Neuropharmacology 108 (2016) 382–393

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Sodium selenate, a protein phosphatase 2A activator, mitigates hyperphosphorylated tau and improves repeated mild traumatic brain injury outcomes

Xin L. Tan [a], David K. Wright [b, c], Shijie Liu [a], Christopher Hovens [d], Terence J. O'Brien [a], Sandy R. Shultz [a, *]

[a] Department of Medicine, The Royal Melbourne Hospital, The University of Melbourne, Parkville, VIC, 3050, Australia
[b] Anatomy and Neuroscience, The University of Melbourne, Parkville, VIC, 3010, Australia
[c] The Florey Institute of Neuroscience and Mental Health, Parkville, VIC, 3052, Australia
[d] Department of Surgery, The Royal Melbourne Hospital, The University of Melbourne, Parkville, VIC, 3050, Australia

ARTICLE INFO

Article history:
Received 12 November 2015
Received in revised form
26 April 2016
Accepted 3 May 2016
Available online 7 May 2016

Keywords:
concussion
chronic traumatic encephalopathy
MRI
diffusion tensor imaging
traumatic brain injury
tauopathy

ABSTRACT

Mild traumatic brain injuries may result in cumulative brain damage and neurodegenerative disease. To date, there is no pharmaceutical intervention known to prevent these consequences. Hyperphosphorylated tau has been associated in this process, and protein phosphatase 2A 55 kDa regulatory B subunit (PP2A/PR55) – the major tau phosphatase – is decreased after a brain insult. Sodium selenate up-regulates PP2A/PR55 and dephosphorylates tau, and may hold promise as a treatment in the mild brain injury setting. Here we investigated sodium selenate treatment in rats given repeated mild traumatic brain injuries. Rats were given three mild fluid percussion injuries or three sham-injuries, and treated with sodium selenate (1 mg/kg/day) or saline-vehicle for three months before undergoing behavioral testing, MRI, and post-mortem analysis of brain tissue. Repeated mild traumatic brain injuries increased the phosphorylation of tau and decreased PP2A/PR55, whilst inducing brain atrophy and cognitive and sensorimotor deficits. Sodium selenate treatment increased PP2A/PR55, and decreased tau phosphorylation, brain damage, and cognitive and motor impairments in rats given repeated mild traumatic brain injuries. Our findings implicate PP2A/PR55 and tau as important mechanisms in the pathophysiological aftermath of repeated mild brain traumas, and support sodium selenate as a novel and translatable treatment for these common injuries.

1. Introduction

Mild traumatic brain injuries (mTBI), including concussions, account for the majority of TBI cases (Blennow et al., 2012; Jordan, 2013). Although individuals typically recover after a single mTBI, repeated mTBIs (rmTBI) can have cumulative and chronic effects, and are associated with neurodegenerative diseases such as chronic traumatic encephalopathy (CTE; Blennow et al., 2012; Jordan, 2013; McKee et al., 2013). While the mechanisms and factors that contribute to these effects are poorly understood, the pathological hallmark of CTE is hyperphosphorylated tau (h-tau; McKee et al., 2013) and there is growing evidence that h-tau is involved in the neurodegeneration and neurological impairments that occur in TBI and CTE (Cheng et al., 2014; Kondo et al., 2015; Shultz et al., 2015c).

Tau is a microtubule-associated protein that is important in the stabilization of microtubules, axonal transport, and neuronal health (Ballatore et al., 2007; Morris et al., 2011). H-tau can occur in neuropathological settings, such as excitotoxicity and oxidative stress (Dias-Santagata et al., 2007; Liang et al., 2009; Zhu et al., 2000), which are common in TBI (Blennow et al., 2012). Consequently, h-tau is unable to bind to the microtubule, the

Abbreviations: ANTS, advanced normalization tools; CTE, chronic traumatic encephalopathy; DTI, diffusion tensor imaging; TE, echo time; FOV, field of view; h-tau, hyperphosphorylated tau; MRI, magnetic resonance imaging; mFPI, mild fluid percussion injury; mTBI, mild traumatic brain injury; PVDF, polyvinyl difluoride; PP2A, protein phosphatase 2A; PP2Ac, protein phosphatase 2A, catalytic C-subunit; PP2A/PR55, protein phosphatase 2A, PR55 B-subunit; ROI, regions of interest; rmFPI, repetitive mild fluid percussion injury; rmTBI, repetitive mild traumatic brain injury; TR, repetition time; SDS, sodium dodecyl sulphate; TBI, traumatic brain injury.

