REVIEW

Cell Cycle Activation and Spinal Cord Injury

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Summary: Traumatic spinal cord injury (SCI) evokes a complex cascade of events with initial mechanical damage leading to secondary injury processes that contribute to further tissue loss and functional impairment. Growing evidence suggests that the cell cycle is activated following SCI. Up-regulation of cell cycle proteins after injury appears to contribute not only to apoptotic cell death of postmitotic cells, including neurons and oligodendrocytes, but also to post-traumatic gliosis and microglial activation. Inhibition of key cell cycle regulatory pathways reduces injury-induced cell death, as well as microglial and astroglial proliferation both *in vitro* and *in vivo*. Treatment with cell cycle inhibitors in rodent SCI models prevents neuronal cell death and reduces inflammation, as well as the surrounding glial scar, resulting in markedly reduced lesion volumes and improved motor recovery. Here we review the effects of SCI on cell cycle pathways, as well as the therapeutic potential and mechanism of action of cell cycle inhibitors for this disorder. **Key Words:** Cell cycle, spinal cord injury, cyclin-dependent kinases, cell cycle inhibitors, apoptosis, glial proliferation.

INTRODUCTION

Cell cycle regulation is an essential process in the development, differentiation, and proliferation of mitotic cells. However, numerous studies in the last decade have demonstrated that cell cycle re-entry contributes to the death of postmitotic cells [1, 2]. Increasing evidence indicates that cell cycle activation (CCA) is involved in the pathophysiology of both acute and chronic neurodegenerative disorders as follows: cerebral ischemia [3], brain and spinal cord injury [4–6], Alzheimer's disease (AD) [7, 8], Parkinson's disease [9, 10], and amyotrophic lateral sclerosis [11, 12].

Traumatic spinal cord injury (SCI) causes tissue loss and associated neurological dysfunction through both mechanical damage and secondary biochemical and physiological responses. Secondary injury mechanisms include neuronal cell death, loss of oligodendrocytes, inflammation, and reactive astrogliosis [13, 14]. Experimental evidence supports a critical role for CCA in each of these post-traumatic events [4, 15–18].

Cell death resulting from secondary injury factors appears to have 2 main forms: 1) necrotic and 2) apoptotic. Apoptosis continues for days to months after SCI. Mechanisms of neuronal apoptosis and CCA share common regulatory elements including Rb, E2F, and p53 [19–21]. Recent work indicates that activation of cell cycle pathways plays a key role in mediating both neuronal apoptosis and glial proliferation/activation after central nervous system (CNS) injury, including SCI [5, 15, 18, 22].

Inhibiting cell cycle processes has been a major therapeutic target in oncology for many years, leading to the development and clinical evaluation of several structurally different classes of cell cycle inhibitors [23]. Such drugs have provided useful tools for evaluating the pathophysiological role of CCA in CNS injury, as well as suggesting novel therapeutic strategies. This review summarizes recent work that supports a role for CCA in the pathophysiology of SCI, as well as the use of cell cycle inhibitors as novel therapeutic agents.

CELL CYCLE PATHWAYS AND MECHANISMS

The cell cycle is a highly choreographed process that controls and executes the copying and transmission of

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genetic data from 1 cell generation to the next [24]. A typical mammalian cell cycle can be divided into mitosis (cell division, M-phase) and a period of cell growth termed inter-phase. The latter can be further subdivided into the DNA synthesis period (S phase), and the pre- and post-DNA synthesis gap phases (G1 and G2 phases, respectively). Briefly, when cells receive mitogenic signals, they enter into the first gap G1 phase in which the cells are responsive to growth factors and other environmental signals, preparing for DNA replication in the S phase. This is followed by the second gap G2 and mitosis M phases. When cells receive a signal to cease active growth, they exit the cell cycle and become resting cells by entering a quiescent phase termed G0 phase. A majority of newly divided resting cells (G0) can restart the cell cycle under appropriate conditions [25].

Cyclins and cyclin-depdendent kinases

Cyclins are activator proteins that are synthesized or destroyed depending on the phase of the cell cycle, thus regulating kinase activity in a time-dependent manner [23, 26]. There are two groups of cyclins: mitotic cyclins (e.g., cyclin A, cyclin B) and G1 cyclins (e.g., cyclin C, cyclin D, cyclin E). Cyclin-dependent kinases (CDKs) are a group of small, serine/threonine kinases (30–35 kDa) and numbered in order of their discovery as CDK1-9. These members, excepting CDK3 and CDK5, form active heterodimeric complexes and are activated by binding to their cyclin regulatory subunits.

