Targeting α7 nicotinic acetylcholine receptors: a future potential for neuroprotection from traumatic brain injury

Traumatic brain injury (TBI) poses a significant socioeconomic burden in the world. The long-lasting consequences in cognitive impairments are often underreported and its mechanisms are unclear. In this perspective, cholinergic dysfunction and therapeuatic strategy targeting this will be reviewed. Novel agents that can target specific subtype of acetylcholine receptors have been developed over the recent years and are at various stages of development, which include AR-R 17779, GTS-21, SSR-180711A, AR-R17779, and PNU-282987. A detailed review on this topic has been previously published (Shin and Dixon, 2015).

Cholinergic system is regarded as an important modulator of cognitive function. It has an important role in learning, memory formation, and attention. Thus, in pathologic neurodegenerative diseases such as Alzheimer's disease (AD), loss of cholinergic functions are believed to be an important contribu- tor to cognitive deficits. Similarly, TBI induces dysregulation of the cholinergic system, and this is believed to be one of the significant underlying mechanisms of cognitive deficits (Zafonte et al., 2012; Shin and Dixon, 2015). With recent advancements in pharmacological science, novel agents that target specific receptor subtypes of the cholinergic system have been developed (Sun et al., 2013; Barbier et al., 2015; Dinley et al., 2015). Specifically, α7 nicotinic acetylcholine receptors (nAChRs) have been shown to have a major role in both the neuronal injury as well as cognitive dysfunction after TBI. Agents that target these specific receptors are promising potential future targets in both animal studies and clinical trials.

Acetylcholine transduces signals by muscarinic and nicotinic acetylcholine receptors. Whereas muscarinic receptors are G-protein coupled receptors, nicotinic receptors are ligand gated ion channels composed of five subunits. Binding of a ligand will induce conformational change to an open state, allowing an outflux of K⁺, and influx of Na⁺ and Ca²⁺ to a minor extent. Various subunits of nAChRs have been characterized over the years. They form heteromeric combinations of α2–10 and α2–4 subunits and α7 homopentamers. As previously reviewed (Dinley et al., 2015), α4β2 subtype is a major nAChR subtype in the brain, whereas α3β4 is a major subtype found in the peripheral nervous system. Specifically, α4β2 and α7 nAChRs in the basal forebrain acetylcholine neurotransmission have major roles in cognitive performance.

Nicotinic receptors are found widely throughout the brain, and their contribution to cognitive function has been tested in several important regions. Specifically, nAChRs in the hippocampus are involved in the regulation of working memory: blockade of nAChRs in ventral or dorsal hippocampus leads to decreased performance in radial-arm maze task (Bancroft and Levin, 2000; Levin et al., 2002). Infusions of nAChR antagonists to habenula (Sanders et al., 2010) or brainstem (Cannady et al., 2009) also lead to memory deficits. As shown in Figure 1, activation of α7 nAChR leads to complex intracellular signaling pathways leading to transcription of genes important for memory formation. The activation of nAChRs also indirectly enhances dopaminergic signaling, as nAChR activation on dopaminergic neurons would lead to dopamine release in the frontal cortex or striatum (Shin et al., 2012) which are also important components of cognitive function. Recovery from dopaminergic deficit after TBI can be indirectly enhanced by nAChR activation as previously shown (Shin et al., 2012).

Recent studies have focused on the role of each nAChR subtype in cognitive function. The α7 receptors are of particular interest due to many studies showing their important contribution to learning and memory. These α7 receptors are found in the central nervous system as well as peripheral organs and immune cells. They have been found in various cell types such as astroglia, microglia, oligodendrocytes, and endothelial cells. Their distribution in the hippocampus as well as high permeability to calcium are consistent with the fact that animal studies show their important role in enhancing cognitive performance (Shin and Dixon, 2015). Activation of α7 receptors also modulates production of inflammatory cytokines (Han et al., 2014).

In vitro studies have shown that cell lines expressing α7 receptors have activation of signal transduction pathways important for learning and memory. Activation of α7 receptors induces the activation of extracellular signal-regulated kinase ½ (ERK ½) (Dickinson et al., 2008). ERK is activated by phosphorylation, leading to the activation of its downstream kinase p90 ribosomal S6 kinase (p90RSK) (Figure 1). This subsequently leads to activation of Ca²⁺ response element-binding protein (CREB), which modulates the transcription of downstream genes that formulate learning and memory. The high permeability of calcium by α7 receptors can also lead to calcium regulated cascades which activate calmodulin and calmodulin regulated kinases II and IV (CamKII, CaMKIV) leading to the activation of CREB. This enhancement of learning and memory has been demonstrated for AR-R 17779, an α7 receptor agonist as previously reviewed (Shin and Dixon, 2015). Other α7 receptor activating agents such as GTS-21, SSR-180711A, AR-R17779, and PNU-282987 have been developed and will be useful future candidates in TBI research as previously reviewed (Shin and Dixon, 2015). Animal models of TBI using controlled cortical impact (Dixon et al., 1991), fluid percussion injury (McLaroch et al., 1987), and weight drop model (Marmarou et al., 1994) are well characterized methods to study the effects of these agents in the setting of TBI. These models often used reliable and reproducible tests of working memory such as Morris Water Maze and tests of motor function such as balance walking, beam walking, and rotarod test (Hamm, 2001). Future studies for these new agents using various models of injury are warranted to characterize the contribution of each nAChR subtypes.

