Chapter

Microglial Plasticity Contributes to Recovery of Bone Marrow Mononuclear Cells during Experimental Stroke

Edna Cristina S. Franco, Marcelo Marques Cardoso, Celice Cordeiro de Souza, Michelle Castro da Silva, Carolina Ramos dos Santos and Walace Gomes-Leal

Abstract

Brain stroke is an acute neural disorder characterized by obstruction (ischemic) or rupture (hemorrhagic) of blood vessels causing neural damage and subsequent functional impairment. Its pathophysiology is complex and involves a multitude of pathological events including energetic collapse, excitotoxicity, oxidative stress, metabolic acidosis, cell death and neuroinflammation. Despite its clinical importance, there is no effective pharmacological therapies available to diminish secondary damage avowing functional deficits. Considering the failure of pharmacological approaches for stroke, cell therapy came as promising alternative. Different cell types have been investigated in different experimental models with promising results. An important issue regarding the transplantation of stem cells into the damaged CNS tissue is how the pathological environment influences the transplanted cells. It has been established that an exacerbated inflammation in the pathological environment is detrimental to the survival of the transplanted stem cells. This prompted us to develop an experimental strategy to improve the therapeutic actions of bone marrow mononuclear cells (BMMCs) transplanted into the acute phase of brain stroke by modulating microglial activation with minocycline. In this chapter, we first review the basic pathophysiology of ischemic stroke with emphasis on the role of microglia to the pathological outcome. We then review the experimental approach of modulating microglia activation in order to enhance therapeutic actions of BMMCS for experimental stroke. We suggest that such an approach may be applied as an adjuvant therapy to control excessive neuroinflammation in the pathological environment allowing acute transplants and improving therapeutic actions of different kind of stem cells.

Keywords: stroke, stem cells, cell therapy, minocycline, neuroinflammation, neuroprotection

1. Introduction

The central nervous system (CNS) is affected by acute and chronic neural disorders. In acute neural disorders, like stroke, spinal cord injury (SCI) and brain

trauma, neuronal and glial loss happens quickly with inexorable cell loss and functional impairment [1–5]. In chronic neurodegenerative diseases, including Parkinson's, Huntington's, Alzheimer's diseases and Amyotrophic Lateral Sclerosis (ALS) progressive cell loss occurs over decades with inexorable functional loss and sensory-motor and/or cognitive declines [1, 5].

Stroke is an acute neural disorder and leading cause of death and functional impairment worldwide [1, 5]. Recent epidemiological data point out that occurred 1.12 million cases of stroke in 2017 in European Union countries, with 9.53 million survivors, approximately half a million deaths, and 7 million people with permanent sequelae [6]. According to this study there will be about 40,000 new stroke cases in Europe by 2047, and an increase of about 27% in the number of people living with sequelae of some type of stroke [6].

Similar data were published by the American Heart Association (AHA), which showed that about 7 million Americans over the age of 20 had strokes between 2013 and 2016 with a prevalence that increases with advancing age in both sexes [7]. The same study shows that more than 3.4 million Americans over the age of 20 will have a stroke by 2030, an increase of 20.5% in prevalence compared to 2012.

Stroke is a vascular disorder characterized by obstruction (ischemic) or rupture of blood vessels (hemorrhagic). Following this primary pathological event, further outcomes are diverse and characterized by a multitude of factors, excitotoxicity, oxidative stress, metabolic acidosis, periinfarct depolarization, apoptosis and uncontrolled neuroinflammation, which contributes to cell death and functional impairment in both experimental animals and humans [1–5, 8–10].

There are no effective pharmacological treatment or cell therapy approved for stroke [2, 5, 8]. Approved clinical therapy is restricted to thrombolysis by using the recombinant tissue plasminogen activator (tPA) for ischemic stroke, which is limited by its narrow therapeutic window [11–13]. In the clinical practice, people with stroke arrive at the hospital usually several hours after the onset of symptoms, outside the therapeutic window for the use of thrombolytic agents (alteplase), mainly in low income countries with a limited public health system.

Numerous experimental studies have shown the inefficacy of several tested neuroprotective agents, including glutamatergic antagonists, calcium antagonists, antioxidants, magnesium for inducing neuroprotection in animals [14, 15]. This fact raised considerable skepticism regarding the possibility of finding an effective neuroprotective agent for neurological human diseases [14, 15].

Considering the limitations of pharmacological approaches, it is believed that cell therapy is considered a promising therapeutic approach for inducing neuroprotection, cell replacement and functional improvement following both acute and chronic neural disorders [16–20]. This is confirmed by several studies using experimental models of neural disorders, including stroke [21].

Different types of stem cells from different sources (umbilical cord blood cells, bone marrow stem cells, neural stem cells, induced pluripotent stem cells) have been tested in different experimental stroke models rendering neuroprotection and functional impairment [16–20].

Although embryonic stem cell transplantation is considered a promising future therapeutic approach for neural disorders, technical and ethical-legal restrictions have hindered its clinical use [16–20]. Stem/progenitor cells derived from adult sources, including bone marrow derived stem cells (BMSCs), have been transplanted in both acute and subacute phase after stroke affording considerable degree of neuroprotection [22–26].

An important issue regarding the transplantation of stem cells into the damaged CNS tissue is how the pathological environment influences the transplanted cells. In disorders like stroke and trauma, an intense inflammatory response is

elicited with both cellular and humoral components belonging to innate and adaptive immune systems.

It has been previously shown that bone marrow mononuclear cells (BMMCs) transplanted into the intact adult rodent brain are rejected by components of the CNS inflammatory response [27]. In addition, it has been shown that brain macrophages impair survival and integration of embryonic stem cells transplanted into the acute phase of brain trauma [28]. This prompted us to develop an experimental strategy to improve the therapeutic actions of BMMCs transplanted into the acute phase of brain stroke by modulating microglial activation with minocycline [22, 23]. Using this approach, we were successful in improving therapeutic actions of BMMC transplanted into both ischemic cortex [22] and striatum {Cardoso, 2013 #27 in adult rats.

In this chapter, we will first review the basic pathophysiology of ischemic stroke with emphasis on the role of microglia to the pathological outcome. We then review the experimental approach of modulating microglia activation in order to enhance therapeutic actions of BMMCS for experimental stroke. We suggest that such an approach may be applied as an adjuvant therapy to control excessive neuroinflammation in the pathological environment allowing acute transplants and improving therapeutic actions of different kind of stem cells.

2. Stroke pathophysiology

2.1 Overview

The pathophysiological events of stroke are extremely complex and involve different mechanisms [1–5, 8]. Following metabolic collapse in the brain function, ischemic injury results in a complex sequence of pathophysiological events that include metabolic acidosis, excitotoxicity, peri-infarction depolarization, oxidative stress, programmed cell death and neuroinflammation [1–5, 8].

Several events are related to cell death after stroke. The interruption of blood flow generates an energy collapse in the cells, followed by ionic imbalance, with intense Ca^{2+} influx, exacerbated release of glutamate and oxidative/nitrosative stress. All of these events are correlated and lead to cell death, triggering an intense inflammatory response in the ischemic environment, which has a dubious role, as it can contribute to both tissue repair and to intensify the injury [9, 10, 29].

The brain tissue requires a high energy demand for its optimal functioning, being responsible for 20% of all the body's oxygen consumption. In addition to the energy needed to maintain cellular homeostasis, the synaptic transmission process requires a large amount of ATP, which is obtained from the oxidation of glucose in the mitochondria oxidative phosphorylation chain. Therefore, the glucose and oxygen reduction in the ischemic environment has severe deleterious effects on the nervous tissue [30].

After ischemia, the alteration of several physiological, biochemical, molecular and genetic mechanisms results in cell death and impaired neuronal function (**Figure 1**). In the ischemic core, cell death occurs predominantly from necrosis minutes after ischemia. Initially, the interruption of blood supply leads to a reduction in oxygen and glucose reaching neurons, which compromises the process of oxidative phosphorylation in mitochondria and drastically reduces the ATP production [1, 5, 30].

