Journal Club

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Tumor Necrosis Factor α as a Potential Mediator of the Effects of Phosphodiesterase 4B Inhibition on Cognition after Traumatic Brain Injury

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Review of Titus et al.

Traumatic brain injury (TBI) is a major public health problem worldwide, causing chronic disability in an estimated 200 million people every year (Dougall et al., 2015). Chronic cognitive impairment is one of the most common consequences of TBI, affecting up to 80% of TBI survivors (Dougall et al., 2015; Titus et al., 2016).

Many mechanisms may underlie post-TBI chronic cognitive dysfunction, including impaired LTP (Titus et al., 2013), cholinergic (Arciniegas, 2003) and catecholaminergic (Jenkins et al., 2016) signaling disturbances, neuroinflammation (Morganti et al., 2015), and dysfunctions of white matter and neural connectivity (Hayes et al., 2016). Although substantial progress has been made in understanding the mechanisms underlying chronic cognitive dysfunction, clinical trials in humans have largely been unsuccessful in improving post-TBI cognition (Zafonte et al., 2012; Dougall et al., 2015).

A recent study in *The Journal of Neuroscience* determined the effects of a phospho-

diesterase (PDE) 4B inhibitor on chronic cognitive dysfunction in a rodent model of TBI (Titus et al., 2016). PDE4 terminates cAMP signaling and is acutely upregulated after TBI in rodent models (Titus et al., 2016). Because cAMP signaling is vital to learning and memory processes, PDE4 may be an important factor in post-TBI cognitive dysfunction. Indeed, inhibition of all four PDE4 subtypes (A–D) with rolipram rescued cAMP signaling, improved LTP, and enhanced cognitive performance in rodent TBI models (Titus et al., 2013). However, rolipram causes significant emesis (Robichaud et al., 2002) and cannot be used in humans. Therefore, Titus et al. (2016) asked whether inhibiting just one PDE4 subtype would improve hippocampal function and cognition. Because PDE4B and PDE4D are involved in processes that mediate outcomes from TBI, and PDE4D is the subtype thought to cause emesis (Robichaud et al., 2002), the authors investigated the effects of the PDE4B-specific inhibitor A33.

First, the authors examined the effects of A33 on hippocampal function. In hippocampal slices obtained from rats 3 months after TBI, basal synaptic transmission and LTP expression were significantly reduced. Both effects were rescued by bath application of A33. Second, the authors tested the ability of A33 to cross the blood–brain barrier (BBB). A33 was present at ef-

fective concentrations in sham and TBI brain tissue 30 min after an intraperitoneal injection. Because PDE4B alters expression of tumor necrosis factor α (TNF α), the authors measured the effects of A33 on brain TNF α levels as a proxy for the effects of A33 on brain PDE4B activity. In the acute period after TBI, A33 significantly reduced brain TNF α levels. Finally, at 3 months after sham or TBI procedures, animals were tested on fear conditioning, spatial memory, and working memory tasks. On each task, TBI animals performed worse than sham-injured animals and deficits were rescued by pretask training treatment with A33.

A33 is a promising treatment for post-TBI cognitive dysfunction. The importance of this work is enhanced because of the efficacy of the drug during the chronic phase of TBI. Although it is hard to measure how many individuals with TBI do not receive prompt treatment, one study found that 35% of young adults who had suffered a mild TBI did not immediately seek medical attention (Demakis and Rimland, 2010). Therefore, a pharmacological intervention will have more impact in human patients if it ameliorates symptoms even when administered months after injury.

Titus et al. (2016) next investigated potential mechanisms of A33-mediated cognitive improvement. They found that TBI reduced levels of phosphorylated CREB (pCREB) and that pCREB levels were res-

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cued by A33 treatment. CREB is a transcription factor that is activated through the cAMP signaling pathway and that is essential for LTP and memory formation (Tully et al., 2003). This experiment therefore indicates that cAMP signaling and CREB activation are likely mediators of A33's effects on cognition. The authors also investigated the effects of TBI and A33 on hippocampal atrophy. TBI caused hippocampal atrophy, but this was not affected by A33 treatment. Because PDE4B has been implicated in inflammatory processes, the authors also examined the effects of A33 on post-TBI neuroinflammation. Although TBI altered microglial morphology, there was no effect of A33 on microglial number or morphology.

