons, some capped with terminal bulbs (Fig. 1B). At 3 and 7 days post-TBI, the extent of β-APP-IR axons in the Och decreased and the injury profile was of swollen axons present at the boundary of the Och (close to optic radiations or adjacent nuclei), rather than at its center.

In the CC, at 8 and 28 h post-TBI, the profiles of β-APP-IR axons were that of swollen axons with some retraction balls (Fig. 2A). At 3 and 7 days post-TBI, the extent of β-APP-IR axons decreased and the injury profile was that of swollen axons as well as dispersed axons with a punctuated dense appearance. The β-APP-IR axonal density was significantly ($p<0.01$) less in the CC at 8 h post-TBI than in the Och at the same time point (Figs. 4A, C).

In both the Och and CC, the RMO14-IR axons appeared as dense bands and dispersed with a small-punctuated dense appearance at 8 h and 28 h post-TBI (Fig. 2B). At 3 and 7 days post-TBI, the RMO14-IR axonal profiles appeared as elongated dense bands, some capped with terminal bulbs (Fig. 3). The RMO14-IR axonal densities across all time points were statistically insignificant between the Och and the CC. In addition, RMO14-IR axons were mainly present at the boundary of the Och, whereas in the CC the RMO14-IR axons were more diffuse in location.

2.2. *Quantitative analysis*

Quantitative analysis of β-APP and RMO14-IR axons over time showed significant changes in the Och. β-APP-IR axons were