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REVIEW ARTICLE

Neuroprotection in Hypoxic-Ischemic Brain Injury Targeting Glial Cells

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Abstract: Brain injury constitutes a disabling health condition of several etiologies. One of the major causes of brain injury is hypoxia-ischemia. Until recently, pharmacological treatments were solely focused on neurons. In the last decades, glial cells started to be considered as alternative targets for neuroprotection. Novel treatments for hypoxia-ischemia intend to modulate reactive forms of glial cells, and/or potentiate their recovery response. In this review, we summarize these neuroprotective strategies in hypoxia-ischemia and discuss their mechanisms of action.

Keywords: brain injury, hypoxia-ischemia, pharmacological treatments, glial cells, neuroprotection

1. INTRODUCTION

Brain injury represents one of the most widespread clinical problems around the world. This disabling condition impacts on multiple domains of health. Depending on the affected brain areas, sequelae involve physical, cognitive, emotional and/or behavioral functioning [1]. Cell death constitutes a key phenomenon in brain injury, occurring in the context of initial necrosis or further apopto-sis [2] as a result of mitochondrial dysfunction, inflammatory responses or neurotransmitter release, as listed in Table **1**.

Hypoxia, which occurs in the brain when oxygen availability drops below the normal level, may induce ischemia and constitutes a major cause of brain injury [3]. Energy failure occurring in hypoxia leads to a radical shift from aerobic to a less efficient anaerobic metabolism, which results in decreased adenosine triphosphate (ATP), lactate accumulation, decreased pH, and overproduction of reactive oxygen species (ROS). Because of reduction in ATP levels, extracellular glutamate concentration increases, resulting in over-activation of N-methyl-D-aspartic acid (NMDA) receptors and a massive influx of calcium (Ca2+) into cells. High cytosolic Ca2+ levels provoke cytoskeletal and extracellular matrix (ECM) proteins degra-dation. Finally, the entire cascade of downstream intracellular processes leads to excitotoxic neuronal damage and cell death. Although re-oxygenation is necessary for survival, it may lead to uneven distribution of blood flow in the brain, favouring the brain stem at the expense of the cortex, where ischemia can occur. In addition, during re-oxygenation period, a sustained overexpression of alternative metabolic pathways may prolong the energy deficit and/or generate further oxidative stress [4].

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Under the neuron damage paradigm, treatments for brain injury were solely focused on neuronal pathophysiology. Currently, glial cells are considered as suitable candidates for research. Astrocytes and microglia respond to brain injury by changing their phenotype and active functions to become reactive cells [5-8]. When ECM homeostasis is altered, astrocytes become reactive by proliferating, ongoing hypertrophy and activating multiple genes. The intensity of astrocytic activation may present two major consequences: either tissue recovery or consolidation of the damage [6, 9-12]. Reactive astrocytes secrete several molecules: pro and anti-inflammatory cytokines, neurotrophic factors, complement factors, chemokines and ROS. These molecules mediate neuroprotective and/or neurotoxic actions. For instance, some astrocyte-derived cytokines, such as tumor necrosis factor (TNF)-α, are involved in neurotoxicity, while others like transforming growth factor (TGF)-β, are considered to promote neuroprotection [6] (Fig. **1**). Despite their crucial role in brain homeostasis, astrocytes constitute a therapeutic target, either by re-establising their normal function or enhancing neuroprotective pathways [6]. Similarly, microglia apparently exerts dual roles. Activated microglia promotes brain recovery by removing cell debris, releasing trophic factors and resolving local inflammation. However microglia may expand tissue damage by releasing destructive pro-inflammatory mediators [4, 13]. These contradictory functions reflect two distinct cellular phenotypes in response to micro environmental cues: the immunomodulator phenotype (M2a) and the reactive phenotype (M1/M2b), respectively. These differential phenotypes, which constitute two extreme activation states, release antagonical molecules as it is illustrated in Fig. **1**. Therefore, therapeutic approaches targeting brain inflammation should shift from broad suppression of microglia towards subtle adjustment of the balance between microglial phenotypes [13]. The aim of this review is to resume the available information about neuroprotective therapies targeting glial cells in hypoxic-ischemic (HI) brain injury. Table **2** summarizes the different treatments for HI that will be exposed.

