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Research Report

Salubrinal reduces oxidative stress, neuroinflammation and impulsive-like behavior in a rodent model of traumatic brain injury

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ABSTRACT

Traumatic brain injury (TBI) is the leading cause of trauma related morbidity in the developed world. TBI has been shown to trigger secondary injury cascades including endoplasmic reticulum (ER) stress, oxidative stress, and neuroinflammation. The link between secondary injury cascades and behavioral outcome following TBI is poorly understood warranting further investigation. Using our validated rodent blast TBI model, we examined the interaction of secondary injury cascades following single injury and how these interactions may contribute to impulsive-like behavior after a clinically relevant repetitive TBI paradigm. We targeted these secondary pathways acutely following single injury with the cellular stress modulator, salubrinal (SAL). We examined the neuroprotective effects of SAL administration on significantly reducing ER stress: janus-N-terminal kinase (JNK) phosphorylation and C/EBP homology protein (CHOP), oxidative stress: superoxide and carbonyls, and neuroinflammation: nuclear factor kappa beta (NFκB) activity, inducible nitric oxide synthase (iNOS) protein expression, and pro-inflammatory cytokines at 24 h post-TBI. We then used the more clinically relevant repeat injury paradigm and observed elevated NFκB and iNOS activity. These injury cascades were associated with impulsive-like behavior measured on the elevated plus maze. SAL administration attenuated secondary iNOS activity at 72 h following repetitive TBI, and most importantly prevented impulsive-like behavior. Overall, these results suggest a link between secondary injury cascades and impulsive-like behavior that can be modulated by SAL administration.

1. Introduction

3.2 million Americans are currently living with disabilities from traumatic brain injury (TBI) (Zaloshnja et al., 2008). Impulsivity is one of the most common and potentially dangerous symptoms associated with brain injury (Schwarzbold et al., 2010; Adhikari et al., 2011; Logsdon et al., 2014; Michael et al., 2015). Impulsivity is a key finding in patients diagnosed with chronic traumatic encephalopathy (CTE) and presents early in disease progression (Banks et al., 2014; Rebetez et al., 2015). Currently, no therapies for impulsivity are available for patients diagnosed with CTE. The underlying mechanisms linking neurotrauma to subacute neuropsychiatric symptoms are still poorly understood (Lucke-Wold et al., 2014a).

The concept of an interrelationship between cellular stress and lasting degenerative changes remains to be elucidated. Endoplasmic reticulum (ER) stress and oxidative stress have emerged as contributors to neurodegeneration and behavioral dysfunction. ER stress has been shown to play a significant role in acute and chronic disease pathology following TBI (Zhang et al., 2012; Abdul-Muneer et al., 2014; Begum et al., 2014; Lucke-Wold et al., 2015a).

Abbreviations: TBI, traumatic brain injury; sTBI, single blast-induced traumatic brain injury; rTBI, repeated blast-induced traumatic brain injury; ER, endoplasmic reticulum; SAL, salubrinal; GFAP, Glial fibrillary acidic protein; FJB, Fluorojade B; JNK, c-jun N-terminal kinase; NFκB, nuclear factor kappa beta; iNOS, intrinsic nitric oxide synthase; BiP, binding immunoglobulin protein; CHOP, C/EBP homology protein; TNFα, tumor necrosis factor alpha; IL-1β, interleukin 1 beta; NOX, NADPH-oxidase

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We recently showed that markers of ER stress were increased in the brains of athletes diagnosed with CTE, and rodents exposed to repetitive blast injury (Lucke-Wold et al., 2016).

Janus-N-terminal kinase (JNK) is a common downstream component of ER stress (Urano et al., 2000), which is activated following TBI (Otani et al., 2002; Szmydynger-Chodobska et al., 2010). JNK activity can influence nuclear factor kappa beta (NFκB) translocation to the nucleus, which upregulates pro-inflammatory mediators (Ruan et al., 2015). It is well known that neural injury accelerates the release of pro-inflammatory cytokines which can signal lasting neuronal cell stress (Hong et al., 2016). If the cellular stress response is severe, or sustained, the neuron will undergo apoptosis (Nakagawa and Yuan, 2000), causing extensive gliosis and neuroinflammation (Harvey et al., 2015). We propose that TBI induces NOX4-mediated oxidative stress and JNK-mediated ER stress, which subsequently contributes to neuroinflammation through NFκB activation.

Simultaneous to ER stress activation, oxidative stress occurs and generates free radicals, which play a role in cell death and disease pathology following TBI (Toklu and Tumer, 2015). Free radicals damage cellular membranes, increase carbonyl formation, and can contribute to cell death and neurobehavioral dysfunction (Ferguson et al., 2010). We previously showed NOX4-mediated oxidative stress increased neuronal apoptosis following traumatic brain injury (Lucke-Wold et al., 2015b). In addition, Wu and colleagues used an *In vitro* model of endothelial injury to causally link NADPH-oxidase (Nox4)-mediated oxidative stress to Janus-N-terminal kinase (JNK)-mediated ER stress (Wu et al., 2014b). The group also silenced JNK-mediated ER stress and observed an attenuation of nuclear translocation NFκB (Wu et al., 2014b). These findings suggest NOX-mediated oxidative stress and JNK-mediated ER stress to be linked to NFκB activation.