This reduction impairs the functioning of ATP-dependent ion pumps, such as the Na+/K+ pump, causing an imbalance in the ionic potential and generating the cell membrane depolarization. In addition, impaired mitochondrial function

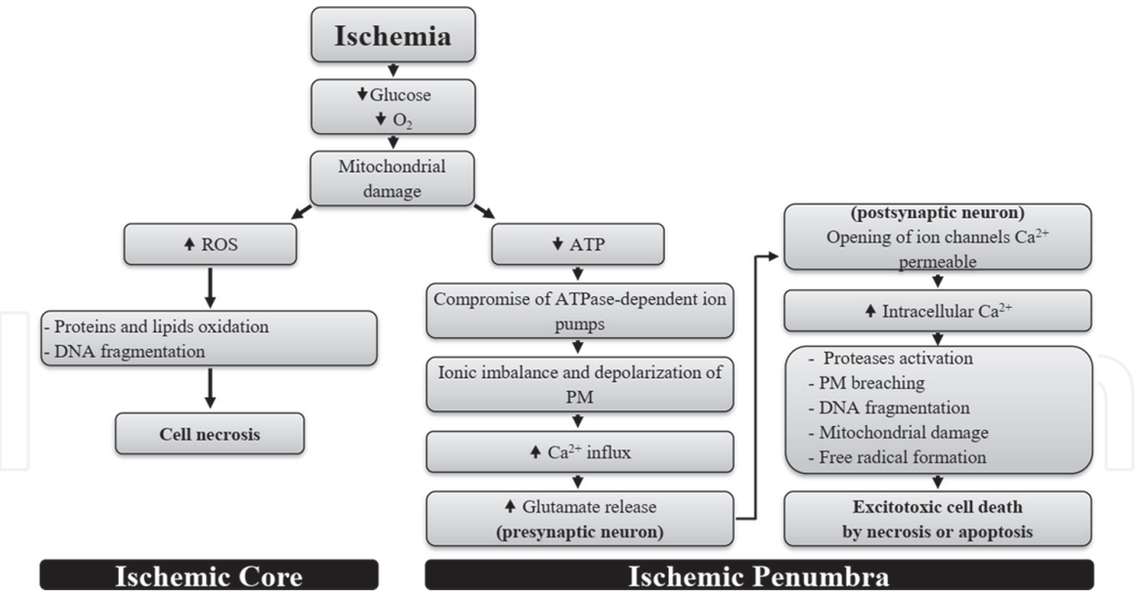

Figure 1.
Overview of stroke pathophysiology. The primary pathological event is abrupt reduction of blood flow resulting in oxygen level shortage, mithochondrial damage and ATP depletion. Metabolical colapse induce formation of reactive oxygen species (ROS) leading to protein and lipid oxidation and cell death. Control f excitatory neurotransmitter levels is lost leading to increased calcium intracelular levels, pathological activation of lipases, proteases and further cell death.

generates the production of superoxide radicals, reducing antioxidant activity and causing oxidative stress, which in turn results in the oxidation of proteins and lipids in the cell membrane and DNA fragmentation, ultimately leading to cell necrosis [30]. On the other hand, the events that lead to cell death in the penumbra area are more complex and can extend for weeks after ischemia [30–32].

In general, the oxygen and glucose reduction generates an imbalance in the ionic potential of the membrane, which causes the intense influx of Ca2+ and the release of glutamate, events that are interspersed in a positive feedback loop and lead to cell death due to excitotoxicity. Since, in this pathological environment in which the mechanisms of intracellular Ca2+ control are compromised, cell death programmed by apoptosis and/or total cell collapse may occur, leading to necrosis [33]. The main events triggered by the scarcity of glucose and oxygen that lead to cell death in the center and in the ischemic penumbra are described in **Figure 1**.

2.2 Cell death after stroke

Several mechanisms of cell death can be triggered after ischemia, including necrosis and apoptosis, which can occur interchangeably according to changes in the ischemic environment. Necrosis, predominant in the ischemic core, is characterize by the cytoplasm vacuolization, cell edema, plasma membrane rupture and pro-inflammatory cytokines release [30–33]. Apoptosis is strictly regulated, demands energy, being predominant in the ischemic penumbra area, and it is characterized by cell retraction, chromatin density and condensation increased nuclear membrane rupture and formation of the apoptotic bodies, but maintaining the membrane cellular integrity [33, 34].

Apoptosis occurs intrinsically, by mitochondrial signaling, or extrinsically, by cell death receptors stimulating, such as tumor necrosis factor α (TNF-α), TRAIL receptors (TNF-related apoptosis-inducing ligand) and FAS (CD95/APO1). In both processes, it is necessary to activate the cysteine-aspartate protease family proteins, called caspases [35]. This activation involves the Bcl-2 family proteins, which includes pro-apoptotic proteins (Bax and Bak) and anti-apoptotic proteins (Bid and

Bcl-2) acting at external mitochondrial membrane maintenance and Ca2+ regulation in the mitochondria and endoplasmic reticulum [33]. After stroke, neuronal death from apoptosis occurs primarily intrinsically due to mitochondrial damage and cytochrome c release in the cytosol [33, 35].

Another process present in stroke is the autophagy, a programmed cell death in which cell degradation is carried out by lysosomes in response to severe cell damage when the cell is submitted to environmental stress. This can occur in three ways: mediated by chaperones, microautophagy and macroautophagy, the most observed in stroke. In general, autophagy is blocked by activated mTOR (target of rapamycin) and induced by AMP-activated protein kinase (AMPK) and rapamycin that inhibits mTOR. The moderate autophagy activation is a beneficial and anti-apoptotic process, including the mitochondria removal from damaged cells. On the other hand, this process becomes deleterious and pro-apoptotic when intensified in the ischemic environment, being related to the inflammatory process [33].

Two mechanisms of cell death present in stroke are not fully described: necroptosis and pyroptosis. Necroptosis has characteristics similar to necrosis, such as cell edema, plasma membrane rupture and pro-inflammatory cytokines release, however, it is not a completely passive process and it is activated through the receptors of cell death, such as TNF-α, and inhibited by the necroptosis-inhibiting factor-1 (Nec-1) [35]. Pyroptosis is trigger by caspase-1, being characterized by DNA damage, plasma membrane rupture and pro-inflammatory factors release [36].

The different mechanisms of cell death that occur after stroke are correlate in a complex process. Although there is a predominance of certain types in the ischemic core and others in the ischemic penumbra, some pathways occur simultaneously in both area, playing a beneficial, harmful or dubious role. Thus, the results of the interaction between these mechanisms is directly relate to the inflammatory process after ischemia and will define the affected cells survival [33].

2.3 Neuroinflammation

Neuroinflammation is an important component of the pathophysiology of acute and chronic neural disorders [9, 10, 22, 37]. After stroke and trauma, an intense inflammatory response is initiated with both humoral and cellular components [9, 10, 23, 38–42].

The cellular components of neuroinflammation belong to both innate and adaptive immune systems [9, 10]. In experimental models of stroke [9, 10, 38, 41, 42] and trauma [40, 43], neutrophils are recruited from blood vessels to the lesion site, peaking at 24 h post-damage onset. In latter survival times, macrophages dominate the pathological environment peaking between 3 and 7 days after trauma [40, 43] or ischemia [23, 38, 41, 42] in adult rodents.

Macrophages are derived from both resident microglia and blood monocytes recruited from the blood stream [44, 45]. An intense microglial activation is observed in the first week after spinal cord trauma [40, 43] and experimental stroke in both cortex [22], striatum [9, 10, 23, 41, 42].

Microgliosis is accompanied by intense astrocytosis that differs in its temporal profile in different compartments of the CNS [39]. In our previous studies, we demonstrated that astrocytes are activated more quickly in the white matter (WM) than in gray matter (GM) after excitotoxic injury to the spinal cord [39].

The inflammatory response in the CNS has a dubious nature, contributing to events of tissue repair and regeneration, as well as contributing to the exacerbation of the injury process [9, 10]. This is most evident when considering the role of microglial/macrophage cells. It has been shown that microglia inhibition with

minocycline induces neuroprotection, decreases axonal loss and programmed cell death after traumatic injury [46–48] or ischemia [23, 49–51].

Considerable neuroprotection is obtained after blocking recruitment of hematogenous macrophages after experimental spinal cord trauma [52]. Some studies suggest that treatment with minocycline is safe and can benefit people in the acute phase of ischemic stroke [53–56]. This fact is even more relevant considering that minocycline, despite having pleiotropic effects, has an important action on microglial activity [23, 49–51] and that, in humans, microglial activation is an important component of neuroinflammatory events [57–59].