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Table 1. Processes triggered after an injury.

Primary injury	Secondary injury
Depolarization of cells membrane	Excitotoxicity mediated by glutamate
Release of excitatory amino acids	Over excitation of neighbor neurons produced by glutamate
Increase in arachidonic acid metabolism	Production of free radicals
Free radicals liberation	Disruption of ionic homeostasis
Neuronal death	Nitric oxide release
Hemorrhage	Inflammatory regulators release
Contusion	Oligodendrocyte injury leading to demyelination
Ischemia	Slow propagation of action potentials along axons

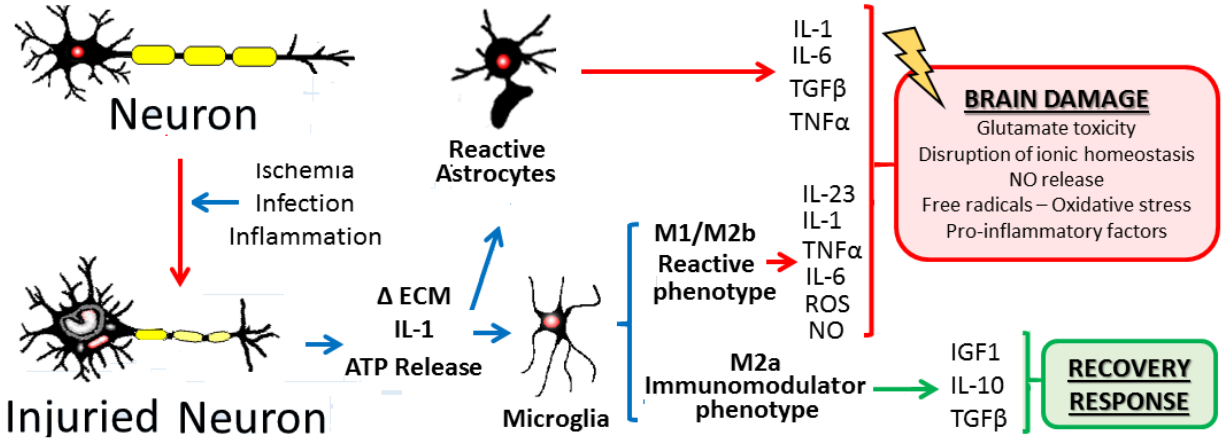

Figure 1. Neurotoxic and neuroprotective actions of glial cells.

2. TREATMENTS IN HYPOXIC-ISCHEMIC BRAIN INJURY TARGETING MICROGLIA

Some novel pharmacological therapies base their strategies in the modulation of reactive microglia phenotype, in order to limit the extent of the ischemic brain area [13]. Soluble factors of pro-inflammatory molecules like lipopolysaccharide (LPS), interleukin (IL)-1β and TNF-α increase gene expression of M1 and M2b microglial markers when added *in vitro*, decreasing the capacity of gene expression of M2a markers, and therefore promoting damage response and an active inflammatory state. However, when IL-4 is added to the culture, an increase in M2a markers is observed, which in turn enhances recovery response and neuron viability [14-17]. Similarly, high levels of IL-4 are associated with a neuroprotective effect in a murine model of focal cerebral ischemia by unilateral occlusion of the left middle cerebral artery (MCA) and the left common carotid artery (CCA) (MCA/CCAo) [17]. Regulation of both phenotypes is a constant and concomitant phenomenon: when M2a markers increase their expression, M1/M2b markers expression decreases, and viceversa [15].