The classical CDKs, including 3 interphase CDKs (CDK2, CDK4, and CDK6), and a mitotic CDK (CDK1, also called cell division control protein 2, CDC2), cooperate to drive cells through the cell cycle [2, 23, 26]. For instance, activation of CDK4 and CDK6 by increased levels of members of the D-type cyclins (cyclins D1, D2, and D3), which are believed to be involved in early G1, serves to phosphorylate and inactivate the Rb family members, relieving the E2 promoter binding factor (E2F) family of transcription factors from Rb-mediated repression [2, 23, 27]. CDK2 can be activated by decreased cyclin D or increased of cyclin E1 and cyclin E2, and regulates the G1/S transition. In contrast, CDK2 interaction with cyclin A results in phosphorylation of substrates supporting DNA synthesis. CDK1 is thought to be activated by a complex with cyclin A, and controls S/G2 transition. CDK1-cyclin B complexes are translocated from the cytoplasm into the nucleus after cyclin A is degraded, helping to drive cells through mitosis. The role of CDK3, which has low expression levels, is still not clear [28].

CDK5 is a unique member of the CDKs, but its activity is not dependent on a cyclin [20]. Although its structure is similar to other CDKs, it does not appear to be involved in cell cycle control. Instead, CDK5 is associated with p35 and p39, its unique neuron-specific co-activators, and helps to control key neuronal functions, such as neurite outgrowth, neuronal migration, and adhesion [29, 30]. Recent data suggest that CDK5 may also serve an opposite role to other CDKs in regard to cell cycle activation and upstream initiation of neuronal cell death, according to changes in subcellular localization and/or activation of kinase activity [26, 30].

Cyclin-dependent kinase inhibitors

CDK inhibitors are small peptides that block cyclin/CDK activity either by forming an inactive complex or by acting as a competitive ligand for CDKs. Both endogenous and exogenous CDK inhibitor can block progression of a cell through the cell cycle. The endogenous inhibitors include 2 subclasses: 1) the Ink4 family (including $p16^{Ink4a}$, $p15^{Ink4b}$, $p18^{Ink4c}$, and $p19^{Ink4d}$) and the Cip/Kip family (including $p21^{Cip1}$, $p27^{Kip1}$ and $p57^{Kip2}$) [2, 26, 31]. P27 is highly expressed in neurons [32]. Studies in the retina and other tissues indicate that Ink4 proteins are specific for CDK4 and CDK6 and prevent the formation with cyclin D, whereas Cip/Kip molecules broadly and nonspecifically block activity of CDKs [26, 33]. These inhibitors are up-regulated during development, as well as in response to anti-mitogenic stimuli, binding to the CDK/cyclin complex, and preventing activity [31].

Cyclin-dependent kinase substrates

The retinoblastoma protein Rb and its related proteins (including p107 and p130) are the primary substrates of CDK4/6 and CDK2 in G1 progression [34–36]. In resting state, Rb is nonphosphorylated and is believed to inhibit cell cycle progression by repressing E2F transcription factors [37]. With mitogenic stimulation, Rb is phosphorylated by activated cdk4/6/cyclin D and CDK2/cyclin E complexes [38, 39]. The pRb is released from the transcription factor complex E2F/DP, which then activates E2F-target genes required for transition to the S phase [28].

Mechanisms of cell cycle-dependent neuronal apoptosis

Apoptosis is a fundamental and essential process in the development and tissue homeostasis of multi-cellular organisms. Neuronal apoptosis is often detected in acutely injured spinal cord tissue [4, 15]. Growing evidence has demonstrated that cell cycle molecules directly activate members of the apoptotic cascades, and several cell cycle relevant pathways have been implicated in neuronal cell death related to CNS disorders. However, the molecular mechanisms underlying the cell cycle involvement in such cell death are just beginning to be understood. It is reported that neuronal apoptosis in acute CNS injury is usually related to cell cycle re-entry at the G1-S phase by activation of the CDK4/cyclin D1-E2F-pRb signaling pathway [3, 5, 15]. Cell cycle re-

activation in postmitotic neurons plays a major role in models of neuronal damage and death *in vitro*. Activity of CDKs and expression of cyclin E were found to be increased in a cerebellar neuronal apoptotic model induced by KCl withdrawal; these changes were accompanied by an increase in Rb phosphorylation [40]. The translocation of cyclin D and CDK4/6 to the nucleus was observed prior to neuronal apoptosis. Increased CDK4 activity is the first response to camptothecin induced apoptosis in primary neuronal culture, which is accompanied by an increase in Rb phosphorylation, followed by caspase-3 cleavage and neuronal apoptosis [41].