Compared to other subtypes of nAChRs, α7 subunits are unique entities in the setting of TBI. In TBI rats studied using quantitative autoradiography, there is a reduction of α7 receptor binding at wide range of areas, whereas α3 or α4 subtypes showed lower magnitude of reduction and in fewer regions (Verbois et al., 2002). Aside from their role in cognitive function, α7 nAChRs are recently noted as possible targets that can lead to neuroprotective effects in the setting of various acute injuries. Several studies using agents that activate α7 nAChRs show improvement in memory and survival of neurons, as well as reduction of inflammatory response to injury (Shin and Dixon, 2015). Microglia expresses α7 nAChRs, and activation of these receptors leads to reduction of inflammatory cytokine release. The neuroprotective effect of α7 receptor activation was also shown in the peripheral nervous system. In the setting of sciatic nerve crush injury, activation of α7 nAChR by PNU-282987 lead to decreased TNF-α level and increased axonal regeneration (Wang et al., 2015).

Another group of agents, known as positive allosteric modulators (PAM) for nAChRs, have been developed in the recent years. Unlike agonists that directly bind to and activate the
receptors, PAMs enhance the amplitude of response or increase the duration of activity when there is a preexisting cholinergic signaling. A newly developed PAM agent, PNU-120596 was shown to protect against ischemic brain injury and improve motor function (Sun et al., 2013; Shin and Dixon, 2015). In subarachnoid hemorrhage model of rats, PNU-282987 improves motor function by reducing inflammation and neuronal loss whereas αβ2 agonist RJR-2403 does not have this effect. However, this neuroprotective effect may be injury specific and cell type specific, as SH-SY5Y cells and rat hippocampal cultures provided with PNU-120596 had decreased viability due to overloading of intracellular Ca2+ leading to cell death (Guer- ra-Alvarez et al., 2015).

Another major agent of interest is CDP-Choline, otherwise known as the effect of Citicholine. It has both α7 nAChRs agonist effect as well as the effect of enhancing neuronal membrane synthesis. When taken as dietary intake, it is metabolized to cytidine and choline before resynthesizing into the CDP-choline in liver and other tissues. However, direct injection of this agent into the local neural tissue can activate α7 nAChRs. It also reduces apoptosis in AD models of animals and improves cognitive performance in schizophrenia, such as working memory, verbal learning, verbal memory, and executive function (Knott et al., 2015). These neuroprotective effects may lead one to expect therapeutic benefit when applied in TBI. Disappointingly, COBRIT, a multicenter, randomized, double-blind, placebo-controlled trial showed no beneficial effect of this agent (Zafonte et al., 2012). This lack of improvement in cognitive function was attributed to the possibility that different levels of injury severity may have different responses to this agent. However, it should also be noted that this agent was taken enterally, and the degree of direct activation of α7 nAChRs in the central nervous system by this route of administration is unclear. Clinical trials of α7 enhancing agents DMXB-A, UCI-4083, and TC-5619 are ongoing in Schizophrenia patients and showed some degree of success in improving cognitive outcome (Freedman, 2014). However, these agents have not been used in human TBI trials. Among the many agents mentioned in this article, clinical trials for some of these agents in TBI will likely take place in the near future.

Asides the agents previously mentioned, pharmacological research still continues in developing various new drugs that are α7 nAChR specific. These even more novel ligands that are α7 receptor specific are currently under research investigation to be used as therapeutic agents or radioligands. Examples are a radioligand [18F]NS10743 (Teodoro et al., 2014) and α7 nAChR partial agonist Encenicline (Barbier et al., 2015) which are currently at development stages. Further validation on dosing as well as their applicability in TBI must be clarified in the next few years to come. However, with such variety of α7 targeting agents already available for animal studies, some of these agents may become key players in the long awaited pharmacological treatment regimen for TBI.

Although there are many beneficial effects of nAChR activation, the possibility of using nAChR modulating agents must be approached with caution. Prior studies have shown various side effects of nAChR activation. High doses of nicotine administration can increase symptoms of anxiety and depression (Newhouse et al., 1988) as well as increased heart rate and blood pressure. This occurs via nonspecific activation of nAChRs throughout the body, since nAChRs are found widely in autonomic nervous system as well as adrenal medulla. Activation of adrenal medulla leads to increase in corticosterone, epinephrine, and norepinephrine levels in blood (Cryer et al., 1976). Higher doses can even induce convulsions (Okamoto et al., 1992) and can be lethal (Okamoto et al., 1994). The synthetic nAChR agonists discussed in this article were designed to have reduced side effect profiles, with less effect on cardiovascular parameters. Commonly studied nAChR agonists in neurodegenerative disease and injury models are central nervous system specific, such as mecamylamine (Shytle et al., 2002).

Moreover, there is a limitation of looking at the functional deficits after TBI only from the perspective of nicotinic receptor dysfunction. The mechanism of TBI is much more complex, involving dysfunction of other neurotransmitters, as well as oxidative stress and inflammation. Dopamine synthesis and release deficit has been characterized after TBI (Shin et al., 2011), as well as alterations in the postsynaptic regulators of dopamine neurotransmission (Bales et al., 2009, 2010, 2011). Therapeutic effects of enhancing other neurotransmitter systems such as the serotonin system, have also been shown to be beneficial in animal models (Kline et al., 2012; Yelleswarapu et al., 2012). Also, therapeutic strategies targeting oxidative stress, such as antioxidant glutathione administration, reduce cell death and inflammation in animals (Roth et al., 2014) and improve post TBI symptoms (Hoffer et al., 2013). In the midst of complex interplay of various pathological cascades, targeting only α7 nAChRs may not be effective. Combination therapies that target multiple pathological mechanisms of TBI have recently gained interest (Margulies et al., 2015), and the use of α7 nAChR activating mechanisms.
agent in combination with other novel agents targeting multiple pathways may be the most effective future treatment regimen for TBI.

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