When microglial cells turn to their activated phenotype in an environment with oxygen glucose deprivation (OGD), they express the enzyme sphingosine kinase (Sphk1), which hints the importance of sphingolipids on early events of neuron cell survival/death. Sphk1 catabolizes the reaction of sphingolipids to sphingosine-1-phospate (S1P), which induces the production of the pro-inflammatory cytokine IL-17A by microglia. Moreover, S1P increases the expression ratio of TNF-α, which further contributes to damage response. However, when the small interfering RNA (siRNA) of Sphk1 is added *in vitro* or *in vivo* (in a middle cerebral artery occlusion (MCAO) murine model), expression rates of IL-17A and TNF-α show a remarkable reduction [18]. In addition, blocking S1P receptors in lymphocytes T helper (LTh) with FTY720 (fingolimod), modulates effectively the inflammatory response in a murine model of LPS- sensitized perinatal HI [19].

Pituitary adenylate cyclase–activating polypeptide (PACAP) is a neuropeptide with important anti-inflammatory properties. When this enzyme is injected into a stem cell (SC), it induces neuroprotective responses and restores the ECM of the ischemic zone. Cells expressing PACAP show a reduction of TNF-α and IL-1β levels and an increase of the immunosuppressive cytokine IL-10 and TGF-β. In contrast, cultures or knockout animals that do not express the enzyme show an exacerbated inflammatory response. Moreover, PACAP facilitates the expression of M2a markers in microglial cells, enabling the neoangiogenic process [20]. In addi-tion, mesenchymal stem cells (MSC) apparently stimulate M2a phenotype by increasing the expression of IL-10 [21].

The administration of ginsenoside Rb1 (GRb1) is also neuroprotective by inhibiting the inflammatory cascade in a murine model of cerebral ischemia by MCAO. This suppression might be understood as a consequence of the reduction in microglial activation in the penumbra and the down-regulation of the binding site between transcriptional factors and DNA of TNF- α and IL-6 genes [22]. Early treatment with propofol in rats subjected to MCAO is apparently effective by decreasing microRNA (miRNA) expression of microglial markers CD68 and Emr1, which determines beneficial effects on infarct volume [23]. Similar results may be obtained with the administration of other compounds like carnosine and deoxys-

Table 2. Summary of treatments for HI and their targets.

Target		Treatment	Effect
↓ DAMAGE RESPONSE	↓ Glutamate toxicity	Blocking EphA4/Ephrin-A3 receptors	Decreases the expression of astrocytic glutamate transporters
		miR-181	Inhibits the astrocytic glutamate transporter
		Blocking AMPA/kainate receptors	Diminishes Ca^{+2} conductance in oligodendrocytes
		α7 nAChR agonists	Stimulates the activation of α7 nAChR
		Hypothermia	Decreases the expression of astrocytic glutamate transporters
	↓ Disruption of ECM	DOR agonists	Interrupts Na^{+} influx through the neuronal membrane
	↓ NO production/release	Diclofenac/Cysteamine conjugation	Decreases (COX)-2 and iNOS rates
	↓ ROS/Oxidative stress	Nfr2 expression	Activates detoxifying and antioxidant enzymes
		Deferoxamine	Scavenges Iron
		Catalpol	Scavenges ROS
		Apocynin	Inhibits ROS pathway
		Sulforedoxins (Srxn1)	Increases levels of GSH, affects apoptosis pathway
		1,5-dicaffeoylquinic acid	Activates Nrf2
		O-1966	Overexpresses CB_2 receptors
	↓ Pro-inflammatory factors ↓ Reactive phenotypes	IL-4 Administration	Turns reactive microglia to its immunomodulator phenotype
		Sphk1 siRNA	Reduces the expression of Sphk1
		Fingolimod (FTY720)	Blocks S1P receptors in LTh
		GnRb1 administration	Inhibits the expression of TNF-α and IL-6
		Propofol	Decreases mRNA expression of Cd68 and Emr1 TF
		Carnosine	Decreases the expression of TNF-α and IL-6

appanone B, which contribute to a decrease in pro-inflammatory markers [24, 25].