In conjunction with these cell stress responses, chronic neuroinflammation has emerged as a possible contributory factor to behavior change (Faden et al., 2015). Preclinical models have shown that TBI is associated with a significant inflammatory burden (Kumar et al., 2014). Furthermore, it has been shown that neuroinflammation can persist years after injury in the brains of retired athletes (Coughlin et al., 2014). Recent clinical evidence ties neuroinflammation to neurobehavioral symptoms (Cho et al., 2013; Wu et al., 2014a). We propose that acute modulation of cellular stress after TBI will positively influence the extracellular inflammatory milieu leading to improved behavioral outcomes.

Salubrinal (SAL) is a modulator of cellular stress known to inhibit protein phosphatase 1, and attenuate global translation (Boyce et al., 2005). Reducing the ER workload promotes proteostasis and cell survival (Hotamisligil, 2010; Tsaytler et al., 2011; Walter and Ron, 2011) SAL has been shown to be neuroprotective in models of protein toxicity (Colla et al., 2012; Huang et al., 2012), stroke (Nakka et al., 2010), excitotoxicity (Sokka et al., 2007), and TBI (Rubovitch et al., 2015). In our model of TBI, have previously shown SAL to reduce ER-mediated apoptosis and to ameliorate impulsive-like behavior (Logsdon et al., 2014). In the present study, we investigated the effects of SAL on reducing neuroinflammation, and impulsive-like behavior following a more clinically relevant repetitive TBI paradigm (Fig.1).

2. Results

2.1. SAL attenuated markers of ER stress after single blast

ER stress is a common secondary cascade implicated in subacute injury expansion following TBI (Farook et al., 2013; Begum et al., 2014). Our previous study showed that markers of the acute phase ER stress were upregulated following sTBI (Logsdon et al., 2014). In this study, we investigated additional markers of ER stress, JNK phosphorylation and CHOP activation, which have been associated with NFκB activity (Deng et al., 2004; Tsai et al., 2012).

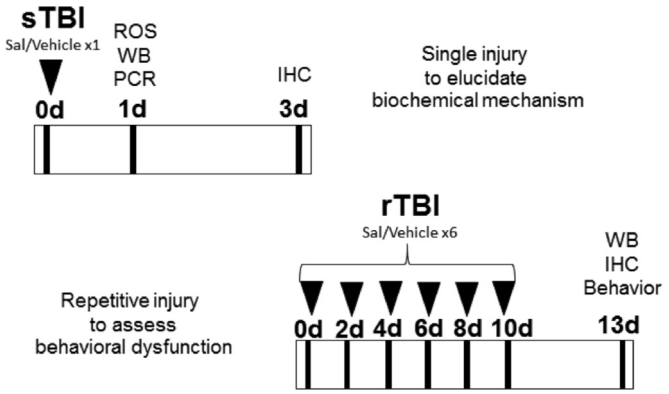

Fig. 1. Experimental design. Detailed experimental timeline showing: sTBI (single blast) animals at top; rTBI (repetitive blast) animals at bottom. SAL (Salubrinal); ROS (Reactive oxygen species); WB (Western blot); PCR (Polymerase chain reaction); IHC (Immunohistochemistry).

Fig. 2(A) indicates a significant difference between experimental groups in JNK activity after sTBI ($F_{(2,9)}=9.04$; $P<0.01$). A significant increase in the ratio between JNK phosphorylation and total JNK expression was observed at 24 h after sTBI compared to control rats ($q=5.79$; $P<0.01$). SAL administration significantly attenuated JNK phosphorylation when compared to vehicle-treated sTBI rats ($q=4.31$; $P<0.05$).

Fig. 2(B) indicates a significant difference between experimental groups in CHOP activation after sTBI ($F_{(2,9)}=8.769$; $P<0.01$). A significant increase in CHOP expression at 24 h was observed in sTBI rats as compared to control rats ($q=5.86$; $P<0.01$). SAL administration significantly attenuated CHOP activation when compared to vehicle-treated sTBI rats ($q=3.673$; $P<0.05$). SAL successfully reduced markers of ER stress when administered acutely after injury.

2.2. SAL reduced markers of oxidative stress after single blast

ER stress activation has been proposed to directly increase oxidative stress particularly in the striatum (Malhotra and Kaufman, 2007). ROS generation is a consequence of TBI mainly through membrane damage and subsequent NOX4 system activation (Zhang et al., 2012; Loane et al., 2013). Activation of the NOX4 system predominantly creates superoxide (Brennan et al., 2009; Lucke-Wold et al., 2015b). A previous report indicated that both NOX4 and superoxide were acutely elevated after TBI (Ansari et al., 2014).

Fig. 3(A) shows a significant difference in carbonyl levels between groups after sTBI ($F_{(2,9)}=10.21$; $P<0.01$). Carbonyl formation is an end product of oxidative stress damage. A significant increase in carbonyl levels was measured in sTBI rats as compared to control rats ($q=6.26$; $P<0.01$). SAL administration significantly reduced carbonyl levels ($q=4.23$; $P<0.05$) when compared to vehicle-treated sTBI rats.

Fig. 3(B) shows a significant difference in superoxide levels between experimental groups after sTBI ($F_{(2,9)}=7.68$; $P<0.05$). A significant increase in superoxide levels was measured in sTBI rats when compared to control rats ($q=4.92$, $P<0.05$). SAL administration significantly reduced superoxide levels as compared to vehicle-treated sTBI rats ($q=4.68$, $P<0.01$). Fig. 3(C) shows no difference in total ROS levels ($F_{(2,9)}=0.16$; $P>0.05$).

Fig. 3(D) shows a significant difference in corrected total cell fluorescence for NOX4 in the striatum at 24 h post-sTBI ($F_{(2,27)}=3.76$; $P<0.05$). A significant increase in NOX4