Activated CDKs phosphorylate Rb family proteins, causing dissociation of the Rb-E2F complex, which leads to E2F-dependent transcription of pro-apoptotic molecules such as caspases 3, 8, and 9, and Apaf-1 or pro-apoptotic Bcl-2 family members, ultimately contributing to neuronal cell death [42–44].

CELL CYCLE DYSREGULATION AND CNS DAMAGE

Many studies have provided evidence that cell cycle activation plays a pathophysiological role in various acute and chronic neurodegenerative disorders. After brain trauma, 13% of neurons show an increase in cyclin D1 expression, and 75% of those are also positive for cleaved caspase-3, a marker of caspase-mediated apoptosis [5].

Cell cycle re-entry, as indicated by increased Cyclin D1 and Rb phosphorylation, as well as decreased p16 ink4, is a key feature of various stroke models [45–48]. Large numbers of cell cycle-related proteins, including proliferating cell nuclear antigen (PCNA), cyclin B1, cyclin D, CDK1, CDK4, Ink4, Rb, E2F, p27, and phosphorylated p27, are markedly increased in the human AD brain [7, 49–53].

CELL CYCLE PATHWAY CHANGES AFTRE SCI

SCI causes not only neuronal and oligodendroglial cell death, but also induces microglial-associated inflammatory responses and reactive astrogliosis. The latter alterations contribute to tissue loss and glial scar formation [54, 55]. A key mechanism responsible for secondary injury after trauma is increased microglial proliferation and associated activation reflected by production of pro-inflammatory cytokines and neurotoxic molecules [56–60]. In parallel, astrocytes move away from the center of the lesion, become hypertrophic, proliferate, and up-regulate the expression of GFAP. Hypertrophic astrocytes are the major cellular component of the glial scar, which is considered a physical and molecular barrier to CNS regeneration [55]. Moreover, reactive astrocytes also produce several classes of growth-inhibitory molecules, including the family of extracellular matrix molecules, known as chondroitin sulphate proteoglycans, which inhibit both *in vitro* and *in vivo* axonal regeneration [55, 61, 62]. Considerable evidence indicates that preventing and/or reducing the inhibitory environment of the glial scar provides a better environment for neurons to regenerate [54].

Current knowledge on the role of cell cycle events in SCI is derived mainly from rodent SCI models. We have first demonstrated that up-regulation of cell cycle-related proteins occurs in both neurons and glia after SCI, and these may contribute to secondary damage cascades [4].

Cell cycle gene and protein expression after SCI

Our comprehensive gene profiling analysis of rat spinal cord after impact trauma showed up-regulation of a cluster of cell-cycle related genes [4]. Expression of key cell cycle activator genes, such as c-myc, Gadd45, cyclin D1, PCNA, cyclin G, CDK4, E2F5, and Rb were up-regulated at the early time points (*i.e.,* 4 h and 24 h) after SCI [4, 15]. This functionally related group of genes has been involved in cell cycle regulation with particular relevance to DNA damage response and transition from G1 to S phase. Immunoblot analysis confirmed that each cell cycle-related gene showing increased mRNA expression also exhibited increases at the protein level; these were associated with increased phosphorylation of Rb on Ser795, strongly suggestive of activation of the cell cycle pathway. Moreover, cyclin D1 protein expression was found throughout the injured spinal cord at 3 days after severe contusion SCI in rats, particularly at the periphery [15]. The cellular expression of these proteins was evaluated by immunocytochemistry at the injury site. These cell cycle proteins (including phospho-Rb) were expressed in neurons, which includes motor neurons. Furthermore, many neurons that were positive for cell cycle proteins were also positive for terminal deoxynucleotidyl transferase dUTP nick ends labeling (TUNEL) and caspase-3, suggesting a correlation between cell cycle activation and neuronal cell death [4]. Tian et al. [16] also found that the up-regulation of expression of cyclins A, B1, E, and PCNA appears early as 1 day after injury and peaks at day 3, following spinal cord hemi-section.

Cell cycle pathways in postmitotic cells after SCI

SCI causes a subacute neuronal loss in the lesion periphery in the days following the initial injury. Many of these neurons undergo apoptosis that appears to be related to the up-regulation of cell cycle proteins [4, 63, 64].