MicroRNAs (miRNAs) make possible to modify gene expression of a pro-inflammatory molecule by binding to its 3′-untranslated region (3'-UTR), mediating its cytosolic degradation. When hypoxia is induced *in vitro* by OGD, there is an activation of Fas ligand (FasL) expression in reactive microglia, which can be suppressed by the overproduction of miR-21. FasL induces neu-ronal apoptosis through microglial cells. Nevertheless, when miR-21 is expressed, there is a reduction in the number of apoptotic neurons [26]. Let-7c-5p is another conserved miRNA sequence, which is associated with M2a phenotype and exerts neuroprotective effects against cerebral ischemia in a murine MCAO model. Lower levels of let-7c-5p are related to M1 phenotype, an increase in TNF-α and a consequent damage response [27].

Designing novel drugs for molecular target sites is another strategy used by researchers. Non-steroidal anti-inflammatory drugs can be conjugated with a ligand resulting in a new compound with stronger properties. Diclofenac and cysteamine administrated *in vivo* and *in vitro*, showed a weak inhibitory action on pro-inflammatory expression. However, the combination of these two molecules in a new compound, has been shown to suppress the cytotoxic action mediated by LPS-activated microglia, decreasing significantly the concentration of TNF-α, IL-6, cyclooxygenase (COX)-2 and inducible nitric oxide synthetase (iNOS) [28]. A paradol derivative compound, 6-paradol, has an important effect in reducing inflammation in the same pro-inflammatory molecules targeted by the cysteamine-diclofenac conjugation [29]. Nitric oxide (NO) is a major pro-inflammatory molecule, which is associated with cytotoxicity at high concentrations. Therefore, the interruption of the NO pathway seems to be a potential target for HI treatment [28-30].

During neuronal injury, microglia responds to the toxic EMC with ATP exocytosis. ATP binds the purinergic microglial ionotropic receptors (P2X4, P2X7 and P2X12) that activate cation selective membrane channels, which in turn contribute to the different cell specific microglial mechanisms involved in their inflammatory phenotype [31]. Moreover, the activation of the P2Y metabotropic receptors is apparently related to cytoskeletal changes that benefit cell motility [32]. Some studies positioned the interruption of these channels expression in microglia as a possible target to minimize tissue damage [33, 34]. In other cases, the stimulation of channels activation, such as the α7 subunit of nicotinic acetylcholine receptor (AchR), plays an important role in neuroprotection, mediating a reduction of oxidative stress and inflammation *in vivo* [35-38].

3. TREATMENTS IN HYPOXIC-ISCHEMIC BRAIN INJURY TARGETING ASTROCYTES

Hypoxic preconditioning (HP) of nervous tissue in *in vivo* models has been a useful tool in the study of HI mechanisms. This technique consists of using a preceding mild HI insult, as a way of inducing ischemic tolerance and protecting brain tissue against future HI insults [39, 40]. For instance, the hypoxia inducible factor

(HIF)-1α is one of the activated genes in this technique, and constitutes a well-known mediator of ischemic tolerance. Astrocytes also play an essential role in the induction of brain ischemic tolerance, suggesting that underlying mechanisms of HP are not exclusively neuron-based. Recent findings have demonstrated that HP may cause activation of astrocytes, without producing any noticeable brain damage. P2X7 receptors are significantly up-regulated in activated astrocytes, and astrocyte-mediated ischemic tolerance is absent in P2X7 receptor knock-out mice. Therefore, P2X7 up-regulation constitutes a key underlying mechanism. In addition, HIF-1α is apparently involved in P2X7-mediated ischemic tolerance [40]. Another set of receptors, the P2Y metabotropic family, seems to be involved in the activation of different proteins needed for astrocytic proliferation [41]. Blocking receptors of EphA4/ Ephrin-A3 in astrocytes is another possible target due to the consequent reduction of glutamate receptors involved in glutamate toxicity [42, 43].

Due to their high specificity in gene modulation and the immediate effect of their regulatory activity, miRNAs seem to constitute a promising treatment. Several astrocytic targets for miRNAs have been studied. One of the four main families is the miR-181, which acts as a regulator of immune cell development. When the level of expression is low, there is an increase in pro-inflammatory molecules. The second family, miR-29, has been described as an apoptosis protector during neuronal migration. The miR-497 family targets two anti-apoptotic genes (BCL-2 and BCL-w), and therefore inhibits programmed cell death. Finally, miR-146a is involved in the regulation of the immune response in astrocytes and the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) pathway [44-48].

