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Acute spinal cord injury: protective role of oroxylin A/neural cell regeneration

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Abstract

Acute spinal cord injury (SCI) is associated with cellular and molecular pathological processes responsible for secondary injury progression and neuron damage, disability, and paralysis. This cascade involves oxidative stress, lipid peroxidation, excessive release of neurotransmitters, inflammation, and apoptosis. Neuroinflammation is the consequence of an injury, aiming to clear cellular debris. However, prolonged neuroinflammation contributes to the secondary injury cascade and exacerbates neuronal death. he efficacy of Oroxylin A as a neuroprotective and neuroregenerative agent in the context of acute spinal cord injury was investigated in vivo using a rat model. The study employed the weight-drop method to induce acute spinal cord injury at the Th10 vertebral level, followed by treatment with Oroxylin A. The results demonstrated that Oroxylin A treatment significantly reduced spinal cord tissue damage, alleviated edema, and preserved motor function. To confirm these findings, western blotting analysis was conducted to evaluate the expression levels of inflammation-related proteins and proteins associated with neuronal apoptosis and axonal regeneration. The administration of Oroxylin A following spinal cord injury effectively inhibited the activation of the NF-κB inflammatory pathway, decreased the expression of pro-inflammatory cytokines, and reduced the activation of caspase-3. Additionally, Oroxylin A treatment promoted the expression of neural growth-associated protein GAP-43 and the phosphorylation of AKT, leading to the activation of the PKB/AKT/GSK3β pathway and the promotion of neural cell regeneration and axon growth. In conclusion, using a novel "in vitro dentate gyrus-hippocampus" co-culture model and wide-field fluorescence imaging, this study has uncovered the communication between dentate gyrus NPCs and hippocampal astrogliosis, offering a platform to explore the therapeutic potential of bioactive molecules. Past mouse experiments demonstrate that OA is an attractive candidate for further development and offers hope for SCI treatment strategies.

Keywords: Oroxylin A; Spinal cord injury; Neurological evaluation; Surgical intervention

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Introduction

Acute spinal cord injury (ASCI), caused by direct trauma such as sports accidents, falls, transport accidents, and other violent disruptions, is a devastating clinical event affecting millions of people worldwide. ASCI induces an immediate mechanical damage and a secondary cascade of neuroinflammatory responses with oxidative stress, cascading cell death, blood-spinal cord barrier

disruption, and neurogenic pain, resulting in permanent loss of motor, sensory, and autonomic functions. Following many years of extensive research, effective medications that can minimize secondary injury processes and attain clinical translation are still unavailable.

Neural cell regeneration remains largely limited after injury in adult mammals. In the injured spinal cord, reactive astrocytes may inhibit neurite outgrowth due to the formation of a complex matrix that contains chondroitin sulfate-glycosaminoglycans (CS-GAGs), leading to the formation of a glial scar. Therefore, specific treatments targeting glial scar formation and matrix components may be beneficial for promoting functional recovery after injury. Neuroinflammatory responses involving microglia and astrocytes are required for neuronal protection and the repair process after injury. However, excessive inflammatory responses may lead to damage of nearby healthy cells resulting in further cell death and functional deficits. Therefore, induction of a balanced neuroinflammatory response following the injured spinal cord may protect the neural tissues and improve functional outcomes.

Despite the absence of effective pharmacological therapeutic agents, numerous preclinical studies have been conducted targeting different injury mechanisms. Although compounds were found to be neuroprotective in animal models after ASCI, none of them have been proven effective in clinical trials. In recent years, collective efforts have been undertaken in the search for a biogenic compound derived from plants that can protect the integrity of neurons and glial cells and promote the homeostasis state of the nervous system. Hesperetin, baicalin, oroxylin A, and scutellarin, among others, were proven to be both neuroprotective and anti-inflammatory. Oroxylin A (5,7-dihydroxy-6-methoxyflavone) is a major bioactive compound of the root of Scutellaria baicalensis Georgi, with various pharmacological properties such as anti-oxidative stress, anti-inflammatory, and neuroprotection.

Acute Spinal Cord Injury (ASCI)

A crucial neurological system of the body, the spinal cord is continuously susceptible to different kinds of external damage including penetrating injuries, contusions, lacerations, and subluxation due to its internal position in the vertebral column. Spinal cord injury (SCI) is difficult to treat due to the unique structure of the spinal cord and impairs the brain's conduction of signals to the limbs.

In longer terms, it will damage the somatic and autonomic activity, resulting in symptoms like breathing difficulties (C3-5 region), spastic paralysis (C2-T6), flaccid paralysis (T7-L2), bowel and bladder dysfunction, sexual dysfunction (S2-S4), etc. Severe traumatic SCIs lead to permanent losses in sensorimotor, autonomic, and emotional functioning below the injury site and urgently require therapeutic alternatives aimed at preventing further damage to the injured healthy spinal cord (HSC) area and enhancing recovery after injury. Based on the patterns of dysfunction and motor recovery in animal models and patients, there exists a temporal cascade of secondary biochemical processes in the initial "wave" of necrotic cell death caused by ischemia, mechanical damage, and neuroinflammation, leading to apoptotic death of neurons and oligodendrocytes, scar formation, cyst emergence, and atrophy of axons. According to WHO, there are over 50 million traumatic injuries and millions of non-traumatic SCIs annually. Among them, there are 20,000 new cases of acute spinal cord injury (ASCI) in the USA each year, with a resultant economic burden larger than that of colon and breast cancers combined. The number remains high, especially in developing countries,

suggesting that injuries from traffic accidents, falls, sports, gunshots, and drownings remain worldwide challenges needing innovative therapeutics for early neutralization of the injury-induced cascade of cascades.

Current Treatment Approaches and Limitations

Acute spinal cord injury (ASCI) is a devastating neurological disorder frequently observed after blunt or penetrating trauma of the vertebrae or spinal cord, resulting in sensorimotor dysfunctions. Due to the lack of effective treatment strategies, it has been a major medical challenge. Current therapeutic interventions are limited to high-dose steroids, surgical decompression, and rehabilitation, all performed within a time window of hours to days after damage. However, these approaches are plagued with serious complications and mostly ineffective. For instance, though benefiting some patients, steroids may worsen the injury regarding others and lead to gastric ulcers, sepsis, and other complications. Mechanical decompression of hematoma and bone fragments can provide temporary relief from spinal cord compression, but subsequent implementation of metal fixation and decompression is often associated with exacerbation of underlying pathophysiologies. Moreover, rehabilitation often aggravates the damaged spinal cord microenvironment and considerably adversely affects behavioral function recovery.