* Corresponding author. Office 1.02, Melbourne Brain Centre, Department of Medicine, The University of Melbourne, Parkville, VIC, 3052, Australia.
E-mail address: sshultz@unimelb.edu.au (S.R. Shultz).

http://dx.doi.org/10.1016/j.neuropharm.2016.05.001
0028-3908/

cytoskeleton destabilizes, cellular functions are disrupted, and h-tau can accumulate and form insoluble neurofibrillary tangles, all of which may have neurotoxic effects (Ballatore et al., 2007; Gendron and Petrucelli, 2009; Morris et al., 2011).

The phosphorylated state of tau is regulated by a series of kinases and phosphatases. Protein phosphatase 2A (PP2A) is a heterotrimeric protein that consists of a core A-subunit, a catalytic C-subunit (PP2Ac), and a regulatory B-subunit (Iqbal et al., 2009; Zheng et al., 2014). PP2A heterotrimers consisting of the PR55 regulatory B-subunit (PP2A/PR55) account for over 70% of tau phosphatase activity in the brain and is essential for the dephosphorylation of tau (Iqbal et al., 2009; Liu et al., 2005; Shi, 2009; Wang et al., 1996, 2007; Xu et al., 2008). Importantly, the down-regulation of PP2A/PR55 occurs after brain insult (Chen et al., 2010; Koh, 2013; Shultz et al., 2015c), and promotes h-tau (Bolognin et al., 2012; Sontag et al., 2004). As such, PP2A/PR55 may be affected by mTBI and contribute to h-tau, and up-regulating PP2A/PR55 may reduce h-tau and improve outcomes in the rmTBI setting. Sodium selenate is a potent PP2A/PR55 activator, and previous findings demonstrate that treatment with sodium selenate up-regulates PP2A/PR55, decreases h-tau, and improves outcome in animal tauopathy models (Corcoran et al., 2010b; Shultz et al., 2015c; Van Eersel et al., 2010).

Due to the relatively mild nature of mTBI, and the progressive and cumulative effects of rmTBI, it is challenging to study the pathobiology and treatment of these injuries in the clinical setting. Animal models allow for the rigorous investigation of pathophysiological changes (e.g., h-tau and PP2A) in mTBI and rmTBI, and for the assessment of novel pharmaceutical interventions that target these mechanisms. The single mild fluid percussion injury (mFPI) model in rats results in transient motor and cognitive deficits (DeRoss et al., 2002; Gurkoff et al., 2006; Hylin et al., 2013; Shultz et al., 2011), as well as abnormalities in sleep (Lim et al., 2013), electrophysiology (Aungst et al., 2014; Johnstone et al., 2014), and other behaviors (Shultz et al., 2011). A single mFPI also induces transient neuroinflammation, axonal injury, and reduced cerebral blood flow (Hylin et al., 2013; Shultz et al., 2012, 2011), but does not result in significant neuronal loss, visible brain contusion, focal lesion, or enduring cognitive and behavioral impairments (Aungst et al., 2014; Gurkoff et al., 2006; Hylin et al., 2013; Shultz et al., 2012, 2011). These findings are consistent with signs, symptoms, and physiological changes reported in mTBI patients. Furthermore, studies have now administered repeated mFPIs (rmFPI) to rats to study the effects of rmTBI, and related conditions such as CTE. Findings from these studies indicate that rmFPIs induce cumulative and persisting cognitive, sensorimotor, and emotional abnormalities that occur in the presence of progressive cortical atrophy and neuronal loss, axonal injury, chronic neuroinflammation, alterations in excitatory synaptic neurotransmission, and impaired long-term potentiation (Aungst et al., 2014; DeRoss et al., 2002; Shultz et al., 2013, 2012; Wang et al., 2013; Webster et al., 2015). Many of these changes resemble the spectrum of abnormalities that have been reported in humans who have suffered rmTBIs. Therefore, here we utilized the mFPI models of mTBI and rmTBI in rats to characterize the influence of these injuries on PP2A/PR55 and h-tau. We then assessed sodium selenate treatment in rats administered rmTBIs and given a three-month recovery time. After the three-month recovery period, rats underwent behavioral testing and magnetic resonance imaging (MRI) before post-mortem analysis of brain tissue.