Stem cells (SC) represent a promising tool for studying a specific-patient target. Its proliferation as a replacement treatment might permit tissue regeneration without cell type limitations. Even more, human stem cell-derived astrocytes enable the study of neu-roprotective pathways mediated by the nuclear erythroid-2-related factor 2 (Nrf2), which exerts antioxidant properties [49]. Nrf2 has demonstrated its neuroprotective benefit *in vitro* through downstream expression of genes that promote detoxification, anti-oxidant and anti-inflammatory actions [50].

4. OTHER TREATMENTS TARGETING GLIAL CELLS

The developing brain is very sensitive to oxidative stress. Reactive oxidants are responsible for the induction of damage from excitotoxicity and inflammatory processes [51]. Among ROS, NO is one of the most studied as a possible treatment target in HI [30, 52].

Glutamate is the main excitatory neurotransmitter in mammals. It is necesary for physiological neurotransmission but in excess it may trigger a pathophysiological response [53]. HI stimulates the release of glutamate from injured cells to the ECM, promoting a glutamate receptor-mediated excitotoxicity process in the hypoxic tissue, with a consequent generation of ROS and NO reactive oxidants. Under normal conditions, glutamate receptors mediate a transient oligodendrocyte Ca^{+2} permeability. During an ischemic insult, higher loads of intracellular Ca^{+2} influx trigger cell mechanisms that may produce cell death. This situation can be reverted by the blockade of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) / kainite (KARs) receptors in oligodendrocytes [54]. Deregulation of zinc ($Zn^{+2)}$ homeostasis might also trigger excitoxicity by AMPA activation [55].

During HI, ROS activate Jun amino-terminal kinases (JNK) and NF-kB pathways, which determine mitochondrial alterations. On the other hand, NO increases cell damage through the alteration of arginine metabolism [53]. Moreover, NO increases the generation of hydrogen peroxide (H_2O_2) and free iron in HI neonatal brain injury [30, 56]. The inhibition of NO and iron scaveng-ing by the iron chelator deferoxamine may determine a decrease in the H_2O_2 ratio and a subsequent diminution of the infarct area [33]. Experiments with catalpol, an irinoid glycoside, also demonstrated this compound might be neuroprotective by ROS scavenging via glutathione peroxidase (GSH-PX), attenuating neuronal apoptosis, total nitric oxide synthase (TNOS) and iNOS activity. Nevertheless, the exact mechanisms involved in these processes are still unknown [54, 57-59].

Neonatal exposition to HI determines an autophagic response as a consequence of oxidative stress. In *in vivo* experiments, the pharmacologic inhibition of ROS pathway derived from nicotinamide adenine dinucleotide phosphate (NADPH) by apocynin or gp91ds-tat is capable of inhibiting the autophagic response [60]. As a physiological reaction, there is an incresase in glutathione (GSH) levels during HI, which contributes to palliate oxidative stress [61]. Thus, the up-regulation of its regeneration and NADPH ratio may present a beneficial effect, as it has been described in Parkinson Disease where sulforedoxins (Srxn1) were associated with a neuroprotective effect through the down-regulation of mitochondrial pathways leading to apoptosis [62-64]. Neuroprotective properties have also been attributed to the caffeoylquinic acid derivative, 1,5-dicaffeoylquinic acid, by the activation of Nrf2 in the GSH pathway [65]. As another example, melatonin has also been reported to have similar effects via scavenging of ROS as well as by promoting myelination after an injury [66].

Nicotinic acetylcholine receptors (nAChRs) are a family of ligand-gated ion channels, which are involved in inflammation. Targeting α7 nAChR activation protects tissue against glutamate and OGD (among other harmful stimuli) in HI both *in vitro* and *in vivo* [35-38, 67]. Other suggested potential targets for modulation of inflammatory processes in retinal ischemia are baicalein [68] and the Wnt/β-catenin pathway [69].