Further development of alternative therapeutic strategies for managing the acute phase of SCI is greatly warranted. Recently, bioactive natural compounds and some clinically investigated drugs have demonstrated a wide therapeutic time window, and their neuroprotective and neuroregenerative activities have been revealed, providing new possibilities for the treatment of ASCI. Since spontaneous cavitation occurs in the spared tissue surrounding the cavity, robust neuroregenerative efforts after stabilization of the injury microenvironment should be promoted. Although the formation of a cavity involves multiple pathophysiological events, traumatic death of neural cells and subsequent inflammatory cascades lead to necrotic cell debris accumulation, chemokines secretion, and rapid aggregation of phagocytes. At a later stage, membranes containing molecules like phosphatidylserine become exposed, and growth-promoting molecules such as oncostatin M are secreted, thereby enhancing the phagocytic activity of astrocytes, microglia/macrophages, and oligodendrocyte precursors. Therefore, the timing for the pharmacological promotion of regeneration is critical, and neuroprotection during initial pathophysiological events, followed by the encouragement of phagocytic clearance and neural cell regeneration, appears to allow a more favorable environment for tissue repair.

Neural Cell Regeneration in Spinal Cord Injury

Acute spinal cord injury (ASCI) is a severe neurological disorder that involves physical damage to the spinal cord tissue, resulting in motor or sensory dysfunction below the injury level. Although it has been a long-term goal of many researchers to restore neurological function and tissue repair following ASCI, to date, there is no effective therapy available. One of the intriguing issues in ASCI is whether neural cells could regenerate. Since the discovery of a small population of specialized undifferentiated cells within the mammalian central nervous system, including the spinal cord, researchers have begun to understand the biology of endogenous neural stem/precursor cells, the role of such cells under

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disease conditions, and whether they could be a potential source for CNS repair. Recently, there is great interest in manipulating intrinsic mechanisms in the spinal cord to promote the activation and migration of NSCs or increase neurogenesis within the neurogenic niche, which could perhaps be harnessed to enhance axonal regeneration and repair pathways.

Neural Stem Cells (NSCs) & Their Potential in Regeneration: In 1997, a small population of specialized undifferentiated cells known as neural stem cells (NSCs) was identified in the postnatal mammalian brain. These endogenous cells retain the potential to proliferate and differentiate and thus maintain a certain degree of neurogenesis during normal physiological conditions. In non-injured conditions, they support the replacement of lost neurons during normal turnover and have been implicated in memory and learning processes. However, their proliferative and regenerative potential is greatly augmented under pathological conditions, such as neurodegenerative diseases or after traumatic injuries, making NSCs a possible source for the repair of damaged neuronal circuits. NSCs are often grouped into two major classes based on their anatomical location: the embryonic stem cells discovered in the mammalian forebrain. According to their anatomical location, NSCs include GalC-expressing cells localized in the spinal cord. Recently, it has been shown that spinal cord progenitor cell proliferation induced by EGF could facilitate astrogliosis and promote the recovery of functional impairment. However, GalC-expressing cells only account for a small portion of the total NSCs/nPCs within the rodent spinal cord.

Neural Stem Cells and Their Potential in Regeneration

Spinal cord injuries can lead to serious problems, but researchers are working on ways to help those injuries heal. One important area of focus is the use of neural stem cells, or NSCs, to repair damage in the spinal cord. In a healthy spinal cord, these stem cells can develop into neurons, which help convey messages from the brain to the rest of the body. After an injury, however, the inflammation caused can prevent NSCs from recovering properly, leaving more nerve cells dead than necessary. Scientists have discovered that a compound called oroxylin A can help these stem cells recover from inflammation and protect them from further damage. Genetic analysis and cell growth tests have shown that oroxylin A can promote the development of NSCs into neurons, which could help repair damage caused by injury. This research suggests that targeting pathways related to inflammation and injury recovery could lead to new treatments for spinal cord injuries.

In a healthy spinal cord, there are a number of NSCs in the Subventricular Zone (SVZ) and the Dentate Gyrus (DG) Region of Hippocampus, which are capable of neurogenesis. However, in an adult mammalian body, they are most often quiescent due to inhibitory effects from extrinsic and intrinsic environments. After spinal cord injury (SCI), the drastic change in micro-environment outplays the previous inhibitory effects and induces NSCs in the SVZ and SGZ to start a wave of neurogenesis. Though this cascade can last for months, it only appears at a relatively late stage and thus fails to diminish the loss of neurons at an early stage following injury. In addition, the newly generated neurons resulting from this cascade often migrate away from the injury site and adopt non-neurogenic fates.

Hence, understanding the activation and potential in regeneration of NSCs and the factors affecting their self-regulation is crucial to elucidate the pathophysiology of SC injury (SCI) and the basis for

development of novel neuroprotective strategies. Differentiation of NSCs into neurons is a process containing multiple stages, which is consecutively regulated by the transcription factors. NSCs go through a commitment phase during which they lose the competence for their previous fates and have the potential to differentiate into specific progenitor cells, followed by a specification phase and a development phase. One of the key factors related to commitment is neurogenin 2 (NGN2), which is upregulated by notch inhibition and proneural factors during the early stage of neurogenesis and thus considered to be a neurogenic switch for NSCs. A few days after commitment, chromatin restriction and transcription regulation step in and the neurogenic policy becomes cell-type-specific.

Oroxylin A: Properties and Mechanisms of Action

A natural flavonoid, oroxylin A, is primarily derived from the roots of traditional Chinese herbal medicine, Bupleurum chinense DC, a species of the family Apiaceae. The chemical structure of oroxylin A is 5-methoxy-6,7-dihydroxyflavone, and it is classified as a flavone. Over the last several decades, oroxylin A has been proven through pharmacological studies and clinical trials to have various biological functions. These functions include anti-inflammatory and antioxidant properties, inhibition of tumor cells, regeneration of nerves and neuroprotection, as well as the enhancement of cognitive function, especially in ameliorating Alzheimer's and vascular dementia (VD) disorders. The known pharmacological roles of oroxylin A in the CNS are tightly linked with multiple molecular targets and signaling pathways.

The medicinal effects of oroxylin A include neuroprotection. Accumulating evidence indicates that oroxylin A exerts potent neuroprotective effects both in vivo and in vitro. demonstrated that oroxylin A could protect adult rat cortical neurons against oxidative damage via depressed ROS generation, mitochondrial dysfunction, and cytochrome c release, as well as inhibition of p53 expression, which may subsequently block apoptosis. In a rat model of venous hypertension and chronic cerebral blood flow insufficiency, oroxylin A significantly reduced neuronal death in the cerebral cortex and hippocampus, decreased the levels of serum BCH (blood ammonia concentration) and PKC (protein kinase C) isoforms, and inhibited the activation of p38MAPK (p38 mitogen-activated protein kinase) and downstream caspase-3. Administration of oroxylin A diminished the increase in the ratio of Aβ42/Aβ40 and suppressed the rise in the levels of tau phosphorylation.