2. Materials and methods

2.1. Subjects

Subjects were 86 young adult male Long-Evans hooded rats that were obtained from Monash animal research services (Melbourne, Australia). At the time of surgery rats were 12 weeks old, weighed 250–300 g, and were naïve to all experimental procedures. After surgery, rats were housed individually for the remainder of the study under a 12:12 light/dark cycle and were allowed access to food and water *ad libitum*. All procedures were approved by the University of Melbourne Animal Ethics Committee (AEC #1112173), and were within the guidelines of the Australian code of practice for the care and use of animals for scientific purposes by the Australian National Health and Medical Research Council (NHMRC).

2.2. Experimental groups

To investigate whether a single mTBI affects PP2A/PR55 and h-tau, and whether rmTBI exacerbates these effects, 45 rats were assigned to receive either one mFPI (1mTBI), two mFPIs (2mTBI), or sham-injuries (SHAM), and were killed at 24 h, one week, and one month post-injury (n = 5/group/time point).

To assess the effects of sodium selenate (SS) treatment (1 mg/kg/day subcutaneously) on long-term rmTBI outcomes, the remaining 41 rats were assigned to one of four experimental conditions: three sham-injuries + saline-vehicle treatment (SHAM + VEH; n = 10), three sham-injuries + SS treatment (SHAM + SS; n = 10), three mFPIs + saline-vehicle treatment (3mTBI + VEH; n = 11), or three mFPIs + SS treatment (3mTBI + SS; n = 10). Previous experiments from our laboratories have demonstrated that a continuous subcutaneous dose of 1 mg/kg/day (delivered via a subcutaneous pump) activates PP2A/PR55, reduces h-tau, and does not have any overt toxic effects in rodents (Liu et al., 2016; Shultz et al., 2015c). Furthermore, similar doses and treatment durations have been well-tolerated in human clinical trials (i.e., up to 90 mg per day for adult patients; Corcoran et al., 2010a), and a continuous dose is necessary to maintain consistent bioavailability because sodium selenate has a short half-life in vivo (approximately 1.2–2.9 h; Corcoran et al., 2010a). The three mFPI model was chosen for the treatment study because three mFPIs, with each injury separated by five days, have been shown to result in chronic behavioral and pathological consequences (Shultz et al., 2013, 2012; Weaver et al., 2015; Webster et al., 2015).

2.3. Surgery and mFPI

All procedures were based on previously described protocols (Johnstone et al., 2014; Shultz et al., 2013, 2012; Webster et al., 2015). Rats were placed in a sealed Plexiglas box into which 4% isoflurane and 2 L/min oxygen flow was introduced for anesthesia. Rats were then placed in a stereotaxic frame via ear bars, with anesthesia maintained at 2% isoflurane and 1 L/min oxygen, and given a subcutaneous injection of analgesic (carprofen 5 mg/kg). A craniotomy (5 mm diameter) centered over the following coordinates with reference to Bregma: anterior/posterior −3.0 mm, medial/lateral 4.0 mm was performed on all rats under anesthetic and aseptic conditions. A hollow plastic injury cap was sealed over the craniotomy using dental cement and a removable plug was inserted into the injury cap to seal the craniotomy at all times except during mFPI or sham injury.

For the study characterizing the effects of SHAM, 1mTBI, or 2mTBI on PP2A/PR55 and h-tau at 24 h, 1 week, and 1 month post-injury, the rats were attached to the FPI device immediately after the craniotomy surgery. At the first response of a hind-limb withdrawal to a toe pinch, rats in the 2mTBI group were administered a mFPI induced by a 1–1.5 atm fluid pulse to the brain that was generated by the FPI device. The rats from the SHAM and 1mTBI groups were administered sham-injuries, which involve identical procedures as those for a mFPI except that the fluid pulse was not