Several studies examined the endocannabinoid system (ECS) as a target to induce neuroprotection. Under normal conditions, glial cells constitutively express cannabinoid receptor type 1 (CB1) at high levels and cannabinoid receptor type 2 (CB2) at low levels. CB1 potentiates synaptic transmission in astrocytes and contributes to anti-inflammatory responses [70, 71]. Treatment with CB1 agonists is associated with higher oligodendrocytes survival in *in vivo* experiments [72]. Under pathological conditions, glial cells increase the expression of CB2 [70, 73]. This process is apparently protective against ischemia damage *in vivo* [74]. Furthermore, CB2 over-expression may determine a decrease in neuronal loss and oxidative stress in Alzheimer, as it has been observed in an experimental model [75]. Similarly, administration of O-1966, a CB2 agonist, mediates a reduction of infarct size in mice [74].

Fatty acid amide hydrolase (FAAH) is the main enzyme in ECS that degrades the endocannabinoid anandamide (AEA) and its congeners, the non-cannabimimmetic compounds palmitoylethanolamide (PEA) and oleoylethanolamide (OEA) [76, 77]. The inhibition of FAAH determines an anti-inflammatory profile, through the increment of AEA, PEA and OEA in plasma cells [76]. AEA shows a neuroprotective activity in the acutely damaged brain because of OGD through the regulation of extracellular signal–regulated kinases (ERK) 1/2 and JNK pathways [70, 78] and the modification in pro-inflammatory levels of NO, IL-2 and TNF-α [79]. PEA and OEA have also demonstrated to exert neuroprotective functions via activation of the peroxisome proliferator-activated receptor alpha (PPAR-α) [80].

As we have stated, HI produces a disruption in ECM homeostasis causing neuronal injury or death. This mechanism may be attenuated by δ-opioid receptors (DOR), which inhibit sodium ($Na^{+)}$ influx through the cellular membrane. Experimental evidence has documented neuronal survival as a consequence of this action [81]. Treatments with compounds of DOR agonists, such as [D-Ala2, D-Leu5] enkephalin (DADLE), reduce the damage response through what has been interpreted as a post-conditioning mechanism against neuron loss in HI [82].

In mammals, the Central Nervous System (CNS) and the endocrine system (ES) have a close relation. Therefore, injuries determine changes in both systems. The female hormone estradiol exerts a protective action against neuroinflammation, modulating the release of pro-inflammatory cytokines and chemokines by glial cells. However, the use of estrogens as treatment for CNS injuries is controversial because it has been associated with cancer development. On the contrary, selective estrogen receptor modulators (SERMs) can act as estrogen agonists or antagonists [83, 84]. Among the most studied SERMs for HI, 17β-estradiol exerts several effects: (a) reduction of reactive gliosis, attenuating ischemic injury in neurons and decreasing oxidative stress and the release of pro-inflammatory molecules [84-87]; (b) prevention from cell death and mitochondrial dysfunction [88]; (c) release of neurotrophic factors which protect neurons and oligodendrocytes; (d) reduction of edema by increasing the expression of aquaporin in astrocytes [89]; (e) up-regulation of glutamate transport and [84, 90], (f) synthesis of progesterone by the reduction of astrogliosis [89, 91]. Raloxifene and tamoxifen are also capable of promoting neuronal survival [92] and reducing astrogliosis in groups of ovariectomized rats at 2, 8 and 18 months of age [93]. Furthermore, raloxifene also induces a strong neuroprotective response by suppressing C-C motif chemokine ligand 20 (CCL20) expression and NF-kB pathway in reactive astrocytes [94].