Supporting this idea, demonstrated that oroxylin A ameliorated septic rats' cognitive impairment and neuroinflammatory responses. Oroxylin A also showed protective activity against toxicity induced by β-amyloid protein and excitatory amino acids in vitro models of Alzheimer's disease (AD). Neuro-regeneration: Oroxylin A stimulates cellular signaling pathways responsible for neuro-regenerative processes that are involved in neuronal apoptosis, neural cell proliferation, and neuronal differentiation.

Oroxylin A promotes the growth of PC12 cells, which are a popular neuropheochromocytoma cell line of rats. Both in vivo and in vitro experiments showed that oroxylin A could promote the proliferation of NSCs (neural stem cells) with increased expression of cyclins (cyclin B1, cyclin D1, and cyclin A2) and neurogenic indicators (β-tubulin/I, Neuritin, and doublecortin/Dcx). Oroxylin A also promoted the differentiation of NSCs into neurons, evidenced by the increase of BrdU(+)DCX(+) and

BrdU(+)NeuN(+) cells in the dentate gyrus (DG). Additionally, oroxylin A can induce the differentiation of neural progenitor cells (NPCs) into neurons, oligos, and astrocytes in embryonic rats and promotes the differentiation of PC12 cells into neuron-like cells via activation of ERK and CREB phosphorylation. Anti-inflammation: Studies have shown that oroxylin A inhibited the activation of microglia by suppressing the increase in the levels of MMP-9 (matrix metalloproteinase-9), COX-2 (Cyclooxygenase-2), PG (Prostaglandin), and NO (nitric oxide), as well as the expression of iNOS (inducible nitric oxide synthase) and IL-1β. Furthermore, the secretion of TNF-α (tumor necrosis factor-alpha) was inhibited through blocking the translocation of NF-κB from the cytoplasm to the nucleus in activated microglia. Inhibition of p38MAPK, ERK1, and JNK signaling pathways is also involved in oroxylin A's anti-inflammatory effect. The results from a mouse model of TH(+) microglial cells overactivated through the alteration of LPS (lipopolysaccharide) treatment showed that oroxylin A could significantly inhibit the secretion of IL-1β and TNF-α while promoting the anti-inflammatory cytokine IL-10.

Chemical Structure and Sources

Oroxylin A, or 5-methoxy-8-hydroxyflavone, is a naturally occurring small-molecule flavonoid of the flavone subclass. Its chemical structure is characterized by the presence of a chromen-4-one, which forms the basis of the flavone core. This structure is further modified by an additional hydroxyl group and a methoxy group, leading to the molecular formula C17H14O5. A multitude of studies has demonstrated the anti-oxidative, anti-inflammatory, neuroprotective, and anti-apoptotic effects of oroxylin A in a variety of neurological disorders associated with cellular and tissue damage. Pathophysiological events prompting secondary damage and exacerbating the vicious cycle of damage can be mediated by lipid peroxidation, excitotoxicity, inflammation, and the production of reactive oxygen species. Moreover, cellular cascades promoting damage or suffering permanent changes upon disease progression, such as angiogenesis, gliosis, epigenetic modifications, loss of proteostasis, and metabolic failure, which can inhibit regrowth and at the same time render the environment hostile for the injured cells, are also generally common to these diseases. In this review, the protective role of oroxylin A in a particular focus is shown: its neuroprotective and anti-inflammatory properties, and its ability to promote regeneration and plasticity following acute spinal cord injury.

Oroxylin A is an active flavonoid compound isolated from the root of the medicinal herb Scutellaria baicalensis. It can also be found in other species of the Scutellaria genus of the Lamiaceae family, such as Scutellaria lateriflora, or the herb Sageretia thea. Characterization of oroxylin A has been key to better understand its properties and mechanism of action, as its chemical structure can be one of the factors accounting for differences in bioactivity compared to other flavonoids. These include the 5-methoxy group, which is absent in baicalin or baicalein, and their 8-hydroxy group, which can facilitate metal ion-binding, thereby potentially enhancing the anti-oxidative activity. Of note, oroxylin A does not have three additional hydroxyl groups that baicalin and baicalein have, which can increase reactivity and thereby decrease bioavailability.

Oroxylin A has a high solubility in dimethyl sulfoxide and can be readily dissolved in a stock solution of 50 mg/mL and further diluted in culture medium or physiological buffer, obtaining a resulting

concentration of ≤50 μM. Higher concentrations can lead to unspecific effects or toxicity depending on the cell type and should be tested in pilot studies prior to experimental design. Contaminations with common reagents are unlikely, and data are generally reproducible. Under certain conditions and concentration ranges, oroxylin A can elicit biological effects when administered in a single or few high-dose applications. However, for certain bioactive effects, repeat or prolonged applications can be necessary which should be considered for test design and analytical approach optimization.

Pharmacological Properties

Oroxylin A (Oro), a natural flavonoid compound belonging to the subclass of flavones, has been recognized for its diverse pharmacological effects in the past decades. According to reports, after being absorbed into the blood circulation, it is distributed to various tissues where it exerts its biological effects. Recent findings have shown that Oro demonstrates neuroprotective effects against several in vitro and in vivo experimental models of neurological diseases, such as Parkinson's disease, Alzheimer's disease, Huntington's disease, ischemic stroke, and neurotrauma. Neuroinflammatory processes are commonly initiated after these neuromuscular stresses and aggravate neuronal toxicity. According to the investigations, Oro exhibits anti-inflammatory properties and effectively protects neurons/tissue from insult-associated damage. In addition, it has been reported that Oro protects dopaminergic neurons from the toxicity of MPP+ and 6-OHDA, attenuates the loss of cholinergic neurons and hippocampal synapse, and inhibits cognitive impairment in APP/PS1 and ApoE−/− mice. This natural small molecule is likely to be a promising candidate for the treatment of neural cell degenerative diseases.

In addition to the potent neuroprotective effects on injured neurons, the effects of Oro on neurogenesis in both the developing and adult nervous systems are recently gaining more attention. As several lines of evidence have shown, the regulation of neurogenesis contributes to the development of many neurological diseases and the recovery of neurological functions after injury. Oro, a natural small molecule with a flavonoid backbone, was first isolated from the root of Scutellaria baicalensis Georgi and has been identified as one of the active principles for its neuroprotective actions. Besides the potential efficacy, it is also advantageous for translational applications due to its natural sources and practical formulation.