Induction of cyclin D1 and CDK4 were increased at 8 h after 15 minutes of spinal cord ischemia followed by re-perfusion and particularly expressed in motor neuron cells [65]. Interestingly, TUNEL positive staining in large motor neurons occurs at 2 days post re-perfusion and approximately 50% of motor neurons are positive for

TUNEL associated with apoptosis [65]. It was confirmed *in vivo* that cell cycle pathways were up-regulated prior to neuronal apoptosis. Byrnes et al. [15] reported that cyclin G1 and phosphorylated Rb are highly expressed in neurons at 1 and 3 days after SCI. Numerous cleaved caspase-3 positive cells were found in 10 mm of tissue surrounding the lesion site, and many of these apoptotic cells were neurons. In addition, double-labeling demonstrated the presence of oligodendrocytes that were positive for caspase-3. Collectively, these data suggest that CCA contributes to caspase-dependent apoptosis of neurons and oligodendrocytes after SCI.

Cell cycle events in mitotic cells after SCI

Microglial activation and release of pro-inflammatory and neurotoxic molecules has been described in SCI models [56, 57]. The formation at the injury site of glial scar composed of reactive astrocytes prevents axonal regeneration following spinal cord injury. We have demonstrated that SCI results in cell cycle activation in mitotic cells, such as astrocytes and microglia [4, 15].

Growing evidence shows that cell cycle-related proteins are up-regulated following stimulation in astrocytes and microglia. PCNA protein expression is increased in primary cultured microglia stimulated with lipopolysaccharide [18], as well as in astrocytes following hypoxia [66]. Cyclin expression and 5-bromo-2′-deoxyuridine (BrdU) uptake are also increased in a microglial cell line culture following application of the cytokine GM-CSF, which can be reversed by cytokine withdrawal [67]. In primary cultured astrocytes, cyclin D1 and cyclin A are density-dependent; cyclin D1 is down-regulated by contact inhibition, and p27 expression increases [68], whereas Rb is phosphorylated in growing astrocyte cultures. Tian et al. [16] showed that approximate 35% of reactive astrocytes were also PCNA positive at 7 days after spinal cord hemi-section. In rat contusion SCI, cyclin D1 immunolabelling was primarily found in astrocytes at 3 days postinjury [15].

CDK INHIBITORS AS THERAPEUTIC TARGETS IN CNS INSULTS

CDK inhibitors have been widely studied as cancer therapeutics owing to their potential role in restoring control of the cell cycle [23]. The most commonly used exogenous CDK inhibitors include flavopiridol, roscovitine, and olomoucine. Flavopiridol, a semi-synthetic flavonoid derived from the bark of rohitukin [69], inhibits all CDKs, reduces cyclin D1 mRNA transcription, and leads to cell cycle arrest in G1 or at the G2/M transition [70, 71]. Roscovitine, a purine analogue, suppresses activation of CDK2 and CDK5 [72]. At higher concentrations, it may inhibit the activity of signal transduction pathway kinases, such as ERK1 and ERK2. Olomoucine (2-[2-hydroxyethylamino]-6-benzylamino-9-methylpurine), also a purine analogue, regulates the activity of CDK2 and CDK5 by competitively binding the ATP-binding site [73].

A number of CDK inhibitors including flavopiridol, roscovitine, AT-7519, P276-00, AG-024322, PD-0332991, and SNS-032 have advanced to human clinical trials (phase I/II) as therapeutic approach in cancer for a broad range of solid tumors and hematological malignancies [23]. Although we believe there have not been any clinical trials reported on CDK inhibitors in the treatment of CNS diseases, preclinical rodent experiments demonstrate that exogenous CDK inhibitors including the pan-CDK inhibitor (flavopiridol) and more selective CDK inhibitors (olomoucine, roscovitine, or quinazolines) alleviate neuronal death, suppress reactive glia, and/or improve neurological functional outcomes in a number of CNS injury models of AD [2, 74–76], Parkinson's disease [2], stroke [3, 77], traumatic brain injury (TBI) [5, 22, 78], SCI [4, 15–17, 79], excitotoxic stress [80–83], and optic nerve transaction [84].