Hypothermia exerts a protective effect against neuronal death, therefore coining the idea that temperature is involved in mechanisms that mediate tissue ischemia [95]. The main pathway affected in temperature treatments is glutamate excitotoxicity [96]. During HI, glutamate transporter (GLT)-1 decreases in astrocytes membrane, preventing the ECM clearance of the released glutamate. Exposition of astroglia and neuron cultures to hypothermic conditions (33°C) facilitates the expression of this receptor. The astrocyte-neuron interaction seems to be necessary for a proper GLT-1 expression [96, 97]. Hypothermia may prevent neuroinflammation by suppressing the release of TNF-α, IL-10 and NO at early stages and up-regulating glutamate signaling cascades [97, 98]. Treatment with hypothermic condition combined with insulin like growth factor (IGF)-1 is apparently beneficial for oligodendrocytes survival by promoting myelination [99]. However, myelination has been also observed in *in vivo* and *in vitro* models exposed to low temperatures, thus positioning hypothermia as an effective treatment by itself to regulate cell metabolism during HI [100-102].

Vascular endothelial growth factor (VEGF) is secreted by cells exposed to hypoxia and it has been reported to stimulate the sprouting of new vessels from the existing ones (angiogenesis) in injured brain tissue [103]. This phenomenon is achieved by the activation of tyrosine kinase vascular endothelial growth factor receptor (VEGFR)-1 and VEGFR-2 in endothelial cells [103]. Several animal experiments that studied brain response to exogenous VEGF administration after stroke have shown better outcomes for treated groups. Nevertheless, VEGF therapy in humans is still under study [104].

CONCLUDING REMARKS

For decades, the CNS was considered to have a limited capacity of regeneration. However, it gradually became clear that brain injury may trigger several repair processes, including neurogenesis, axonal sprouting, synaptogenesis, angiogenesis, oligodendrogenesis and remyelination [13]. The stimulation of any of these endogenous processes might be neuroprotective. For instance, the neuropeptide PACAP exerts neuroprotection by promoting the neoangiogenic process in HI [20]. In addition, this peptide facilitates the expression of M2a markers [20], which also represent a neuroprotective pathway as it has been demonstrated in several studies [14-17, 21, 27]. Molecules that inhibit the expression of M1/M2b markers constitute complimentary neuroprotective agents [18, 19, 22, 28], suggesting that subtle modulation of microglial phenotype represents a key target for neuroprotection [13, 15]. Microglia presents the ability to acquire diverse activation states or phenotypes, mediating different facets of neuroinflammation (cytotoxicity, repair, regeneration and/or immunosuppression) [15]. This might be the reason for the apparent discrepant effects of broad microglial down-regulation [14]. Further research should contribute to a comprehensive study of classical and novel microglial phenotypic markers *in vitro* [15].

Regulation of astrocytic glutamate transport [42, 43] and/or immune response in astrocytes [44-48] represent alternative neuroprotective targets for HI. HP might also be neuroprotective by inducing astrocyte-mediated ischemic tolerance. The underlying mechanisms of this process might be P2X7 up-regulation and HIF-1α activation [40]. In addition, human stem cell-derived astrocytes represent a useful tool for studying several genes, such as the anti-oxidant and anti-inflammatory Nrf2 [49, 50]. Recent studies have reported that the blockade of AMPA / KARs receptors in oligodendrocytes might revert excitotoxicity and cell death in HI [54, 55], suggesting that these glial cells may also constitute promising neuroprotective targets.

Finally, some neuroprotective strategies are particularly promising because of their capability of targeting several glial cells. For instance, therapeutic hypothermia, which is already applied in humans, may promote oligodendrocytes survival and myelination [99], and facilitate the expression of GLT-1 in the astrocytes membrane, contributing to diminish excitoxicity in HI [96, 97]. Endocannabinoids may also contribute to oligodendrocytes survival in HI [72], besides regulating both microglial response against injury and the activity of astrocytic hemichannels [70], and potentiating synaptic transmission through stimulation of astrocytes [71]. SERMs also exert multiple effects on glial cells in HI: reduction of reactive gliosis [84-87], release of neurotrophic factors in order to protect oligodendrocytes, and augmentation in the expression of aquaporin in astrocytes, with a consequent reduction of edema [89]. Future studies should contribute to study novel neuroprotective strategies for HI targeting glial cells, and to determine the most effective treatment for this serious disabling condition.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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