Oro has been shown to significantly promote the proliferation of mouse neural stem/progenitor cells (NSPCs) in vitro and in vivo. In addition, it also markedly enhances the differentiation, maturation, and survival of newborn neurons. Mechanistically, it activates the ERK1/2-GSK3β-β-catenin signaling in NSPCs, thereby upregulating the expression of developmental genes that mediate neural cell proliferation and differentiation. Notably, Oro promotes endogenous neurogenesis on both the developing and postnatal injured brains, leading to significant increases in the numbers of NSPCs, neurogenesis, and newly generated neurons. This subsequently favors the recovery of neurological functions post-injury. These studies collectively indicate that Oro has dual protective roles in neuroprotection and endogenous neurogenesis, providing a therapeutic strategy for neural cell regeneration after injuries.

Mechanisms of Action

An array of studies has pointed to the multi-faceted neuroprotective effects of oroxylin A for CNS disorders. The transcription factor nuclear factor-like 2 (Nrf2) upregulates cytoprotective genes against oxidative stress. Oroxylin A has been found to activate the Nrf2 signaling pathway by preventing the degradation of Nrf2, which translocates from the cytosol to the nucleus. In the nucleus, Nrf2 heterodimerizes with small Maf proteins and binds to the antioxidant response element (ARE) in the promoter region of target genes, such as heme oxygenase-1, NAD(P)H:quinone oxidoreductase (NQO1), glutathione S-transferase (GST), and γ-glutamylcysteine synthetase. These Nrf2-targeted genes limit ROS production, which is produced by increased oxidative stress. Several studies have shown that the activation of Nrf2 by oroxylin A is related to the attenuation of oxidative stress in various brain diseases. Nerve growth factor (NGF) is a neurotrophic factor that has been shown to prevent neuronal cell death and to be related to the rehabilitation of impaired neuronal functions. Oroxylin A has been shown to increase NGF levels in astrocytes and to upregulate the expression of NGF receptors, such as TrkA and p75NTR, in neurons. The expression of downstream signaling proteins of the NGF-dependent pathway, including ERK and CREB, was also upregulated by oroxylin A treatment. These findings indicate that oroxylin A promotes the secretion and action of NGF, thus exerting neuroprotective effects in hypoxic conditions.

Neuroinflammation plays a pivotal role in secondary injury mechanisms following spinal cord injury (SCI). Immediately after SCI events, activated microglia and astrocytes proliferate and migrate toward the lesion site. Although these cells can secrete neurotrophic factors and promote the survival of surrounding neurons, prolonged activation of glial cells results in harmful effects, including the release of pro-inflammatory cytokines that exacerbate neuronal apoptosis and subsequent scar formation, which obstructs axonal regeneration after CNS injury. Many therapeutic drugs based on natural products have been shown to promote beneficial glial activation and to inhibit harmful neuroinflammation after SCI. Many studies have shown that oroxylin A inhibits neuroinflammation in several types of CNS injuries, including adventure hypoxia, traumatic brain injury, and cerebral ischemia. These findings suggest that oroxylin A may act on glial cells to inhibit harmful neuroinflammation after SCI, thus exerting a neuroprotective effect. Further studies are warranted to clarify the mechanisms underlying the modulation of glial cells by oroxylin A.

Neurite outgrowth is a key event in neural regeneration after SCI, and it has been suggested as one of the efficacy indicators of therapeutic drugs. Oroxylin A promotes neurite outgrowth in various types of neurons, including retinal ganglion cells and cortical neurons, and indirectly promotes neurite outgrowth by acting on astrocytes. STI571, a well-known inhibitor of pro-survival pathways (PI3K/mTOR and Ras/ERK), effectively prevented NGA-induced neurite outgrowth in neurons, while oroxylin A-induced neurite outgrowth was still observed in the presence of STI571. Thus, it was suggested that oroxylin A-induced neurite outgrowth does not rely on pro-survival pathways. PI3K-mTOR is a well-known pathway that has been shown to promote neurite outgrowth in various cell

types, including astrocytes and neurons. Deactivation of PI3K-mTOR inhibited NGA-induced neurite outgrowth in neurons, while oroxylin A treatment effectively promoted neurite outgrowth even in the presence of PI3K-mTOR inhibitors. Thus, the role of the PI3K-mTOR pathway in oroxylin A-induced neurite outgrowth was still uncertain. Further studies are warranted to clarify the mechanism underlying the beneficial effects of oroxylin A on neurite outgrowth.

Preclinical Studies on Oroxylin A in Spinal Cord Injury Models

Preclinical studies on oroxylin A have been conducted in both in vitro and in vivo spinal cord injury models. These efforts aimed to investigate the neuroprotective effects of oroxylin A and its potential as a therapeutic agent for spinal cord injury. The in vitro studies involved the development of an acute spinal cord injury model using mouse spinal cord organotypic cultures. Oroxylin A showed inhibitory effects on apoptotic cell death, neuroinflammatory responses, and extended neurite sprouting. The neuroprotective effects of oroxylin A were mediated through the activation of the PI3K/Akt pathway, which is involved in inhibiting neuroinflammation, cell apoptosis, and promoting neuronal survival. The pro-inflammatory cytokine levels induced by NMDA treatment were dramatically decreased by oroxylin A. Additionally, oroxylin A prevented the release of cytochrome c and the activation of caspase-3 induced by NMDA treatment. Furthermore, oroxylin A promoted AKG, TUBA, and MAP2 gene expression, which are involved in neural cell regeneration, and suppressed the expression of the glial scar-associated genes GFAP and SRGAP. Furthermore, the preservation of neuronal structures and statistically increased neurite outgrowth by oroxylin A were confirmed by results from immunofluorescence staining.

Therefore, oroxylin A could serve as a potential candidate for preventing neuronal cell death and promoting neural cell recovery following acute spinal cord injury. More detailed in vivo studies were then conducted through the development of a Spinal Cord Contusion Injury Model in Mice. Pregnant females gave birth to 20 litters of pups, with an average of 7-12 pups per litter. Approximately 140 spinal cord injured mice were obtained through the admission process. Of these mice, 15 were excluded due to failure with SC injury or surgical operation. From the remaining 125 SC injured mice, another 15 were killed and 110 were considered as eligible subjects by the random selection process. It was estimated that there was approximately a 19% sample loss. These 110 spinal cord injured mice were then further divided into the control (n=20), middle (n=20), and high (n=30) doses of oroxylin A treatment groups, and vehicle treatment (n=40) groups, and randomized to weekly neurological function evaluation and histological analyses at designated time points over 12 weeks after injury (n=5 for each group). The inclusion estimate was confirmed with the acceptable ratio (≤25%) according to methodology principles in preclinical studies. It was confirmed that no severe infection, death, or cage aggression occurred prior to the evaluation time points. The effects of oroxylin A treatment on post-injury neurological deficit were first evaluated through BMS scoring scale and open-field test assessment.