Despite the above data, it is known that microglial/macrophage cells can induce neuroprotection after trauma [60–63] or ischemia [64–67]. Recently, we suggested that this dubious action is influenced by the pathological environment and that ligands can activate different receptors in the microglial membrane, activating their harmful and/or protective actions [9, 10].

3. The dual role of microglia after stroke

Neuroinflammation is one of the main components of the pathophysiology of CNS diseases [9, 10, 68–70]. After the stroke, a complex range of humoral and cellular responses occurs, with different consequences for the neuropathological development [9, 10, 68–70]. Neutrophils, lymphocytes and macrophages are recruited to the lesion site, in addition to the concomitant activation of microglia and astrocytes [9, 10]. Concomitantly, an intricate network of humoral responses is developed, characterized by the release of several pro and anti-inflammatory cytokines, with specific roles, depending on the moment after the injury [71].

Neuroinflammation has beneficial and harmful effects after stroke and other neural disorders [9, 10]. The main component of the inflammatory response that occurs after acute neural disorders are the microglia cells, the macrophages residing in the CNS, myeloid cells derived from progenitors of the Yolk sac embryonic structure [72, 73].

Microglial cells are components of the innate immune system that patrol the CNS in normal situations using stochastic movements of its thin and long branches in order to protect it from harmful events [74, 75], movements that depend on endogenous ATP [76].

During development, these cells phagocytose in excess synapses, contributing to the maturation of neural circuits, an action that depends on interleukin 33 released by astrocytes [77]. Like cells of the innate immune system, microglial cells are the first line of defense of the CNS against viruses, bacteria and other pathogens, removing them during phagocytosis infection or by releasing powerful pro-inflammatory agents, nitric oxide, proteases, free radicals in addition to other lytic agents [78–80].

It is well established that after stroke, trauma and other diseases of the CNS, microglial cells have a dubious action, contributing to exacerbation of the injury and repair [9, 10]. The inhibition of microglial activation with tetracycline minocycline decreases the infarction area neuroinflammation, both in the cortex and in the striatum, after experimental occlusion of the middle cerebral artery (MCAO) [51]. Modulation of microglial activation improves the therapeutic effects of bone marrow mononuclear cells, transplanted intravenously after focal ischemic lesion in the cortex [23] or striatum [23]. Paradoxically, the presence of microglial cells of the BV2 cortical lineage in organotypic culture reduces neuron death after glucose and oxygen deprivation [65]. In this same experimental model, microglial cells are highly beneficial for phagocytosing polymorphonuclear cells [66]. After MCAO in

mice, ablation of microglial proliferation worsens the inflammatory process and induces higher levels of programmed cell death after focal ischemia [67].

We have proposed that "friendly fire hypothesis" to explain the dual role of microglia after stroke and other neural disorders [9–10]. According to the friendly fire hypothesis microglia used their biochemical machinery normally used to fight infections during sterile inflammation in neural disorders like stroke, trauma and even over the course of chronic neurodegenerative diseases [9, 10]. According to this notion danger signals released by stressed, damaged or dying cells might bind the same pattern recognition receptors (PRRs), or even different receptors, normally activated by pathogen-associated molecular patterns (PAMPs) present in the microglia cell membrane culminating in secondary cell damage [9, 10]. This is supported by our preliminary findings showing that in the presence of bacterial infection ischemic damage is larger than in the absence of infection [9].

4. Stem cell therapy for stroke

Currently, there are no effective pharmacological treatments for stroke [14, 15]. Conventional therapy is restricted to thrombolysis by using the recombinant tissue plasminogen activator (tPA) [11, 13]. Few patients with ischemic stroke are benefited from thrombolytic therapy, mainly because of its narrow therapeutic window [11, 13]. In clinical practice, people with stroke arrive at the hospital, usually several hours after the onset of symptoms, outside the therapeutic window for the use of thrombolytic agents (alteplase), mainly in low-income countries with deficient the health systems.

Numerous experimental studies have shown promising results of experimental drugs, including glutamatergic antagonists, calcium antagonists, antioxidants, magnesium and many others as neuroprotective agents in experimental animals [14]. However, despite the experimental success of these drugs, their application as neuroprotective agents in humans has been totally ineffective [14, 15]. This fact gave rise to great skepticism regarding the possibility of finding an effective neuroprotective agent for neurological diseases in humans [2, 81].

Considering the failure of translational research for achieving an effective neuroprotective agent, cell therapy came up as promising approach for inducing neuroprotection, cell replacement and functional improvement after acute and chronic neural disorders, depending on cell type [8, 17, 21, 26].

Different types of stem cells from different sources (umbilical cord blood cells, bone marrow stem cells, mesenchymal stem cells, neural stem cells, immortalized cell lines, induced pluripotent stem cells (IPSCs) were used in experimental stroke models to afford neuroprotection, cell replacement and subsequent functional recovery [8, 17, 21, 26]. Although embryonic stem cell transplantation is considered a promising treatment for neurodegenerative diseases, technical and ethical-legal restrictions have hindered its clinical use [8, 17, 21, 26].

An alternative source of cell therapy involves the use of adult progenitor cells derived from bone marrow, including mesenchymal stem cells or their faction – the bone marrow mononuclear cells [22–25, 82]. Both mesenchymal and mononuclear bone marrow cells (BMMCs) are highly anti-inflammatory and neuroprotective in experimental models of stroke [22–25, 82, 83].

5. Bone marrow mononuclear cells and stroke

BMMCs are adult stem cells that can also be divided, basically, into two types: hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs), originating

hematopoietic and mesenchymal lineages, respectively [84, 85]. Both groups represent cellular sources that can be easily obtained and isolated from bone marrow aspirates, in addition to be an autologous source for therapies [22–24].

While HSCs originate in blood cells, MSCs can differentiate into various cell types of mesenchymal origin, including osteocytes, chondrocytes, adipocytes and myocytes [86]. In addition, the latter type of stem cells has an important supporting role (stroma) for HSCs in the bone marrow [86]. The mechanisms of action of HSCs and MSCs when they come into contact with injured tissue are not yet fully understood. It is currently suggested that these cell types can differentiate into glial and even neural lines [86]. Some studies mention that they can form glial and neuronal cells from various inducing mechanisms, such as chemical, genetic and physiological manipulations [86].

Other studies emphasize the trophic functions of these two cell types. It is known that HSCs can secrete neurotrophic growth factors such as angiopoietin-1, which has an angiogenic function [87]. There are also reports that MSCs may have an immunosuppressive function, which can reduce the acute inflammatory response, as well as reduce the reactivity of activated microglia/macrophages and astrocytes [22, 23, 88]. In addition, MSCs can promote axonal regeneration or positively influence functional plasticity through the modulation of an inflammatory medium that allows axonal growth [89]. They can synthesize some neurotrophic cytokines that stimulate neural growth, including BDNF (brain-derived neurotrophic factor), NGF (neural growth factor and VEGF (vascular endothelial growth factor).

Mesenchymal stem cells and bone marrow mononuclear cells promote improvement of functional deficits in animal models of stroke when administered intravenously, intra-arterially and intra-cerebrally [22, 23, 88, 90, 91], although most the injected cells to non-neural organs, mainly spleen [92–96].

Evidence from preclinical studies indicates that the main mechanism of cell therapy does not correspond to cell replacement directly, but to the trophic, anti-inflammatory and immunomodulatory effects that occur in the acute phase and that persist until the transplanted cells die [22, 23, 88, 90, 91].

The route of administration of these cells can be determined by choosing the time for transplantation, according to the therapeutic purpose. For example, intravascular transplants may require earlier delivery as the cells use acute inflammatory signals to reach the injured area [92–96]. On the other hand, intra-parenchymal injection could be beneficial in a later administration in order to favor the survival of these cells since the acute inflammatory environment causes damage to the transplanted cells [92–96].

6. Minocycline and neuroprotective actions

Minocycline is a second generation semi-synthetic tetracycline, commonly used as an antibiotic, but which has a considerable anti-inflammatory and neuroprotective effect in experimental models of stroke and trauma [46, 50, 51, 97, 98]. This has been first demonstrated by Yrjanheikki and colleagues using experimental models of both global [50] and focal [51] ischemia. Following MCAO in rats, minocycline treatment induced a 65% decrease in the cortical infarct area and a 45% reduction in the primary ischemic area [51]. The authors attributed these effects mainly to inhibition of microglial activation. From these initial studies, several other studies have shown the neuroprotective effects of minocycline after ischemia and several other diseases in the CNS [98–100].