As mentioned above, the authors determined the effects of A33 on levels of the inflammatory cytokine TNF α in the brain. TNF α plays an important role in mediating outcomes from TBI; it contributes to neuronal apoptosis, astrogliosis, and permeability of endothelial cell layers, which is likely involved in BBB dysfunction (Chodobski et al., 2011; Baratz et al., 2015). Preclinical studies have indicated that TNF α inhibitors significantly improve neuromotor and cognitive outcomes from TBI (Cheong et al., 2013; Baratz et al., 2015; Perez-Polo et al., 2016). Furthermore, some studies have shown that TNF α inhibition improves motor function in TBI patients and improves cognition in stroke patients even when administered months or years after injury (Tobinick, 2011; Tobinick et al., 2012). TNF α also plays an essential role in mediating sleepiness and cognition (Jewett and Krueger, 2012). High levels of TNF α increase fatigue and sleepiness in humans and increase non-rapid eye movement sleep in animals (Jewett and Krueger, 2012). TNF α may increase sleep by modulating levels of wake-promoting neuropeptides, such as hypocretin (Zhan et al., 2011), or somnogens, such as prostaglandin D2 (Terao et al., 1998). Increased sleepiness is in turn associated with poor cognitive performance (Thomas et al., 2000). TNF α may also affect cognitive performance more directly by decreasing LTP through activation of the p38 MAPK pathway (Butler et al., 2004) and by impairing neurogenesis (Iosif et al., 2006; Cheong et al., 2013). Thus, the ability of A33 to decrease TNF α levels may contribute to the improvements in cognition observed by Titus et al. (2016).

Although the authors found that A33 administration reduced brain TNF α levels in the acute period after TBI, they found that TNF α was undetectable in the cortex or hippocampus 3 months later, when cognition

was assessed. Therefore, they concluded that A33 did not act through modulating levels of TNF α . However, TNF α levels are not only determined by time since injury; they are also determined by circadian rhythms. Like many other cytokines, brain TNF α levels display drastic diurnal variation, especially in the cortex and hippocampus (Floyd and Krueger, 1997; Jewett and Krueger, 2012). In animals maintained on a 24 h light-dark cycle (exposed to 12 h of light followed by 12 h of darkness), cortical and hippocampal levels of TNF α are very low at the end of the light period. However, at the beginning of the light period, cortical and hippocampal levels of TNF α are much higher: between 200 and 300 pg/g in a healthy rat (Floyd and Krueger, 1997). As in nearly all papers measuring cytokines after TBI, Titus et al. (2016) do not report what time of day the animals were tested or killed. If testing occurred near the beginning of the light period and death occurred near the end of the light period, TNF α levels may have been high during testing and then declined to very low levels by the time of death. Thus, given this circadian variation and the ability of A33 to lower brain TNF α levels, it seems that there is insufficient evidence to rule out TNF α as a potential mechanism of action of A33.

Even if TNF α levels do not differ between sham and TBI groups at several months after surgery, reducing physiological levels of TNF α may still improve cognition. Indeed, at 3 months after injury, the authors observed increased levels of A33 in the brain in injured compared with noninjured animals (Titus et al., 2016), possibly resulting from persistent BBB dysfunction. Although this difference did not reach statistical significance ($p = 0.069$), it may still be physiologically relevant, especially given that sample size was small ($n = 3$). Furthermore, glymphatic flow is chronically altered in cases of TBI (Iliff et al., 2014), which may reduce the clearance of substances from the brain, resulting in greater persistence of this TNF α inhibitor in the brain.

In conclusion, the compound A33 is a promising new pharmacotherapy for post-TBI chronic cognitive dysfunction, which should be further developed and potentially translated into clinical research. This research contributes to a greater dialogue about the roles of various PDE4 subtypes in mediating outcomes from TBI. However, TNF α reduction cannot yet be ruled out as one of the possible mechanisms of action of A33. The link between A33, TNF α , and post-TBI cognition warrants further investigation.

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