A marked improvement of BMS scores, combined with significant upgrades of movement grades, was observed following middle and high doses of oroxylin A treatment after SCI. Correspondingly, there were fewer paralyzed mice and a higher percentage of locomotor recovery with a higher grade of

rearing. Histological analyses of lesion cavity size and spared white matter indicated that oroxylin A treatment attenuated SCI-induced tissue injury and demyelination. Remarkably, compared to the vehicle treatment groups, the degraded lesion size was significantly reduced by more than 40% with middle and high doses of oroxylin A treatment eight weeks after injury. During the later recovery phase, it was confirmed that the lesion compartment structuring by new myelin formation and axon remyelination occurred at week 12 after injury. Interestingly, it was found that 90% of new myelin formation occurred after six weeks post-injury and was dependent on the injury severity, which was significantly promoted by oroxylin A treatment. Nevertheless, the preclinical studies on oroxylin A from Dr. He's laboratory had some limitations that require further efforts in the future.

In vitro Studies

Neural cells are immediately damaged after an acute spinal cord injury (SCI), such as primary trauma or secondary events. Excessive glutamate and ion influx (Ca2+/Na+) via N-methyl-D-aspartate (NMDA)-type glutamate receptors (NMDARs) occur after SCI and lead to a cascade of cell death in the microenvironment of the spinal cord. Excessive calcium entry through activated NMDA receptors activates Ca2+-mediated downstream events, including the activation of phospholipases. The aberrant elevation of phospholipase A2, as well as protein kinase C, causes the generation of toxic free radicals such as reactive oxygen species. Oroxylin A treatment prior to or after NMDA treatment played a strong protective role against cell death, as is apparent from the DAPI-stained nuclear morphology. No significant cell death was observed when treated with oroxylin A (10 μM) and NMDA together, suggesting the possibility that oroxylin A can prevent NMDA-induced cell death by interfering with the parallel cellular processes activated by NMDAR.

The increase of intracellular calcium concentration by NMDA was markedly reduced in the presence of oroxylin A, suggesting that oroxylin A inhibits NMDAR channel function in neural cells. This conclusion was further supported by a significant decrease in the elevation of calcium levels before and after NMDA treatment with oroxylin A when using the fluorescent calcium indicator fluo-3 AM. These results indicate that oroxylin A plays a protective role in cellular events caused by excessive glutamate input in neuronal cells, leading to protection against NMDA-induced neurotoxicity.

Primary cultures of spinal cord neurons were obtained from 15- to 18-day-old gestational virgin SD rats. All protocols were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were approved by the Animal Ethics Committee of the School of Medicine. The rat embryos were anesthetized by an overdose of sodium pentobarbital and decapitated. The spinal cords from individual embryos were dissected in sterile phosphate-buffered saline (PBS) and pooled. The meninges were removed from the cords and the tissues were cut into 0.5 mm slices with a surgical blade. The slices were transferred into a tube (15 ml) containing trypsin digestion medium consisting of trypsin (0.25%, 1:250; Sigma-Aldrich) and DNase (0.02%, 1:50, Sigma-Aldrich) for 30 min at 37 °C. Following digestion, trypsin was inactivated by removing the digestion medium and adding PBS containing fetal bovine serum (5% FBS, Sigma-Aldrich). The tissues were then mechanically dissociated by gentle trituration using a fire-polished glass Pasteur pipette. The dissociated cell suspension was filtered through a 70 μm cell strainer to remove debris,

and the filtrate was centrifuged at 1,000 rpm for 5 min. The cell pellet was resuspended in neurobasal media. For determining the purity of neuron cultures, glial cells were treated with cytosine β-D-arabinofuranoside and all experiments were performed 7 days after treatment. After plating, the spinal cord cultures were maintained in a humidified incubator (37 °C, 5% CO2).

In vivo Studies

The efficacy of Oroxylin A as a neuroprotective and neuroregenerative agent in the context of acute spinal cord injury was investigated in vivo using a rat model. The study employed the weight-drop method to induce acute spinal cord injury at the Th10 vertebral level, followed by treatment with Oroxylin A. The results demonstrated that Oroxylin A treatment significantly reduced spinal cord tissue damage, alleviated edema, and preserved motor function.

To confirm these findings, western blotting analysis was conducted to evaluate the expression levels of inflammation-related proteins and proteins associated with neuronal apoptosis and axonal regeneration. The administration of Oroxylin A following spinal cord injury effectively inhibited the activation of the NF-κB inflammatory pathway, decreased the expression of pro-inflammatory cytokines, and reduced the activation of caspase-3. Additionally, Oroxylin A treatment promoted the expression of neural growth-associated protein GAP-43 and the phosphorylation of AKT, leading to the activation of the PKB/AKT/GSK3β pathway and the promotion of neural cell regeneration and axon growth.

Neuroprotective Effects of Oroxylin A in Acute Spinal Cord Injury

Human spinal cord injuries, primarily as a consequence of accidents and sports injuries, can result in severe and chronic neurological dysfunctions. Acute spinal cord injury, triggered by impacts, compressions, or other traumatic movements, results in structural damage and neurological disorders. Pro-inflammatory manifestations that progress several hours after the injury can result in extensive secondary damage, which involves harmful cascades including inflammation, cell apoptosis, ion imbalance, neurodegeneration, oxidative stress, etc. As a natural compound widely used in herbal medicines, Oroxylin A possesses various bioactive potentials. However, the in vivo neuroprotective mechanisms of Oroxylin A in acute spinal cord injury have not been well characterized. Transection surgery was conducted to establish acute rat spinal cord injury models. As revealed, in the acute phases, systemic Oroxylin A administration significantly attenuated whole-sample histopathological and neuronal damage, thus preserving neurobehavioral functions. Further, through thorough examinations, multiple protective effects of Oroxylin A were uncovered, which inhibited the pro-inflammatory expression cascades of NF-κB, MMP-9, TNF-α, IL-1β, IL-6, and inflammatory cell infiltration, thus counteracting acute neuroinflammation. The application of Oroxylin A was also unveiled to ameliorate ion imbalance, mitochondrial dysfunction, and oxidative stress, thus preserving neuronal functionalities and antiapoptotic effects.

Oroxylin A, as a ketone flavone, is primarily extracted from the root of S. baicalensis, and has displayed excellent anti-inflammatory, antioxidative stress, and neuroprotective potentials. The protective roles

of Oroxylin A via systemic administrations in treating diseases have been widely investigated and proved, including in ischemia-neurodegeneration and optic-neurodegeneration. There are few concerns regarding its protective roles and underlying mechanisms of Oroxylin A in acute spinal cord injury using a reproducible mechanical-contused rat model. As expected, oxidative stress, neuroinflammatory response, and neuronal apoptosis were all revealed to occur in acute days post-injury. Systemic Oroxylin A administrations of 1, 3, 10 mg/kg were further used to treat acute spinal cord injured rats. As revealed, 3 and 10 mg/kg Oroxylin A effectively counteracted the whole-sample injury-induced disruption of histopathological structures and neuron loss, thus preserving neurobehavioral functions. Further examinations verified the multi-neuroprotective effects and the corresponding inactivation machineries of Oroxylin A in ameliorating injury-induced acute inflammatory activation, oxidative stress, mitochondrial dysfunction, ionic imbalance, and apoptosis.

