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Neuroprotective Agents in Brain Injury: A Partial Failure?

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ABSTRACT

Brain injury leads to inflammation, stress, and cell death. Neurons are more susceptible to injury than astrocytes, as they have limited antioxidant capacity, and rely heavily on their metabolic coupling with astrocytes to combat oxidative stress. Both normally and after brain injury, astrocytes support neurons by providing antioxidant protection, substrates for neuronal metabolism, and glutamate clearance. Although astrocytes are generally more resilient than neurons after injury, severe damage also results in astrocyte dysfunction, leading to increased neuronal death. This mini review provides a very insightful and brief overview on a few examples of promising neuroprotective compounds targeting astrocyte function, with specific attention on how these treatments alter astrocyte response or viability, and how this may be critical for neuronal survival following brain injury.

KEYWORDS: astrocytes, brain injury, clinical trials, neurons, neuroprotection

INTRODUCTION

Traditionally, research has been focused on neurons and often neglected other cell types in the brain, such as astrocytes. It is increasingly evident that they are vital to normal central nervous system (CNS) functioning and also play important roles in neuropathological conditions. Although astrocytes form an inhibitory glial scar following brain injury, they also perform functions necessary for neuronal survival and well being, such as maintaining extracellular glutamate levels and providing antioxidant protection. Because they have multifaceted functions, astrocytes are attractive candidates as therapeutic targets. By striving to shift astrocytes toward a pro-reparative, neuronal-supportive phenotype following brain injury, future clinical therapies will be more successful in protecting neurons from damage and promoting repair. This mini review provides a very insightful and brief overview on a few examples of promising neuroprotective compounds targeting astrocyte function, with specific attention to how these treatments alter astrocyte response or viability, and how this may be critical for neuronal survival following brain injury.

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We thank Dr. Garcia-Segura for permission to use Figure 1.

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NEUROPROTECTIVE COMPOUNDS

Edaravone

Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one) is an approved clinical drug for acute cerebral ischemia [1], but has not previously been used to treat other brain injuries. Experimental studies have shown that edaravone inhibits production of free radicals, protects against apoptotic neuronal death, and improves cerebral function after brain injury [2, 3]. A possible mechanism mediating this protective effect might rely on the enhancement of prostacyclin production, inhibition of lipoxygenase metabolism of arachidonic acid by trapping hydroxyl radicals, inhibition of alloxan-induced lipid peroxidation, and quenching of active oxygen, leading to protection of various cells, such as astrocytes and neurons, against damage by reactive oxygen species (ROS). More studies are needed to better address how edaravone might directly affect astrocytic functions toward an enhancement of neuronal survival.

Simvastatin

Simvastatin has been shown to significantly enhance neuronal survival and attenuate activation of astrocytes after brain injury. Indeed, recent studies indicate that protective effects of simvastatin may be mediated by reducing the astrocytic production of IL1-beta, increasing in phosphorylation of Akt and its downstream signaling targets, and increasing antiapoptotic signaling in both astrocytes and neurons [4–6].

Metallothionein

Metallothioneins (MT) play an important role in the astrocyte–neuron response to injury. Previous studies indicate the transfer of MT from astrocytes to neurons in vitro [7], thus providing additional antioxidant support, suggesting a close and intrinsic astrocytes–neuronal cross-talk. In addition, MTs are rapidly expressed by reactive astrocytes and their secretion and subsequent interaction with neurons leads to improved neuroregeneration following brain injury [7, 8]. Mice overexpressing metallothionein-I present regulated inflammatory response, decreased oxidative stress, and proapoptotic signaling [9], again, demonstrating that these intracellular proteins widely expressed by astrocytes are important for neuronal protection. The astrocytic MT are thought to act via intracellular free radical scavenging and heavy metal regulation, and in particular zinc [7].

Neurosteroids

Astrocyte–neuron cross-talk is an essential component of the mechanisms involved in the neuroprotective actions of neurosteroids. This group of hormones with actions in the brain includes estradiol, testosterone, progesterone, and their derivatives. These neurohormones may exert rapid signaling events in astroglia via the regulation of the activation of kinase signaling pathways, such as the mitogen-activated protein kinase pathway [10, 11] or the phosphatidylinositol 3-kinase/Akt signaling pathway [12]. For instance, estradiol may regulate glia-to-glia and glia-to-neuron communication by the modification of intracellular calcium levels in astrocytes [13–15]. Estradiol and testosterone regulate reactive astroglia in different models of acute neurodegeneration. For instance, the hormone decreases astrocyte proliferation and glial scar formation after a stab wound injury in the cerebral cortex and hippocampus of young (see Figure 1) and old animals, even after long-term deprivation of this ovarian hormone [16–18]. It is important to point that control of gliosis may be only one of the mechanisms involved in the neuroprotective effects of estradiol postinjury. Similarly, progesterone exerts neuroprotective effects following traumatic brain injury [19], possibly reducing excessive excitotoxicity and inflammation while maintaining normal levels of cell proliferation and apoptosis [20].

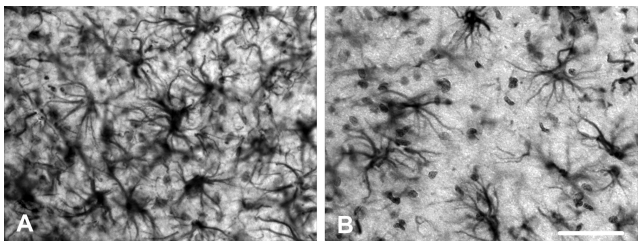

Figure 1. Estradiol modulates astrocytes activation following stab wound injury in the rat brain. The panels illustrate vimentin immunoreactive astrocytes in the CA1 stratum radiatum at a distance of approximately 100–200 μm from the lateral border of the wound. Animals were treated with (A) vehicle and (B) 1 mg/kg estradiol on days 0–2 following stab wound in the brain and sacrificed on day 3 postinjury. Scale bar, 50 μm.