The treatment of rodents submitted to acute SCI with minocycline reduced secondary oligodendrocyte degeneration, increased axonal regeneration and

modulated cell death due to apoptosis [46]. Minocycline treatment increases endogenous neurogenesis in the adult brain after experimental stroke [101]. We have shown that minocycline protects striatal white matter following acute excitotoxic brain injury [102] and that modulation of microglial activation enhances the therapeutic actions of BMMCs into the acute phase of experimental stroke [22, 23].

Part of the success of minocycline may be associated with the chemical structure of this drug [103]. The molecular organization of minocycline allows it to be up to 5 times more lipophilic than the other tetracyclines [99, 103]. This facilitates the molecule to easy crosses the blood brain barrier (BBB) [99, 103]. In addition, minocycline is quickly and easily absorbed, well tolerated in high doses and has an average half-life superior to other drugs with similar biological action [56, 99, 103]. These characteristics make minocycline a therapeutic promise for several CNS diseases, including ischemic stroke {Yong, 2004 #917. These characteristics make minocycline a therapeutic promise for several CNS diseases, including ischemic stroke {Yong, 2004 #917},]104].

Although the mechanism of action of minocycline in ischemic stroke is not fully elucidated, the drug appears to exert influence on different points of the inflammatory response and apoptosis [56, 99, 104]. Minocycline blocks leukocyte activation and infiltration, attenuates the permeability of BBB, inhibits matrix metalloproteinase (MMPs), induced nitric oxide enzyme (iNOS), modulates inflammatory mediators, reduces microglial activation and proliferation [56, 99, 104, 105]. In addition, it has been reported that minocycline inhibits microglial activation by a specific action in a cytokine-like mediator called high-mobility group box-1 (HMGB-1) [106].

In the apoptotic cascade, minocycline can play a role on the extracellular availability of death ligands and/or in the presence of neurotrophic factors in the extracellular medium that activate survival receptors in the cell [107–109]. Intracellularly, the main target of minocycline is the mitochondria. In this organelle, the drug stabilizes the mitochondrial membrane and prevents the release of the enzyme cytochrome-c and downstream caspase-3 activation [107–110].

7. Modulation of microglia activation with minocycline to enhance neuroprotection after BMMC transplants

There is an issue on what is the best time window to transplant stem/progenitor cells after acute neural disorders as the intense inflammatory present in the pathological environment might impair survival of the transplanted cells [27–28]. It has been shown that an exacerbated immune/inflammatory response may impair survival of stem cells transplanted in both normal [27] and pathological tissue [28]. This has been observed in non-neural tissue, as in the case for transplants of exogenous stem cells for myocardial repair [111].

Recent studies using a neuronal relay approach for spinal cord injury (SCI) have considered possible detrimental effects of inflammatory response on fetal [112], embryonic [113] and even induced-pluripotent stem cells (IPSCs) [114]. In this experimental paradigm authors transplant the stem/progenitor cells only 10 days after experimental trauma to avoid the detrimental effects of inflammatory reaction on the transplanted neural progenitor cells [112–118].

It has been confirmed that uncontrolled activated microglia may be detrimental contributing to bystander neuronal damage after stroke [9, 10]. We raised the hypothesis that modulation of microglial activation in the ischemic environment would enhance the therapeutic effects of BMMCs transplanted into the acute phase of both cortical [22] and striatal [23] stroke. We then transplanted BMMCs into

the acute phase of stroke in adult rats and concomitantly treated ischemic rats with minocycline during six days after BMMC transplants at 24 h from stroke induction [22–23]. We compared ischemic animals concomitantly treated with BMMCs and minocycline with animals treated with minocycline or BMMCs [22–23].

The results have shown that concomitant treatment of ischemic animals with BMMCs and minocycline afforded better neuroprotection and functional recovery (**Figures 2–3**) than single treatment with BMMCs or minocyline alone [22–23]. In this experimental paradigm, modulation of microglial activation with minocycline into the acute phase stroke improved the therapeutic actions of both BMMCs and minocycline indicating a therapeutic synergism. The results also suggest that exacerbated microglial activation may impair the therapeutic actions of stem cells transplanted into the acute phase of stroke [22–23].

We further confirmed the suitability of both BMMCs and minocycline as neuroprotective agents using an intracerebral route of transplantation in an experimental models of striatal stroke [24]. We have shown that the direct brain injection of BMMCs into the acute phase of striatal stroke induces better neuroprotection and functional recovery than the intravenous route, although this experimental approach is less invasive the surgical intra-striatal injection [22–24]. In the same study, we have obtained very important information on the peculiarities of minocycline and BMMCs as neuroprotective agents [24]. Both BMMCs and minocycline reduced the number of ED1+ cells, but BMMCs were more effective in reducing it. BMMCs also induced a more pronounced reduction in the number of apoptotic cells (active caspase+ cells) than minocycline. Both treatments were equally effect in reducing neuronal loss [24].

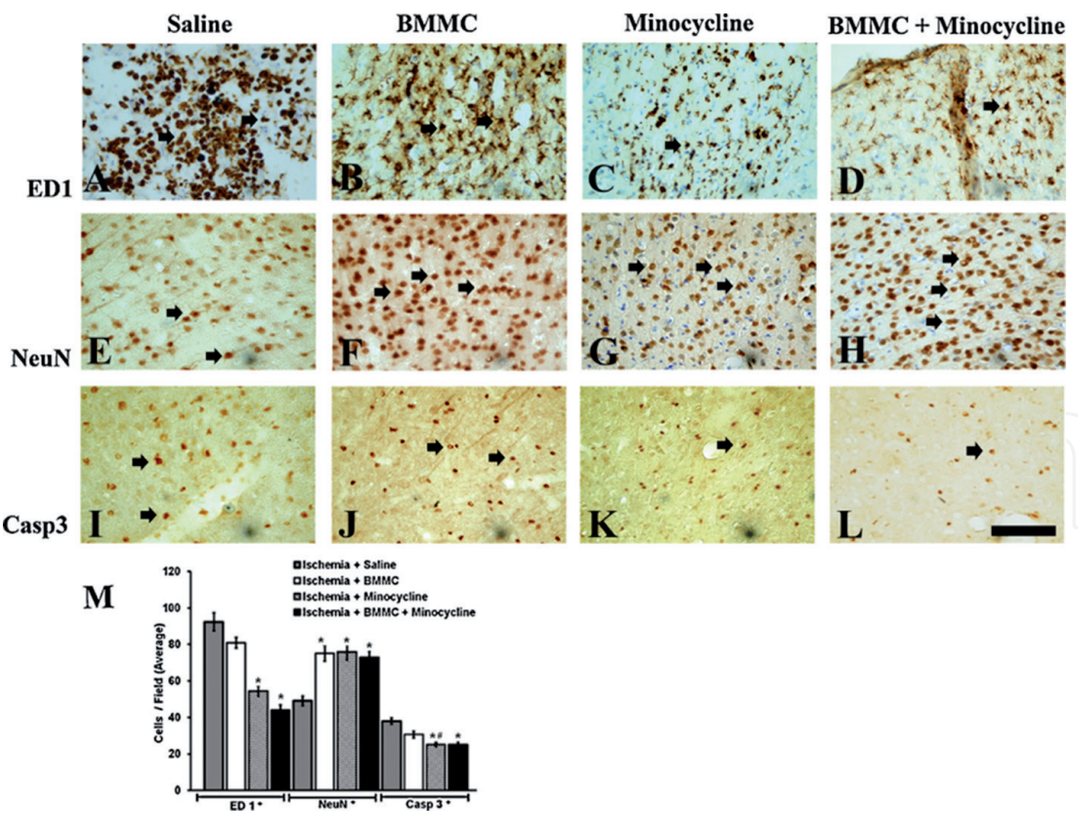

Figure 2.
Modulation of microglia activation with minocycline enhances therapeutic actions of BMMCs transplanted into the acute phase of cortical stroke. Concomitant treatment BMMC/minocycline (D, H, L) reduces the number of activated microglial (ED1+), apoptotic cells (caspas-3+) and increases the number of adult neurons (NeuN+) compared to saline (A, E, I) minocycline (C, G, K), BMMC (B, F, J) at 7 days post-injury. (P < 0.05, ANOVA-Bonferroni, as compared to vehicle or other groups#). Sections B, C, E and F were counterstained with cresyl violet. Arrows indicate immunolabeled cells. Scale bar: 100 m. From reference [22].*