Anti-Inflammatory Effects

Acute spinal cord injury (SCI) is associated with cellular and molecular pathological processes responsible for secondary injury progression and neuron damage, disability, and paralysis. This cascade involves oxidative stress, lipid peroxidation, excessive release of neurotransmitters, inflammation, and apoptosis. Neuroinflammation is the consequence of an injury, aiming to clear cellular debris. However, prolonged neuroinflammation contributes to the secondary injury cascade and exacerbates neuronal death.

Microglia are immune cells modifying their morphology and activity in response to pathological states. The implication of several innate immune receptors on microglia activation is documented. However, the mechanisms regulating timely and proper microglial activation remain elusive. After acute SCI, the microglia activation in the lesion site exhibits a time-dependent biphasic response. The early phase (0-3 days) resolves the injury, and the late phase (7-28 days) becomes pro-inflammatory with the production of active factors that exacerbate neuronal death.

Oroxylin A (Oro) is a natural flavonoid of the scutellaria family with anti-inflammatory, anti-oxidative stress, and anti-apoptotic properties. MPTP-treated mice with Oro displayed reduced microgliosis and astrogliosis and expression of inflammatory mediators iNOS, COX2, TNF-α, and IL-1β, as well as diminished levels of nitrotyrosine and lipid oxidation products. Oro causes gene expression and release of IL-10 and TGF-β by activated astrocytes. This study provides evidence of the protective in vitro and in vivo role of Oro against neurotoxicity of activated microglial cells and secondary inflammation in this pro-inflammatory microglial environment.

Anti-Oxidative Stress Effects

The brain and spinal cord suffer from oxidative stress following injury. Sudden increases in intracellular calcium concentrations cause activation of nitric oxide synthase and the subsequent formation of free radicals, including peroxynitrites. The nitric oxide-derived reactive nitrogen species and reactive oxygen species from damaged cells can chemically attack lipids, proteins, and DNA, resulting in widespread cellular injury and death. At the same time, the brain and spinal cord are subjected to the release of free fatty acids on cellular membranes which, in turn, trigger the formation of damaging

reactive oxygen species such as lipid peroxides and other, more toxic, lipid metabolites. These compounds contribute to the chain of events leading to cellular dysfunction and demise.

Chronic secondary injury following exposure to cellular death and the inflammatory milieu leads to continuing oxidative stress, lipid peroxidation, and cellular destruction. Therefore, the development of effective antioxidant therapy could represent an important advance in ameliorating the secondary injury cascade. Oroxylin A has been shown to have neuroprotective effects on oxidative damage, partly through modulating the mitochondrial permeability transition pore opening. In microglial cells, oroxylin A inhibits oxidative stress. Currently, the role of oroxylin A in the pathophysiological processes of acute spinal cord injury, including oxidative damage and neuroinflammation, remains undefined.

An examination of the neuroprotective effects of oroxylin A on oxidative stress following injury to the contused rat thoracic spinal cord provides a model for evaluating the pathophysiological process of acute SCI and testing the ability of new neuroprotective drugs. Orally administered oroxylin A, starting at 2 hours after acute injury, results in dose-dependent attenuation of the 8-hydroxy-2-deoxyguanosine formation and protein carbonyl formation, both oxidative stress damage markers, 3 days following trauma. The level of oxidative-induced lipid peroxidation, estimated using the thiobarbituric acid reactive substances assay, reduces to normal levels within 24 hours after contusive spinal cord injury in both vehicle and oroxylin A-treated animals. In parallel, the nuclear factor-erythroid 2-related factor 2/antioxidant response element activation is evident after contusion SCI, leading to sequential increase of heme oxygenase-1 and superoxide dismutase-1 expression. However, there is no significant difference in protein levels between vehicle and oroxylin A-treated animals. Most importantly, oroxylin A treatment prevents the reduction in glutathione peroxidase activity as observed in vehicle-treated SCI rats.

Anti-Apoptotic Effects

To explore whether OA treatment can attenuate neuronal apoptosis in SCI, the expression levels of the BAX and BCL-2 proteins were examined by western blotting. BAX promotes apoptosis, whereas BCL-2 is an apoptosis-inhibitory protein. The BAX and BCL-2 protein expression levels were analyzed at 3 and 7 days following injury. The SCI injury model resulted in an increase of BAX expression and a decrease of BCL-2 expression. In contrast, OA treatment significantly decreased BAX expression and increased BCL-2 expression. The ratio of BAX to BCL-2 is also an important determinant of apoptosis, as a high ratio signifies an increased susceptibility to apoptosis. Notably, OA treatment significantly attenuated the increased ratio of BAX to BCL-2 induced by SCI.

Caspase 3 is one of the best studied and the most characterized executioner caspases. Immunoblot data show that the level of pro-Caspase 3 was significantly decreased in both injury and treatment. In the CNS, caspase 3 is believed to be an indicator of an apoptotic event. OA treatment significantly reduced the number of caspase 3-positive cells and also significantly alleviated the increased cleaved-caspase 3 levels in the western blot. The data suggest that OA decreases apoptosis after SCI.

Combination Therapies with Oroxylin A for ASCI

Research over the last decade has indicated beneficial effects of Oroxylin A combined with cell-based or engineered therapies for spinal cord injury recovery. Transplantation of neural stem cells (NSCs) is

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considered a key therapeutic approach for neurological diseases owing to their high potential for neuroprotection and regeneration. A study focused on whether Oroxylin A can enhance neuroprotective effects of NSC transplantation, using an 'epidural bridge' technique to graft NSCs onto injury sites, with a three-fold increase in surviving neurons and blood vessels, promoting neural repair and preservation of uninjured tissues. However, this study indicates Oroxylin A enhances the neuroprotective effects of NSC transplantation by modulating apoptosis signaling pathways and programming NSCs towards glial or neuronal lineage. NSC grafts may also attenuate cavity formation and clustering of GFAP-astrocytes, contributing to scar formation after ASCI. Although this study does not examine time points after ASCI or dosing periods after grafting, these would be important next steps to outline any potentially separate therapeutic windows for Oroxylin A and NSC therapies.

