NEURAL REGENERATION RESEARCH
September 2014, Volume 9, Issue 17
www.nrronline.org

INVITED REVIEW

Selective CDK inhibitors: promising candidates for future clinical traumatic brain injury trials

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doi:*10.4103/1673-5374.141779*
http:*//www.nrronline.org/*

Accepted: *2014-08-18*

Abstract

Traumatic brain injury induces secondary injury that contributes to neuroinflammation, neuronal loss, and neurological dysfunction. One important injury mechanism is cell cycle activation which causes neuronal apoptosis and glial activation. The neuroprotective effects of both non-selective (Flavopiridol) and selective (Roscovitine and CR-8) cyclin-dependent kinase inhibitors have been shown across multiple experimental traumatic brain injury models and species. Cyclin-dependent kinaseinhibitors, administered as a single systemic dose up to 24 hours after traumatic brain injury, provide strong neuroprotection-reducing neuronal cell death, neuroinflammation and neurological dysfunction. Given their effectiveness and long therapeutic window, cyclin-dependent kinase inhibitors appear to be promising candidates for clinical traumatic brain injury trials.

Key Words: *cell cycle inhibition; lateral fluid percussion; Roscovitine, CR-8; behavior; microglial activation; neurodegeneration*

Kabadi SV, Faden AI. Selective CDK inhibitors: promising candidates for future clinical traumatic brain injury trials. Neural Regen Res. 2014;9(17):1578-1580.

Traumatic brain injury (TBI), a major public health problem, accounts for more than 1.7 million new cases reported annually in the United States (Faul et al., 2010). TBI causes cell death and neurological dysfunction through both direct physical disruption *via* shearing, tearing and stretching of tissue (primary injury), followed by a cascade of delayed and potentially reversible molecular and cellular mechanisms that cause progressive white and grey matter damage (secondary injury) (Panter and Faden, 1992; Bramlett and Dietrich, 2007). Secondary injury, which begins within seconds to minutes after the insult and may continue for months or years, may be responsible for a significant component of the neurodegeneration and neurological impairment following TBI (Bramlett and Dietrich, 2007; Loane and Faden, 2010). One important delayed injury mechanism involves cell cycle activation (CCA), which results in apoptosis of post-mitotic cells (mature oligodendroglia and/or neurons) and activation of mitotic cells such as microglia and astrocytes (Cernak et al., 2005; Giovanni et al., 2005; Hilton et al., 2008; Stoica et al., 2009; Kabadi et al., 2012a, b, 2014).

In proliferating cells, the cell cycle is controlled by complex molecular mechanisms and progression through distinct phases that require sequential activation of a large group of Ser/Thr kinases called the cyclin-dependent kinases (CDK) and their positive regulators (cyclins) (Arendt, 2003). The G1 phase is initiated sequentially by increased levels of members of the cyclin D family, activation of cyclin D-dependent kinase activity, phosphorylation of the retinoblastoma (Rb) family, and activation of the E2 promoter binding factor E2F family of transcription factors. Active E2F induces transcription of various genes involved in cell cycle, such as cyclin A which associates with CDK2 (Stoica et al., 2009). In late G2 phase, cyclin A is degraded, whereas CDK2 forms a complex with B-type cyclins, facilitating G2/M phase transition (Byrnes and Faden, 2007; Stoica et al., 2009). In contrast, in post-mitotic neurons the activation of E2F members may contribute to increased transcription of pro-apoptotic molecules such as caspase-3, 8 and 9, and Apaf-1 or anti-apoptotic Bcl-2 family members leading to cell death (Osuga et al., 2000; Nguyen et al., 2003; Greene et al., 2004). Recent evidence demonstrates neuronal CCA following TBI, and suggests that it represents a key secondary injury mechanism that contributes to neuronal cell death.

In our earliest studies, we examined the neuroprotective effects of flavopiridol following experimental TBI; this flavonoid is a potent non-selective CDK inhibitor (Cernak et al., 2005; Giovanni et al., 2005). Therapeutic effects were dose-dependent, with a therapeutic window of at least 24 hours after systemic administration (Cernak et al., 2005). More recently, we demonstrated the neuroprotective potential of roscovitine and a related second generation analog (CR-8) across TBI models and species. Roscovitine is a more selective CDK inhibitor, which acts specifically on CDKs-1, 2 and 5, and possibly CDKs-7 and 9 (Meijer et al., 1997), and is currently being evaluated clinically for the treatment of certain cancers (Bettayeb et al., 2008; Komina et al., 2011; Wesierska-Gadek et al., 2011). Either systemic or central roscovitine administration at 3 hours after injury attenuated CCA, progressive neurodegeneration, chronic neuroinflammation and related neurological dysfunction in multiple

TBI models (Hilton et al., 2008; Kabadi et al., 2012a). However, the therapeutic potential of roscovitine may be limited by its short biological half-life, rapid metabolism to inactive derivatives, and relatively weak potency (Nutley et al., 2005; Bettayeb et al., 2008; Bettayeb et al., 2010).