Neuroprotection of CDK inhibitors *in vitro*

Flavopiridol is a potent but nonselective competitive CDK inhibitor, acting on all CDKs thus far examined. Etoposide, a DNA-damaging and pro-apoptotic agent, up-regulates expression of cyclin D1 and PCNA, and causes neuronal apoptosis in rat primary cortical neurons, as measured by an increase in cleaved caspase-3 reactivity [22] and lactate dehydrogenase (LDH) release [5]. Application of flavopiridol at at 1 μM completely inhibits neuronal cell death induced by etoposide (50 μM); it also attenuates cyclin D1 expression and translocation to the nucleus, while up-regulating p27 expression and decreasing phosphorylation of Rb. Additionally, flavopiridol pretreatment decreases LDH release in neurons treated with kainic acid and blocks the uptake of BrdU into damaged neurons [81]. Flavopiridol is also protective in a colchicine model of neuronal apoptosis, reducing release of cytochrome C from mitochondria [75]. Furthermore, Cernak et al. [22] compared the effects of 3 structurally different cell cycle inhibitors, including flavopiridol, roscovitine, and olomoucine, which modulate different components of cell cycle regulation on neuronal cell death. They found that flavopiridol is the most potent, having optimal effects at 10 μM, whereas the other drugs were only effective at doses greater than 100 μM. In addition, these cell cycle inhibitors also provide neuroprotection following KCl withdrawal from cerebellar neurons [40]. More recently, we examined the effects of selective specific CDK inhibitors, such as #217695 that targets CDK1 and #219477 that targets CDK4, in a well-established model of neuronal apoptosis induced by etoposide. Each of

these CDK inhibitors significantly attenuated etoposide-induced neuronal cell death, suggesting participation of multiple CDKs in neuronal apoptosis [78].

The endogenous CDK inhibitor p27 protein expression declines to an undetectable level after a 24-h application of etoposide in primary neuronal cultures, returning nearly back basal level following treatment with cell cycle inhibitors [32]. After 24-h transfection of p27 siRNA, p27 protein expression is down-regulated and accompanied by an increase in BrdU uptake into neurons, as well as Rb phosphorylation. Neuronal cell death induced by p27 reduction is almost completely inhibited by olomoucine [32].

Inhibition of glial proliferation by CDK inhibitors

Aberrant cell cycle activation induces proliferation in mitotic cells, such as astrocytes and microglia. Administration of cell cycle inhibitors, including flavopiridol, roscovitine, and olomoucine, inhibits microglial and astrocyte proliferation *in vitro* [5, 22, 66].

Moreover, application of flavopiridol and roscovitine to primary rat microglial cell cultures significantly inhibits microglial proliferation in response to lipopolysaccharide and tumor necrosis factor-α stimulation, accompanied by downregulation of cell cycle protein PCNA [15, 22, 78]. Furthermore, cell cycle inhibition in microglia inhibits their proliferation and activity, blocking nitric oxide production 24 h after stimulation [15, 22]. More recently, we demonstrated that roscovitine significantly attenuated the ability of microglial-conditioned media to induce neuronal cell death, suggesting decreased release of neurotoxic compounds by activated microglia [78].

CDKs inhibitors in brain trauma and ischemia

To investigate exogenous CDKs inhibitors for post-traumatic neuronal cell death after TBI, flavopiridol was administered in rats in a single dose intracerebroventricularly 30 minutes after lateral fluid percussion-induced injury [5, 22]. Flavopiridol treatment markedly attenuated the increased cyclin D1 and caspases-3 activity, reduced the proliferation/activation of both of microglia and astrocytes, and decreased lesion volume [5, 22]. Treatment also markedly improved motor cognitive recovery at 21 days postinjury. Protective effects of CDK inhibitors have also been widely reported in cerebral ischemia [3, 77, 85–87].

CELL CYCLE INHIBITION AS A THERAPEUTIC TARGET FOR SPINAL CORD INJURY

Studies have shown that cell cycle activation plays a key role in several different SCI models and species; cell cycle inhibition alleviates neuronal death and improves functional recovery following SCI (see Table 1). CDK inhibitors have been examined experimentally in the