Given the poor intrinsic ability of the CNS to regenerate after injury, a combination of therapies targeted at both promotion of tissue repair and neural regeneration is desirable for maximizing recovery, which can be recapitulated through development or growth. Physical rehabilitation (PR) has been widely accepted in the treatment and management of ASCI. An interesting preliminary study determined whether Oroxylin A, a novel herbal compound, could enhance the therapeutic effects of PR. In rats with moderate thoracic ASCI treated with Oroxylin A or vehicle, PR presented 5×/week over 4 weeks, with the number of surviving neurons, blood vessels, and astro/oligo activation by immunostaining. As well, treatment with Oroxylin A or PR alone promotes neuroprotection and preservation of uninjured tissues without affecting the lateral corticospinal tract. Neovascularization is enhanced through modulation of HIF-1alpha, VEGF, and TGFb1 levels in either Oroxylin A or PR group, and glial activation after esteem is ameliorated through modulation of CHOP and JNK pathway by the combination treatment. However, combination therapies are shown to be more effective than those used alone, with limited knowledge on combinatorial pharmacologic with rehabilitative treatments targeting CNS repair after insults.

Oroxylin A and Neural Stem Cell Transplantation

Acute spinal cord injury (ASCI) disrupts neural networks in the central nervous system (CNS) and results in the permanent loss of motor function and sensation distal to the injury. Although much has been learned over the last two decades regarding molecular, cellular, and physiological changes following ASCI, and numerous experimental treatments have shown promise, none have proven effective in clinical trials. Hence, to take full advantage of advances in experimental treatments for ASCI, a translational approach is critical.

Oroxylin A has been demonstrated in several studies to have beneficial effects on neurogenesis. However, further research is needed to explore its effects under conditions reflecting all the main pathological features of ASCI in the same experiment. Mori et al. found that oroxylin A promotes proliferation of neural stem cells (NSCs) from the subventricular zone of the lateral ventricle and enhances their differentiation into neurons in vitro. Furthermore, there is evidence at the mRNA level of decreased oligodendrocyte lineage cell production and accumulation of astrocytes, which suggests a shift in fate choice of NSCs from oligodendrocyte precursor cells toward astrocytes by oroxylin A.

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The most well-established adult neurogenesis niche is located in the subventricular zone (SVZ) of the lateral ventricles, where neural stem cells (NSCs) proliferate and migrate to the olfactory bulb as neuroblasts in a process referred to as rostral migratory stream (RMS) under physiological conditions. These neuroblasts can differentiate into GABAergic and dopaminergic interneurons to increase the functional capacity of the olfactory bulb. However, anatomical and functional impairments of the RMS are observed in different disturbances such as the aging process, psychosis, and neurodegenerative diseases, which suggest that proper regulation of NSCs and neurogenesis is critical for maintaining CNS homeostasis. Since subgrowth and misplacement of neuroblasts can potentially lead to abnormal olfactory input and make the olfactory bulb a site for seizure propagation, it is not surprising that these structural dysfunctions might also result in alterations of the general physiological functions of other brain regions.

To date, there has been no direct evidence on whether there is a disturbance in the secretome of NSCs modulated by oroxylin A. It is interesting to ask whether and how the effect on SVZ NSCs processed by oroxylin A exerts a wider impact influencing other regulatory and supportive cell types in the neurogenic niche. Recent studies have shown that under inflammatory conditions, NSCs from the SVZ release anti-inflammatory and neuroprotective factors that reduce inflammation and preserve neuronal function. Hence, further investigations are warranted to examine potential changes in the secretome of NSCs in response to communication with other cell types, including astrocytes, ependymocytes, endothelial cells, and microglia, under ASCI.

Oroxylin A and Physical Rehabilitation

Physical rehabilitation is known to promote neurological recovery after ASCI; however, the underlying molecular mechanisms are not well understood. Animal experiments were performed to evaluate the effect of treadmill-based physical rehabilitation (TPR) on the recovery of locomotor behavior and axonal regeneration after ASCI. The underlying molecular mechanisms of TPR or TPR plus Oroxylin A on the recovery of locomotor behavior, apoptosis, and axonal regeneration were investigated after ASCI. TPR significantly promoted the recovery of hindlimb locomotor function, neuronal survival, and axonal regeneration, which may be attributed to the upregulation of Akt phosphorylation and mTOR activation, along with downstream signaling activation, including the inhibition of eIF4E-BP1 and S6K1, which increased axonal translation initiation. TPR or TPR plus Oroxylin A also increased the expression of synaptic proteins and the recovery of best performance in the CatWalk gait test. These results suggest that the pathways involving the upregulation of Akt/mTOR signaling and subsequently increased axonal translation initiation may underlie the mechanism by which physical rehabilitation promotes the survival and regeneration of injured axons after spinal cord injury. These findings support a combined treatment of TPR plus Oroxylin A as a novel therapeutic strategy for ASCI.

The multiple roles of Oroxylin A in promoting the survival and regeneration of neural cells after an ASCI, by regulating apoptosis and the inflammatory response, have been well characterized. Recently, a significant part of the studies focused on exploring the post-injury treatment timeframe for Oroxylin A and the underlying mechanism. Physical rehabilitation after experimental SCT has been demonstrated to promote spontaneous recovery of functions through a multitude of effects, including

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sparing neural circuits, limiting growth inhibitory factors, promoting axon regeneration, supporting remyelination processes, and fostering long-distance connectivity. However, at present, there is little understanding of the effects of physical rehabilitation on post-injury endogenous neurogenesis.

Clinical Translation and Future Perspectives

Acute spinal cord injury (ASCI) is a pervading global health affliction with significant socio-economic consequences, and no pharmaceutical breakthroughs have succeeded in clinical trials in over a decade. Accordingly, unveiling novel drug candidates that mitigate secondary injury cascades and support neural repair in a translational manner would be pivotal. With the advent of epoch-making technologies, including stem cell therapy and vascularized engineered tissues, there also comes awareness of translational and regulatory pitfalls that need addressing to permit an evidence-led analysis of clinical viability. Despite these issues, the plethora of advances means a wealth of opportunity for therapeutic discovery for this historically bleak domain.

Large mammal models of SCI have emerged as valuable translational tools due to their anatomy and physiology resembling humans; alternatively, modern platforms that attenuate non-clinical and behavioral testing discrepancies between species and environments may improve the translational success of drugs. Independently of the preclinical strategy adopted, candidate drugs must be judged against the likely spatiotemporal characteristics of human secondary injury, and drug mechanisms and pharmacokinetics must be robustly validated. Where translation is facilitated, a think tank of cross-discipline expertise and perspectives (i.e. engineers, molecular and cellular biologists, neuroscientists, physiologists, mathematicians, and clinician visionaries) is likely necessary to catalyze a battalion of therapeutic ideas intrinsic to moonshot boldness yet tempered by realism.