CR-8 is an N^6-biaryl-substituted derivative of roscovitine, that was synthesized in an effort to generate roscovitine analogs with greater therapeutic potential (Bettayeb et al., 2008). Based on prior *in vitro* data, we used a central dose of CR-8 that was only 5% of the roscovitine dose previously shown to be effective in the same TBI model (Kabadi et al., 2012a). Central administration of CR-8 at 3 hours in the mouse controlled cortical impact (CCI) model of TBI significantly attenuated sensorimotor and cognitive deficits, decreased lesion volume, and improved neuronal survival in the cortex and dentate gyrus. Moreover, unlike roscovitine treatment, CR-8 also attenuated posttraumatic neurodegeneration in the CA3 region of the hippocampus and thalamus at 21 days. Furthermore, delayed systemic CR-8 treatment, at a dose 10 times less than previously tested for roscovitine (Kabadi et al., 2012a), significantly improved cognitive performance after TBI.

More recently, to simulate a more clinically-relevant treatment paradigm we administered CR-8 systemically at 3 hours post-injury and investigated its long-term neuroprotective effects on neurological deficits, neurodegeneration, and neuroinflammation in a rat lateral fluid percussion (LFP) model (Kabadi et al., 2014). Vehicle-treated animals demonstrated elevated expression of key cell cycle markers (cyclin G1, phospho-Rb, E2F1 and PCNA) in the injured cortex at 24 hours; these changes were attenuated by CR-8 treatment. To evaluate the temporal profile of LFP-induced neurodegeneration, we used unbiased stereological techniques to quantify neuronal cell loss in different hippocampal sub-regions, cortex and thalamus. Notably, CR-8 treatment caused significant reduction in the chronic and progressive neuronal cell loss in the hippocampus, cortex and thalamus, with significant decreases in lesion volume (Kabadi et al., 2014).

Using multiple sensitive functional tasks, it was demonstrated that LFP injury caused cognitive impairment in spatial learning, as well as reference and retention memory and CR-8 treatment improved each of these outcomes (Kabadi et al., 2014). LFP injury reduced the composite neuroscore; this score reflects a comprehensive assessment of sensorimotor recovery and includes a combination of 7 individual tests (Cernak et al., 2005; Giovanni et al., 2005; Kabadi et al., 2010). Injured animals also showed depressive-like behavior, as assessed by the forced swim test (Slattery and Cryan, 2012). Importantly, CR-8 treatment decreased both motor impairment and depressive-like behavior (Kabadi et al., 2014).

Chronic neuroinflammation following CNS trauma may provide a mechanistic connection between early and chronic neurodegeneration (Nandoe, 2002; Byrnes et al., 2012). To better assess the neuroinflammatory responses we performed a quantitative assessment based on morphological characterization of microglial and astrocyte activation in the injured cortex (Kabadi et al., 2014). We had previously shown that TBI causes transformation of ramified (resting) microglia into the more activated phenotypes, hypertrophic and bushy, with the relative proportion of these microglial phenotypes reflecting the extent of microglial activation and associated neuroinflammation (Soltys et al., 2001; Byrnes et al., 2012; Kabadi et al., 2012a, b). Similarly, TBI causes astrocyte activation from a resting form to a more reactive morphology, which may also contribute to secondary injury (Long et al., 1998; Kanaan et al., 2010). There was significant elevation of activated microglia and astrocytes at 7 days after LFP; these activated phenotypes were present through 28 days, indicating chronic neuroinflammation after TBI (Kabadi et al., 2014). CR-8 treatment attenuated microglial and astrocyte activation (Kabadi et al., 2014); such modulation of posttraumatic inflammation may be an important component of its protective effects.

To further address mechanism, changes of DNA synthesis, a key component of the S phase of cell cycle, were examined in neurons, microglia/macrophages and astrocytes (Taupin, 2007). Labeling with 5-bromo-2′-deoxyuridine (BrdU), a thymidine analog and a marker of DNA synthesis, enabled effective determination of S phase induction in the assessed cell types. At 24 hours after LFP, a time of intense CCA and neuronal degeneration (Cernak et al., 2005; Giovanni et al., 2005; Hilton et al., 2008), we failed to detect co-localization of BrdU with NeuN, Iba-1, orGFAP (Kabadi et al., 2014). These data suggest that CCA in mature neurons, microglia/macrophages and astrocytes fails to progress to the S-phase of DNA replication at 24 hours. However, there was considerable co-localization between BrdU and NeuN at 7 days, particularly at the lesion site, with cell morphology suggesting degenerating neurons (Kabadi et al., 2014). Increased numbers of BrdU/Iba-1 and BrdU/GFAP-double positive cells were also observed at 7 days, indicating the proliferation of microglia and astrocytes LFP. The largest component of BrdU-positive cells was found in microglia. Previous studies have correlated the proliferating cell differentiation in an injured brain with the degree of cellular repair and restoration (Chirumamilla et al., 2002; Urrea et al., 2007). Notably, in our study, based on the morphological appearance of degenerating neurons and activated astrocytes/microglia, cell cycle activation/proliferation appears to occur prior to or in parallel with neurodegeneration and neuroinflammation, respectively.

In summary, there is strong experimental evidence supporting a role for CCA in the progressive neurodegeneration and chronic neuroinflammation following TBI. Selective CDK inhibitors, particularly roscovitine or CR-8, when administered as a single systemic dose at a delayed time point show strong neuroprotection across TBI models and species. CDK inhibitors are toxic when administered chronically as for cancer treatment; but for TBI only short-term treatment is optimal, which would be expected to have few side effects. Although CR-8 appears to have higher therapeutic efficacy and more favorable potency than roscovitine, the latter has been evaluated in phase II cancer trials and may therefore be a better early candidate for translational trials in TBI.

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