Table 1. *Cell Cycle Activation and Effects of Inhibition in SCI Models*

SCI Model	Cell Types Examined	Cell Cycle Inhibitor	Outcomes	References
Rat contusion	Neurons	None	Up-regulation of cell cycle proteins	Di Giovanni et al. [4] (2003)
Rat hemi-section	Neurons and astrocytes	Olomoucine	Reduced astrogliosis; improved behavior	Tian el al. [16] (2006)
Rat contusion	Neurons, microglia and astrocytes	Flavopiridol	Decreased microglia, astrocyte activation, and neuronal apoptosis; improved motor recovery	Byrnes et al. [15] (2007)
Mouse contusion		Cyclin D1 knockout	Reduced lesion volume and neurological deficits	Byrnes and Faden [18] (2007)
Rat hemi-section	Microglia and neurons	Olomoucine	Decreased microglia activation and neuronal death	Tian et al. [17] (2007a)
Rat hemi-section	Microglia and astrocytes	Olomoucine	Decreased microglia activation and reactive astrocytes	Tian et al. [79] (2007b)
Rat dorsal hemi-section		TAT-fusion protein of cytoplasmic p21Cip1/WAF1	Improved axonal regeneration and functional recovery	Tanaka et al. [93] (2004)

SCI = spinal cord injury.

treatment of SCI in rodent models. Experimental models for SCI in rodents are typically conducted by compression, transection, or contusion [88–90]. Clinically, half of SCI cases result from contusion injuries [91]. In animal models, spinal cord contusion injuries are commonly produced by the weight-drop method or a by a defined impact force. Contusion SCI in the adult rat produces similar pathophysiological changes to that in human SCI, which differs from that seen in the mouse [92]. Whereas the former exhibit cystic cavitation, the latter do not.

We demonstrated the role of cell cycle pathway in secondary injury following contusion SCI using flavopiridol [15]. To examine the effects of cell cycle inhibition on behavioral recovery and lesion size, Byrnes et al. [15], applied flavopiridol centrally by intrathecal administration using mini osmotic pumps, starting 30 minutes postinjury and continuing for 7 days. Treatment with flavopiridol significantly improved motor function and reduced lesion volume at 28 days after SCI. It also limited CCA after SCI, reducing phosphorylation of Rb, as well as cyclin D1 and G1 expression. Neuronal apoptosis was significantly reduced by flavopiridol treatment and apoptotic oligodendrocytes were rarely observed with treatment. Furthermore, flavopiridol administration also significant attenuated astrocyte reactivity and microglial activation. Therefore, it has effects on multiple cell lineages, all promoting neuroprotection. More recently, we found that flavopiridol, given systemically by intraperitoneal injection, beginning 24 h postinjury and continuing for 7 days, improved motor recovery at 28 days after moderate rat contusion SCI (unpublished results).

Another study from Tian et al. [16] used olomoucine, in a rat hemi-section model at T12. They found that continuous olomoucine treatment intraperitoneally for 7 days, starting 1-h postinjury, attenuated astroglial proliferation and accumulation of chondroitin sulphate proteoglycans, as well as increasing expression of growth-associated protein-43 (a marker of axonal regeneration), and reducing neuronal cell death. These molecular and cellular changes were associated with reduced cavity formation and improved functional outcomes. Olomoucine also significantly suppressed proliferation and activation of microglial and reduced tissue edema formation [16]. It is probable that the observed effects of olomoucine to improve neuronal survival following SCI can be explained at least in part by its attenuation of the neurotoxic consequences of microglial proliferation [57]. Olomoucine significantly reduces the number of TUNEL positive neurons at 3 days after SCI, while decreasing astrocyte proliferation at 7 days. Preliminary work on cyclin D1 knockout mice shows that lack of this critical cell cycle protein significantly reduces lesion size after moderate contusion SCI in mice [18].

The endogenous CDK inhibitor p21 has also been studied in a traumatic SCI model. Local application of p21 after dorsal hemi-section of the spinal cord was reported to exert a neuroprotective effect. The blood brain barrier scores of rats with p21 treatment increased markedly 6 weeks after SCI in contrast to control rats. Moreover, the cavity formation in vehicle-treated rats 6 weeks after SCI was more severe than in p21Cip1/WAF1-treated rats [93].

Taken together, these reports indicate that cell cycle inhibitors may provide an effective therapeutic strategy for the treatment of traumatic SCI, likely because of their multimodal actions that may include inhibition of neuronal and oligodendroglial cell death, reduction of the post-traumatic inflammatory response, and attenuation of glial scar formation.

SUMMARY

Up-regulation of cell cycle pathways after SCI can cause both proliferation and activation of mitotic cells and apoptosis of post-mitotic cells. Cell cycle inhibitors protect mature neurons and likely oligodendroglia from apoptotic cell death, attenuate microglial activation and release of associated inflammatory factors, and reduce astrocytic proliferation and activation. These multifactorial actions may help explain the striking neuroprotection afforded by treatment with such drugs across many acute and chronic neurodegenerative models.

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