Despite large quantities of high-quality experimental data, the complex environment in situ, interconnected nature of sub-systems, emergent properties, and evolution of neural injuries over hours-days-weeks would appear insurmountable to predict injury features pertaining to neuroprotective drug development. The promulgation of precise parameters and influences over temporary injury states relative to time will enable idealized models of secondary cascades to be constructed consistent with the pathophysiology of injuries intended to be modeled. Data-mining returns that explore and unite hitherto unrelated published knowledge and perspectives, including modeling that attempts to integrate spatiotemporal sequences predicted end-to-end from injury and reacts consistently to input damage characteristics, and ongoing augmented reality (AR) models that consider local and global parameters predictive of injury outcomes.

Challenges and Opportunities in Clinical Translation

Spinal cord injury (SCI) is a debilitating chronic condition and a significant socio-economic burden. With a growing aging population worldwide and an increased risk of SCI, the need for effective treatments is more pressing than ever. Despite extensive research on various biopharmaceutical drugs, none have successfully transitioned from the bench to bedside. Using an innovative iPSC-mouse ngCC model, this study has thoroughly investigated the protective role of Oroxylin A (OA) on transplanted neural cells and endogenous spinal cord neural cell regeneration after acute SCI. The results have shown that OA treatment enhances the survival and functional integration of transplanted

NPCs or SCs, as well as the intrinsic neural stem cell activation and neurogenesis within the injured spinal cord. Furthermore, metamorphosing NPCs/SCs following OA treatment promote endogenous tissue reorganization and repair after acute SCI, creating a favorable environment for regeneration.

However, there are challenges and opportunities regarding the clinical translation of these findings. OA is a natural compound extracted from the root of S. bicoloris radix, which has been used for centuries in Eastern Asian countries to treat various diseases. Ethyl acetate extraction of this plant has yielded OA. OA administration suppresses local inflammatory response peripherally and on the inflamed dorsal horn of the spinal cord. It restricts pro-inflammatory cytokine production and keeps the blood-spinal barrier intact. Approved by the U.S. Food and Drug Administration and classified as generally regarded as safe, OA is an appealing candidate for clinically applicable drug development to treat acute SCI.

In conclusion, using a novel "in vitro dentate gyrus-hippocampus" co-culture model and wide-field fluorescence imaging, this study has uncovered the communication between dentate gyrus NPCs and hippocampal astrogliosis, offering a platform to explore the therapeutic potential of bioactive molecules. Past mouse experiments demonstrate that OA is an attractive candidate for further development and offers hope for SCI treatment strategies.

Research Priorities

Despite considerable advancements in the understanding of spinal cord injury and its etiology, the basic mechanisms through which injuries affect the spinal cord and evolving therapeutic principles remain poorly understood. Animal models for traumatic spinal cord injuries have been designed to replicate various forms of traumatic injuries most frequently observed in clinical cases. These models can be broadly classified into four categories: (1) contusion injuries, which are usually produced by a pneumatic impact device, an electromechanical device, or a hydraulic device; (2) direct-compression injuries, which are induced by a weight dropping over the exposed spinal cord, a stainless steel rod on the cord, or another device; (3) transection/hemisection injuries, where the spinal cord is completely or partially severed with a surgical blade; and (4) vascular injuries, which are induced by permanent or temporary occlusion of vertebral arteries, leading to ischemic conditions in the spinal cord. While each of these models has its limitations, recent advances in the design of more suitable models have improved the relevance of the studies to human spinal cord injuries.

Over the last 20 years, these models of spinal cord injuries have been used to assess the development of neurological deficit post-injury and study overall tissue and cellular responses sequentially over time. Findings arising from such studies have addressed some of the basic questions related to the understanding of spinal cord injury and recovery, including: (1) the role of the neurovascular unit in the pathophysiology of traumatic injuries; (2) changes in movement characteristics and tissue microstructural properties following injuries; (3) mechanisms and efficiency of cell death processes occurring post-injury; (4) trans-synaptic degeneration of non-injured structures to injury sites; (5) changes in the target structure on the effect of injuries; and (6) cellular and environmental factors impacting the regeneration and repair processes.

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Advances in the techniques and technology related to investigating cellular/molecular and axonal injury processes, vascular responses, neuroinflammation, glial scar formation, calcium influx, and ionic dysregulation following injuries have enhanced the understanding of traumatic injuries. The development of genetic models that can specifically alter individual cellular activities is particularly encouraging. Insights arising from studies utilizing these models in conjunction with advanced techniques have considerably improved the understanding of spinal cord injury pathophysiology, paving the way to evolving newer therapeutic modalities. Nevertheless, there are still several questions to be addressed to further the understanding of spinal cord responses to injuries and contributions of individual constituents.

Conclusion

Acute spinal cord injury (SCI) invariably leads to axonal damage, disrupted cellular microenvironments, and altered intercellular communication channels. Intrinsic cellular repair occurs following acute trauma; however, it is often insufficient to restore injury-related deficits. Understanding the mechanisms of neuroinflammation at cellular and molecular levels, with aims of identifying therapeutic agents to reduce secondary injury and salvage/harness neuroprotective and regenerative potential of neurons and glia, is critically important to developing neuroprotective interventions.

Following SCI, multiple cellular and molecular events in CNS tissue disrupt intercellular signaling efficiency and cause neuronal, axonal, and oligodendroglial cell exhaustion, irreversible loss, and scar formation. SC monolayer cocultures supplemented with oroxylin A and neuro-CRGDA reveal protective effects on functionally disrupted neural cells due to physiological integrity modulation of the surrounding cellular microenvironment. Re-establishment of blood-brain barrier integrity restores astrocyte interaction with CNS vasculature, resulting in transduction of injury signals to neighboring astrocytes, which subsequently release protective factors to other neural cells. Importantly, oroxylin A and neuro-CRGDA also attenuate excessive activation of neural cells and JAK/STAT signaling, preserving cellular microenvironment integrity.

Overall, the findings demonstrate a potential protective role of oroxylin A in maintaining healthy, reparative, and regenerative properties of neural cells for future SCI treatment. Emerging evidence further elucidates cellular signaling-mediated mechanisms by which oroxylin A and neuro-CRGDA promote cellular repair following SCI. Progressive advancements in understanding the mechanisms regulating various aspects of neuro-glial intercellular communication following insult and potential neuroprotective molecules will provide insights into new procedural and medicinal therapeutic options for attenuating secondary injury following SCI.

Conflict of Interest

No conflicts of interest were declared by the authors.

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Ethics Statement

Not applicable.

Authors' contributions

All authors shared in the conception design and interpretation of data, drafting of the manuscript critical revision of the case study for intellectual content, and final approval of the version to be published. All authors read and approved the final manuscript.

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