Ceftriaxone

Uptake of the neurotransmitter glutamate is affected primarily by transporters expressed on astrocytes, and downregulation of these transporters leads to seizures and neuronal death. Some drugs used in clinics have been shown to protect neurons acting directly on astrocytes. Ceftriaxone was previously reported to increase glutamate transporter 1 (GLT1), the main astrocytic glutamate transporter, by threefold in animals treated on a daily basis before injury. The preadministration of ceftriaxone led to an enhanced neuronal survival and decreased astrogliosis following ischemic brain injury [21], reinforcing that enhancing the mechanisms of glutamate uptake in astrocytes may directly reflect on neuronal survival. However, possible neuroprotection exerted by ceftriaxone after traumatic brain injury needs to be further explored.

Glutathione Peroxidase

Glutathione peroxidase (GPx) has been shown to reduce neuronal damage [22] and modulate recovery in the injured brain, thus showing a clear demonstration of the importance of astrocytes for neuronal survival.

TRANSLATIONAL RESEARCH: PARTIAL FAILURE?

Nowadays, all recent efforts have been devoted to effectively attenuate, or even reverse, the motor and functional deficits that likely occur in bran injury. While most clinical trials have failed, or are faded to be partially successful, many reasons are raised, and the most

TABLE 1. Few clinical trials on traumatic brain injury have shown to be successful. From a batch of 304 studies currently registered on www.clinicaltrials.org, only one had promising results. Treatment with progesterone in patients following traumatic brain injury has proven to be successful in patients following traumatic brain injury. This neurosteroid showed to improve neurological outcome in phases I and II trials, and nowadays a randomized double-blind phase III clinical trial is being conducted

Drug	Recruitment	Effect	Outcome
Genotropin	Terminated	Growth hormone replacement	The study was terminated on Dec 15, 2008, due to an inability to recruit the protocol-specified patient population.
Naratriptan HCl	Terminated	Serotonin agonist	Terminated October 2009 (in 4th year) due to low enrollment and anticipated drug expiration November 2009.
Sertraline	Completed	Antidepressant: selective serotonin reuptake inhibitor	Results not published
Progesterone	Completed	Antiapoptotic and antiinflammatory	Patients treated with progesterone showed "encouraging signs of improvement"

critical issue is the time window between disease onset and further clinical assistance. Although it may be possible to predict the disease event based on clinical signs or biochemical aspects, and plan a preclinical strategy accordingly, this is not applied as it should be in most cases. Therefore, most therapies are applied chronically in patients, and these limitations may be considered as critical for recovery. A good example is illustrated in Table 1. From 304 registered clinical trials on traumatic brain injury (available at www.clinicaltrials.org), three trials have been precociously terminated or completed, and only one had showed promising results (see Table 1).

There are few explanations of why these trials were not successful. To begin with, because experimental brain injury is relatively homogeneous concerning size, territory, and etiology, and consequently inflammation is consistently elicited. However, human brain injury is extremely heterogeneous [23], with different size and territories involving different mechanisms. In addition, these mediators are known to affect many organ systems beyond the CNS, as such, systemic administration of such agents may have left patients partially immunocompromised, thereby confounding the outcome. Further discrepancies often involve the treatment window, and many neuroprotective trials have failed because the experimental efficacy was only proven in preclinical trials using the compound prior to the insult or immediately afterwards. During human studies the drug could not be delivered within the time it was proven efficacious in preclinical experiments and failed to show benefit in patients.

Inflammation and astrocyte response are likely closely connected. Although there is little evidence on the direct relationship between neutrophils and astrocytes, it has been shown that mice with a blunted inflammatory response exhibit increased loss of glial fibrillary acidic protein (GFAP)-positive astrocytes after cortical stab injury [24]. Because astrocytic glial scar formation is important in the protection of spared tissue from further damage [25], it is possible that treatments that drastically attenuate inflammation lead to a stunted astrocyte response that is deleterious to recovery. In addition, studies that have beneficial effects on both neuronal and glial cells are thus likely to be more successful than treatments that target neurons alone.

Another issue of discrepancy between animal studies and clinical trials is the way outcome and functional status is assessed. Animal studies often use histological outcomes. For example, a compound proven to reduce edema in an animal study may not show benefit in clinical outcomes, since edema volume correlates poorly with functional outcome. Functional assessment in animals is usually limited to simple tasks of limb pacing, and beam ad grid walking, while many outcome measures in clinical trials assess social function and activities of daily living.

CONCLUSIONS

Despite hundreds of clinical trials have been completed in the last several years, none of these have proven to provide effective treatment for human brain injury. This lack of success may be because most treatments are aimed at targeting neuronal populations. Although neuronal death is the main outcome that we wish to ameliorate after brain damage, studies have shown that astrocytes play key roles in both normal and pathological CNS functioning, making them viable targets for manipulation after brain injury. Neuroprotective efforts targeting the functional integrity of astrocytes may constitute a superior strategy for future neuroprotection. Although large gaps exist with regard to our understanding of how brain injury affects the neuronal supportive function of astrocytes, it is likely that astrocytes

malfunctioning may contribute to the massive loss of neurons in brain injury.

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