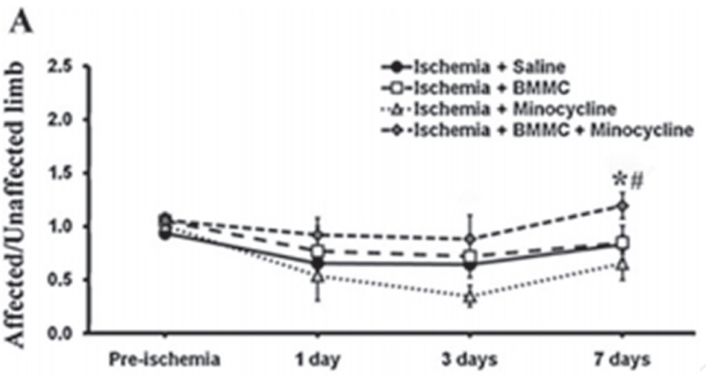

Figure 3.
Concomitant minocycline/BMMC treatment enhances the functional recovery induced by BMMCs into the acute phase of cortical stroke. BMMC-minocycline-treated animals showed better performance in the modified sticky-tape test than animals treated with BMMC or minocycline alone at 7 days post-injury ($P < 0.05$, ANOVA-Bonferroni, as compared to vehicle control animals). From reference [22].

The results suggest that modulation that minocycline and BMMCs are promising neuroprotective agents for experimental stroke and their concomitant use affords better neuroprotection and functional recovery than their single used [22–23]. In addition, intracerebral injections afford better therapeutic actions for BMMCs, although this experimental procedure is more invasive than the intravenous route [24]. The therapeutic synergism of the concomitant use of minocycline and BMMC is an important rationale to be explored in future investigations and a promising therapy for human stroke. It points out to the fact that a proper modulation of an exacerbated neuroinflammation in the ischemic environment is a suitable approach to enhance neuroprotection following transplants of stem cells into the acute phase of stroke and trauma.

8. Conclusion

In this chapter, we reviewed the pathophysiology of experimental stroke and the use of BMMCs as a promising approach to afford neuroprotection and functional recovery after transplants into the acute phase of brain ischemia. We have emphasized that transplanted progenitor/stem cells are affected by the pathological environment, including an exacerbated neuroinflammation. We have shown that a proper modulation of microglial activation of minocycline enhances both neuroprotection and functional recovery of BMMCs transplanted at 24 h after both cortical and striatal experimental stroke [22–23]. This approach can be used as an adjuvant therapy to enhance survival and efficacy of different kind of stem cells transplanted into the acute phase of stroke. In addition, this would reduce the time window of transplantation, which can be very important in the case of stroke, an acute neural disorder in which damage develops quickly.

Acknowledgements

This work was supported by the Brazilian National Council for Scientific and Technological Development (CNPq) and Fundação de Amparo A Pesquisa do Estado do Pará (FAPESPA). Authors thanks to Rosalia Mendez-Otero (UFRJ) and Carlos Alexandre Netto (UFSC) for technical support.

Conflict of interest

The authors declare no conflict of interest.

Author details

Edna Cristina S. Franco[1], Marcelo Marques Cardoso[2], Celice Cordeiro de Souza[3], Michelle Castro da Silva[4], Carolina Ramos dos Santos[2] and Walace Gomes-Leal[2*]

1 Pathology Unit, Evandro Chagas Institute, Belém, Pará, Brazil

2 Laboratory of Experimental Neuroprotection and Neuroregeneration, Institute of Collective Health, Federal University of Western Pará, Brazil

3 University Center of Pará (CESUPA), Belém, Para, Brazil

4 Laboratory of Experimental Neuroprotection and Neuroregeneration, Federal University of Pará, Para State University, Belem, Para, Brazil

*Address all correspondence to: wgomesleal@gmail.com

References

[1] Lo EH, Dalkara T, Moskowitz MA: Mechanisms, challenges and opportunities in stroke. Nat Rev Neurosci 2003; 4: 399-415. 10.1038/nrn1106:

[2] Chamorro A, Lo EH, Renu A, van Leyden K, Lyden PD: The future of neuroprotection in stroke. J Neurol Neurosurg Psychiatry 2020. 10.1136/jnnp-2020-324283:

[3] Kaiser EE, West FD: Large animal ischemic stroke models: replicating human stroke pathophysiology. Neural Regen Res 2020; 15: 1377-87. 10.4103/1673-5374.274324:

[4] Powers WJ: Acute Ischemic Stroke. N Engl J Med 2020; 383: 252-60. 10.1056/NEJMcp1917030:

[5] Moskowitz MA, Lo EH, Iadecola C: The science of stroke: mechanisms in search of treatments. Neuron 2010; 67: 181-98. 10.1016/j.neuron.2010.07.002:

[6] Wafa HA, Wolfe CDA, Emmett E, Roth GA, Johnson CO, Wang Y: Burden of Stroke in Europe: Thirty-Year Projections of Incidence, Prevalence, Deaths, and Disability-Adjusted Life Years. Stroke 2020; 51: 2418-27. 10.1161/STROKEAHA.120.029606:

[7] Virani SS, Alonso A, Benjamin EJ, Bittencourt MS, Callaway CW, Carson AP, Chamberlain AM, Chang AR, Cheng S, Delling FN, Djousse L, Elkind MSV, Ferguson JF, Fornage M, Khan SS, Kissela BM, Knutson KL, Kwan TW, Lackland DT, Lewis TT, Lichtman JH, Longenecker CT, Loop MS, Lutsey PL, Martin SS, Matsushita K, Moran AE, Mussolino ME, Perak AM, Rosamond WD, Roth GA, Sampson UKA, Satou GM, Schroeder EB, Shah SH, Shay CM, Spartano NL, Stokes A, Tirschwell DL, VanWagner LB, Tsao CW, American Heart Association Council on E, Prevention Statistics C, Stroke Statistics S: Heart Disease and Stroke Statistics-2020 Update: A Report From the American Heart Association. Circulation 2020; 141: e139-e596. 10.1161/CIR.0000000000000757:

[8] Jiao Y, Liu YW, Chen WG, Liu J: Neuroregeneration and functional recovery after stroke: advancing neural stem cell therapy toward clinical application. Neural Regen Res 2021; 16: 80-92. 10.4103/1673-5374.286955:

[9] Gomes-Leal W: Why microglia kill neurons after neural disorders? The friendly fire hypothesis. Neural Regen Res 2019; 14: 1499-502. 10.4103/1673-5374.255359:

[10] Gomes-Leal W: Microglial physiopathology: how to explain the dual role of microglia after acute neural disorders? Brain Behav 2012; 2: 345-56. 10.1002/brb3.51:

[11] Carpenter CR, Keim SM, Milne WK, Meurer WJ, Barsan WG, Best Evidence in Emergency Medicine Investigator G: Thrombolytic therapy for acute ischemic stroke beyond three hours. J Emerg Med 2011; 40: 82-92. 10.1016/j.jemermed.2010.05.009:

[12] Kablau M, Alonso A, Hennerici MG, Fatar M: Treatment with tPA predicts better outcome even if MCA occlusion persists. Int J Stroke 2013; 8: 496-502. 10.1111/j.1747-4949.2011.00750.x:

[13] Kablau M, Kreisel SH, Sauer T, Binder J, Szabo K, Hennerici MG, Kern R: Predictors and early outcome of hemorrhagic transformation after acute ischemic stroke. Cerebrovasc Dis 2011; 32: 334-41. 10.1159/000331702:

[14] Ginsberg MD: Current status of neuroprotection for cerebral ischemia: synoptic overview. Stroke 2009; 40: S111-4. 10.1161/STROKEAHA.108.528877:

[15] Ginsberg MD: Expanding the concept of neuroprotection for acute

ischemic stroke: The pivotal roles of reperfusion and the collateral circulation. Prog Neurobiol 2016; 145-146: 46-77. 10.1016/j.pneurobio.2016.09.002:

[16] Lindvall O, Kokaia Z: Stem cell therapy for human brain disorders. Kidney Int 2005; 68: 1937-9. 10.1111/j.1523-1755.2005.00623.x:

[17] Kokaia Z, Darsalia V: Human Neural Stem Cells for Ischemic Stroke Treatment. Results Probl Cell Differ 2018; 66: 249-63. 10.1007/978-3-319-93485-3_11:

[18] Kokaia Z, Llorente IL, Carmichael ST: Customized Brain Cells for Stroke Patients Using Pluripotent Stem Cells. Stroke 2018; 49: 1091-8. 10.1161/STROKEAHA.117.018291:

[19] Gronning Hansen M, Laterza C, Palma-Tortosa S, Kvist G, Monni E, Tsupykov O, Tornero D, Uoshima N, Soriano J, Bengzon J, Martino G, Skibo G, Lindvall O, Kokaia Z: Grafted human pluripotent stem cell-derived cortical neurons integrate into adult human cortical neural circuitry. Stem Cells Transl Med 2020. 10.1002/sctm.20-0134:

[20] Palma-Tortosa S, Tornero D, Gronning Hansen M, Monni E, Hajy M, Kartsivadze S, Aktay S, Tsupykov O, Parmar M, Deisseroth K, Skibo G, Lindvall O, Kokaia Z: Activity in grafted human iPS cell-derived cortical neurons integrated in stroke-injured rat brain regulates motor behavior. Proc Natl Acad Sci U S A 2020; 117: 9094-100. 10.1073/pnas.2000690117:

[21] Lindvall O, Kokaia Z: Stem cells for the treatment of neurological disorders. Nature 2006; 441: 1094-6. 10.1038/nature04960:

[22] Franco EC, Cardoso MM, Gouveia A, Pereira A, Gomes-Leal W: Modulation of microglial activation enhances neuroprotection and functional recovery derived from bone marrow mononuclear cell transplantation after cortical ischemia. Neurosci Res 2012; 73: 122-32. 10.1016/j.neures.2012.03.006:

[23] Cardoso MM, Franco EC, de Souza CC, da Silva MC, Gouveia A, Gomes-Leal W: Minocycline treatment and bone marrow mononuclear cell transplantation after endothelin-1 induced striatal ischemia. Inflammation 2013; 36: 197-205. 10.1007/s10753-012-9535-5:

[24] Souza CC, da Silva MC, Lopes RT, Cardoso MM, de Souza LL, Santos AG, Dos Santos IR, Franco ECS, Gomes-Leal W: Comparative Therapeutic Effects of Minocycline Treatment and Bone Marrow Mononuclear Cell Transplantation following Striatal Stroke. Oxid Med Cell Longev 2017; 2017: 1976191. 10.1155/2017/1976191:

[25] Chumnanvej S, Chumnanvej S: Autologous bone-marrow mononuclear stem cell therapy in patients with stroke: a meta-analysis of comparative studies. Biomed Eng Online 2020; 19: 74. 10.1186/s12938-020-00819-7:

[26] Kawabori M, Shichinohe H, Kuroda S, Houkin K: Clinical Trials of Stem Cell Therapy for Cerebral Ischemic Stroke. Int J Mol Sci 2020; 21. 10.3390/ijms21197380:

[27] Coyne TM, Marcus AJ, Woodbury D, Black IB: Marrow stromal cells transplanted to the adult brain are rejected by an inflammatory response and transfer donor labels to host neurons and glia. Stem Cells 2006; 24: 2483-92. 10.1634/stemcells.2006-0174:

[28] Molcanyi M, Riess P, Bentz K, Maegele M, Hescheler J, Schafke B, Trapp T, Neugebauer E, Klug N, Schafer U: Trauma-associated inflammatory response impairs embryonic stem cell survival and integration after implantation into injured rat brain. J

Neurotrauma 2007; 24: 625-37. 10.1089/neu.2006.0180:

[29] Dos Santos IRC, Dias MNC, Gomes-Leal W: Microglial activation and adult neurogenesis after brain stroke. Neural Regen Res 2021; 16: 456-9. 10.4103/1673-5374.291383:

[30] Puig B, Brenna S, Magnus T: Molecular Communication of a Dying Neuron in Stroke. Int J Mol Sci 2018; 19. 10.3390/ijms19092834:

[31] Fang Y, Gao S, Wang X, Cao Y, Lu J, Chen S, Lenahan C, Zhang JH, Shao A, Zhang J: Programmed Cell Deaths and Potential Crosstalk With Blood-Brain Barrier Dysfunction After Hemorrhagic Stroke. Front Cell Neurosci 2020; 14: 68. 10.3389/fncel.2020.00068:

[32] Datta A, Sarmah D, Mounica L, Kaur H, Kesharwani R, Verma G, Veeresh P, Kotian V, Kalia K, Borah A, Wang X, Dave KR, Yavagal DR, Bhattacharya P: Cell Death Pathways in Ischemic Stroke and Targeted Pharmacotherapy. Transl Stroke Res 2020; 11: 1185-202. 10.1007/s12975-020-00806-z:

[33] Sekerdag E, Solaroglu I, Gursoy-Ozdemir Y: Cell Death Mechanisms in Stroke and Novel Molecular and Cellular Treatment Options. Curr Neuropharmacol 2018; 16: 1396-415. 10.2174/1570159X16666180302115544:

[34] Yuan J: Neuroprotective strategies targeting apoptotic and necrotic cell death for stroke. Apoptosis 2009; 14: 469-77. 10.1007/s10495-008-0304-8:

[35] Liu C, Zhang K, Shen H, Yao X, Sun Q, Chen G: Necroptosis: A novel manner of cell death, associated with stroke (Review). Int J Mol Med 2018; 41: 624-30. 10.3892/ijmm.2017.3279:

[36] Bergsbaken T, Fink SL, Cookson BT: Pyroptosis: host cell death and inflammation. Nat Rev Microbiol 2009; 7: 99-109. 10.1038/nrmicro2070:

[37] Iadecola C, Buckwalter MS, Anrather J: Immune responses to stroke: mechanisms, modulation, and therapeutic potential. J Clin Invest 2020; 130: 2777-88. 10.1172/JCI135530:

[38] Dos Santos CD, Picanco-Diniz CW, Gomes-Leal W: Differential patterns of inflammatory response, axonal damage and myelin impairment following excitotoxic or ischemic damage to the trigeminal spinal nucleus of adult rats. Brain Res 2007; 1172: 130-44. 10.1016/j.brainres.2007.07.037:

[39] Gomes-Leal W, Corkill DJ, Freire MA, Picanco-Diniz CW, Perry VH: Astrocytosis, microglia activation, oligodendrocyte degeneration, and pyknosis following acute spinal cord injury. Exp Neurol 2004; 190: 456-67. 10.1016/j.expneurol.2004.06.028:

[40] Gomes-Leal W, Corkill DJ, Picanco-Diniz CW: Systematic analysis of axonal damage and inflammatory response in different white matter tracts of acutely injured rat spinal cord. Brain Res 2005; 1066: 57-70. 10.1016/j.brainres.2005.10.069:

[41] Lima RR, Santana LN, Fernandes RM, Nascimento EM, Oliveira AC, Fernandes LM, Dos Santos EM, Tavares PA, Dos Santos IR, Gimaraes-Santos A, Gomes-Leal W: Neurodegeneration and Glial Response after Acute Striatal Stroke: Histological Basis for Neuroprotective Studies. Oxid Med Cell Longev 2016; 2016: 3173564. 10.1155/2016/3173564:

[42] Souza-Rodrigues RD, Costa AM, Lima RR, Dos Santos CD, Picanco-Diniz CW, Gomes-Leal W: Inflammatory response and white matter damage after microinjections of endothelin-1 into the rat striatum. Brain Res 2008; 1200: 78-88. 10.1016/j.brainres.2007.11.025:

[43] Schnell L, Fearn S, Klassen H, Schwab ME, Perry VH: Acute inflammatory responses to

mechanical lesions in the CNS: differences between brain and spinal cord. Eur J Neurosci 1999; 11: 3648-58. 10.1046/j.1460-9568.1999.00792.x:

[44] Tanaka R, Komine-Kobayashi M, Mochizuki H, Yamada M, Furuya T, Migita M, Shimada T, Mizuno Y, Urabe T: Migration of enhanced green fluorescent protein expressing bone marrow-derived microglia/macrophage into the mouse brain following permanent focal ischemia. Neuroscience 2003; 117: 531-9. 10.1016/s0306-4522(02)00954-5:

[45] Kronenberg G, Uhlemann R, Richter N, Klempin F, Wegner S, Staerck L, Wolf S, Uckert W, Kettenmann H, Endres M, Gertz K: Distinguishing features of microglia- and monocyte-derived macrophages after stroke. Acta Neuropathol 2018; 135: 551-68. 10.1007/s00401-017-1795-6:

[46] Stirling DP, Khodarahmi K, Liu J, McPhail LT, McBride CB, Steeves JD, Ramer MS, Tetzlaff W: Minocycline treatment reduces delayed oligodendrocyte death, attenuates axonal dieback, and improves functional outcome after spinal cord injury. J Neurosci 2004; 24: 2182-90. 10.1523/JNEUROSCI.5275-03.2004:

[47] Beattie MS: Inflammation and apoptosis: linked therapeutic targets in spinal cord injury. Trends Mol Med 2004; 10: 580-3. 10.1016/j.molmed.2004.10.006:

[48] Casha S, Zygun D, McGowan MD, Bains I, Yong VW, Hurlbert RJ: Results of a phase II placebo-controlled randomized trial of minocycline in acute spinal cord injury. Brain 2012; 135: 1224-36. 10.1093/brain/aws072:

[49] Koistinaho J, Yrjanheikki J, Kauppinen T, Koistinaho M: Tetracycline derivatives as anti-inflammatory agents and potential agents in stroke treatment. Ernst Schering Res Found Workshop 2004; 101-15. 10.1007/978-3-662-05426-0_6:

[50] Yrjanheikki J, Keinanen R, Pellikka M, Hokfelt T, Koistinaho J: Tetracyclines inhibit microglial activation and are neuroprotective in global brain ischemia. Proc Natl Acad Sci U S A 1998; 95: 15769-74. 10.1073/pnas.95.26.15769:

[51] Yrjanheikki J, Tikka T, Keinanen R, Goldsteins G, Chan PH, Koistinaho J: A tetracycline derivative, minocycline, reduces inflammation and protects against focal cerebral ischemia with a wide therapeutic window. Proc Natl Acad Sci U S A 1999; 96: 13496-500. 10.1073/pnas.96.23.13496:

[52] Popovich PG, Guan Z, Wei P, Huitinga I, van Rooijen N, Stokes BT: Depletion of hematogenous macrophages promotes partial hindlimb recovery and neuroanatomical repair after experimental spinal cord injury. Exp Neurol 1999; 158: 351-65. 10.1006/exnr.1999.7118:

[53] Fagan SC: Drug repurposing for drug development in stroke. Pharmacotherapy 2010; 30: 51S–4S. 10.1592/phco.30.pt2.51S:

[54] Fouda AY, Newsome AS, Spellicy S, Waller JL, Zhi W, Hess DC, Ergul A, Edwards DJ, Fagan SC, Switzer JA: Minocycline in Acute Cerebral Hemorrhage: An Early Phase Randomized Trial. Stroke 2017; 48: 2885-7. 10.1161/STROKEAHA.117.018658:

[55] Lampl Y, Boaz M, Gilad R, Lorberboym M, Dabby R, Rapoport A, Anca-Hershkowitz M, Sadeh M: Minocycline treatment in acute stroke: an open-label, evaluator-blinded study. Neurology 2007; 69: 1404-10. 10.1212/01.wnl.0000277487.04281.db:

[56] Malhotra K, Chang JJ, Khunger A, Blacker D, Switzer JA, Goyal N, Hernandez AV, Pasupuleti V, A lexandrov AV, Tsivgoulis G: Minocycline

for acute stroke treatment: a systematic review and meta-analysis of randomized clinical trials. J Neurol 2018; 265: 1871-9. 10.1007/s00415-018-8935-3:

[57] Radlinska BA, Ghinani SA, Lyon P, Jolly D, Soucy JP, Minuk J, Schirrmacher R, Thiel A: Multimodal microglia imaging of fiber tracts in acute subcortical stroke. Ann Neurol 2009; 66: 825-32. 10.1002/ana.21796:

[58] Thiel A, Heiss WD: Imaging of microglia activation in stroke. Stroke 2011; 42: 507-12. 10.1161/STROKEAHA.110.598821:

[59] Thiel A, Radlinska BA, Paquette C, Sidel M, Soucy JP, Schirrmacher R, Minuk J: The temporal dynamics of poststroke neuroinflammation: a longitudinal diffusion tensor imaging-guided PET study with 11C-PK11195 in acute subcortical stroke. J Nucl Med 2010; 51: 1404-12. 10.2967/jnumed.110.076612:

[60] Shechter R, London A, Varol C, Raposo C, Cusimano M, Yovel G, Rolls A, Mack M, Pluchino S, Martino G, Jung S, Schwartz M: Infiltrating blood-derived macrophages are vital cells playing an anti-inflammatory role in recovery from spinal cord injury in mice. PLoS Med 2009; 6: e1000113. 10.1371/journal.pmed.1000113:

[61] Shechter R, Schwartz M: Harnessing monocyte-derived macrophages to control central nervous system pathologies: no longer 'if' but 'how'. J Pathol 2013; 229: 332-46. 10.1002/path.4106:

[62] Kigerl KA, Gensel JC, Ankeny DP, Alexander JK, Donnelly DJ, Popovich PG: Identification of two distinct macrophage subsets with divergent effects causing either neurotoxicity or regeneration in the injured mouse spinal cord. J Neurosci 2009; 29: 13435-44. 10.1523/JNEUROSCI.3257-09.2009:

[63] Rapalino O, Lazarov-Spiegler O, Agranov E, Velan GJ, Yoles E, Fraidakis M, Solomon A, Gepstein R, Katz A, Belkin M, Hadani M, Schwartz M: Implantation of stimulated homologous macrophages results in partial recovery of paraplegic rats. Nat Med 1998; 4: 814-21. 10.1038/nm0798-814:

[64] Neumann H, Kotter MR, Franklin RJ: Debris clearance by microglia: an essential link between degeneration and regeneration. Brain 2009; 132: 288-95. 10.1093/brain/awn109:

[65] Neumann J, Gunzer M, Gutzeit HO, Ullrich O, Reymann KG, Dinkel K: Microglia provide neuroprotection after ischemia. FASEB J 2006; 20: 714-6. 10.1096/fj.05-4882fje:

[66] Neumann J, Sauerzweig S, Ronicke R, Gunzer F, Dinkel K, Ullrich O, Gunzer M, Reymann KG: Microglia cells protect neurons by direct engulfment of invading neutrophil granulocytes: a new mechanism of CNS immune privilege. J Neurosci 2008; 28: 5965-75. 10.1523/JNEUROSCI.0060-08.2008:

[67] Lalancette-Hebert M, Gowing G, Simard A, Weng YC, Kriz J: Selective ablation of proliferating microglial cells exacerbates ischemic injury in the brain. J Neurosci 2007; 27: 2596-605. 10.1523/JNEUROSCI.5360-06.2007:

[68] Rayasam A, Hsu M, Kijak JA, Kissel L, Hernandez G, Sandor M, Fabry Z: Immune responses in stroke: how the immune system contributes to damage and healing after stroke and how this knowledge could be translated to better cures? Immunology 2018; 154: 363-76. 10.1111/imm.12918:

[69] Xu Y, Jin MZ, Yang ZY, Jin WL: Microglia in neurodegenerative diseases. Neural Regen Res 2021; 16: 270-80. 10.4103/1673-5374.290881:

[70] Zhang W, Tian T, Gong SX, Huang WQ , Zhou QY, Wang AP, Tian Y: Microglia-associated neuroinflammation is a potential therapeutic target for

ischemic stroke. Neural Regen Res 2021; 16: 6-11. 10.4103/1673-5374.286954:

[71] Lambertsen KL, Finsen B, Clausen BH: Post-stroke inflammation-target or tool for therapy? Acta Neuropathol 2019; 137: 693-714. 10.1007/s00401-018-1930-z:

[72] Ginhoux F, Garel S: The mysterious origins of microglia. Nat Neurosci 2018; 21: 897-9. 10.1038/s41593-018-0176-3:

[73] Ginhoux F, Greter M, Leboeuf M, Nandi S, See P, Gokhan S, Mehler MF, Conway SJ, Ng LG, Stanley ER, Samokhvalov IM, Merad M: Fate mapping analysis reveals that adult microglia derive from primitive macrophages. Science 2010; 330: 841-5. 10.1126/science.1194637:

[74] Nimmerjahn A: Monitoring neuronal health. Science 2020; 367: 510-1. 10.1126/science.aba4472:

[75] Nimmerjahn A, Kirchhoff F, Helmchen F: Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science 2005; 308: 1314-8. 10.1126/science.1110647:

[76] Davalos D, Grutzendler J, Yang G, Kim JV, Zuo Y, Jung S, Littman DR, Dustin ML, Gan WB: ATP mediates rapid microglial response to local brain injury in vivo. Nat Neurosci 2005; 8: 752-8. 10.1038/nn1472:

[77] Vainchtein ID, Chin G, Cho FS, Kelley KW, Miller JG, Chien EC, Liddelow SA, Nguyen PT, Nakao-Inoue H, Dorman LC, Akil O, Joshita S, Barres BA, Paz JT, Molofsky AB, Molofsky AV: Astrocyte-derived interleukin-33 promotes microglial synapse engulfment and neural circuit development. Science 2018; 359: 1269-73. 10.1126/science.aal3589:

[78] Barichello T, Generoso JS, Simoes LR, Goularte JA, Petronilho F, Saigal P, Badawy M, Quevedo J: Role of Microglial Activation in the Pathophysiology of Bacterial Meningitis. Mol Neurobiol 2016; 53: 1770-81. 10.1007/s12035-015-9107-4:

[79] Shastri A, Bonifati DM, Kishore U: Innate immunity and neuro-inflammation. Mediators Inflamm 2013; 2013: 342931. 10.1155/2013/342931:

[80] Spanos JP, Hsu NJ, Jacobs M: Microglia are crucial regulators of neuro-immunity during central nervous system tuberculosis. Front Cell Neurosci 2015; 9: 182. 10.3389/fncel.2015.00182:

[81] Sacchetti ML: Is it time to definitely abandon neuroprotection in acute ischemic stroke? Stroke 2008; 39: 1659-60. 10.1161/STROKEAHA.107.505024:

[82] Jaillard A, Hommel M, Moisan A, Zeffiro TA, Favre-Wiki IM, Barbieux-Guillot M, Vadot W, Marcel S, Lamalle L, Grand S, Detante O: Autologous Mesenchymal Stem Cells Improve Motor Recovery in Subacute Ischemic Stroke: a Randomized Clinical Trial. Transl Stroke Res 2020; 11: 910-23. 10.1007/s12975-020-00787-z:

[83] Mendez-Otero R, de Freitas GR, Andre C, de Mendonca ML, Friedrich M, Oliveira-Filho J: Potential roles of bone marrow stem cells in stroke therapy. Regen Med 2007; 2: 417-23. 10.2217/17460751.2.4.417:

[84] Lucas D: Structural organization of the bone marrow and its role in hematopoiesis. Curr Opin Hematol 2020. 10.1097/MOH.0000000000000621:

[85] Hurwitz SN, Jung SK, Kurre P: Hematopoietic stem and progenitor cell signaling in the niche. Leukemia 2020. 10.1038/s41375-020-01062-8:

[86] Krabbe C, Zimmer J, Meyer M: Neural transdifferentiation of mesenchymal stem cells--a critical review. APMIS 2005; 113: 831-44. 10.1111/j.1600-0463.2005.apm_3061.x:

[87] Takakura N, Watanabe T, Suenobu S, Yamada Y, Noda T, Ito Y, Satake M, Suda T: A role for hematopoietic stem cells in promoting angiogenesis. Cell 2000; 102: 199-209. 10.1016/s0092-8674(00)00025-8:

[88] Giraldi-Guimardes A, Rezende-Lima M, Bruno FP, Mendez-Otero R: Treatment with bone marrow mononuclear cells induces functional recovery and decreases neurodegeneration after sensorimotor cortical ischemia in rats. Brain Res 2009; 1266: 108-20. 10.1016/j.brainres.2009.01.062:

[89] Zhukareva V, Obrocka M, Houle JD, Fischer I, Neuhuber B: Secretion profile of human bone marrow stromal cells: donor variability and response to inflammatory stimuli. Cytokine 2010; 50: 317-21. 10.1016/j.cyto.2010.01.004:

[90] de Vasconcelos Dos Santos A, da Costa Reis J, Diaz Paredes B, Moraes L, Jasmin, Giraldi-Guimaraes A, Mendez-Otero R: Therapeutic window for treatment of cortical ischemia with bone marrow-derived cells in rats. Brain Res 2010; 1306: 149-58. 10.1016/j.brainres.2009.09.094:

[91] Vasconcelos-dos-Santos A, Rosado-de-Castro PH, Lopes de Souza SA, da Costa Silva J, Ramos AB, Rodriguez de Freitas G, Barbosa da Fonseca LM, Gutfilen B, Mendez-Otero R: Intravenous and intra-arterial administration of bone marrow mononuclear cells after focal cerebral ischemia: Is there a difference in biodistribution and efficacy? Stem Cell Res 2012; 9: 1-8. 10.1016/j.scr.2012.02.002:

[92] Keimpema E, Fokkens MR, Nagy Z, Agoston V, Luiten PG, Nyakas C, Boddeke HW, Copray JC: Early transient presence of implanted bone marrow stem cells reduces lesion size after cerebral ischaemia in adult rats. Neuropathol Appl Neurobiol 2009; 35: 89-102. 10.1111/j.1365-2990.2008.00961.x:

[93] Barbosa da Fonseca LM, Gutfilen B, Rosado de Castro PH, Battistella V, Goldenberg RC, Kasai-Brunswick T, Chagas CL, Wajnberg E, Maiolino A, Salles Xavier S, Andre C, Mendez-Otero R, de Freitas GR: Migration and homing of bone-marrow mononuclear cells in chronic ischemic stroke after intra-arterial injection. Exp Neurol 2010; 221: 122-8. 10.1016/j.expneurol.2009.10.010:

[94] Correa PL, Mesquita CT, Felix RM, Azevedo JC, Barbirato GB, Falcao CH, Gonzalez C, Mendonca ML, Manfrim A, de Freitas G, Oliveira CC, Silva D, Avila D, Borojevic R, Alves S, Oliveira AC, Jr., Dohmann HF: Assessment of intra-arterial injected autologous bone marrow mononuclear cell distribution by radioactive labeling in acute ischemic stroke. Clin Nucl Med 2007; 32: 839-41. 10.1097/RLU.0b013e318156b980:

[95] Rosado-de-Castro PH, Schmidt Fda R, Battistella V, Lopes de Souza SA, Gutfilen B, Goldenberg RC, Kasai-Brunswick TH, Vairo L, Silva RM, Wajnberg E, Alvarenga Americano do Brasil PE, Gasparetto EL, Maiolino A, Alves-Leon SV, Andre C, Mendez-Otero R, Rodriguez de Freitas G, Barbosa da Fonseca LM: Biodistribution of bone marrow mononuclear cells after intra-arterial or intravenous transplantation in subacute stroke patients. Regen Med 2013; 8: 145-55. 10.2217/rme.13.2:

[96] Yang B, Strong R, Sharma S, Brenneman M, Mallikarjunarao K, Xi X, Grotta JC, Aronowski J, Savitz SI: Therapeutic time window and dose response of autologous bone marrow mononuclear cells for ischemic stroke. J Neurosci Res 2011; 89: 833-9. 10.1002/jnr.22614:

[97] Dai S, Hua Y, Keep RF, Novakovic N, Fei Z, Xi G: Minocycline attenuates

brain injury and iron overload after intracerebral hemorrhage in aged female rats. Neurobiol Dis 2019; 126: 76-84. 10.1016/j.nbd.2018.06.001:

[98] Naderi Y, Panahi Y, Barreto GE, Sahebkar A: Neuroprotective effects of minocycline on focal cerebral ischemia injury: a systematic review. Neural Regen Res 2020; 15: 773-82. 10.4103/1673-5374.268898:

[99] Yong VW, Wells J, Giuliani F, Casha S, Power C, Metz LM: The promise of minocycline in neurology. Lancet Neurol 2004; 3: 744-51. 10.1016/S1474-4422(04)00937-8:

[100] Liao TV, Forehand CC, Hess DC, Fagan SC: Minocycline repurposing in critical illness: focus on stroke. Curr Top Med Chem 2013; 13: 2283-90. 10.2174/15680266113136660160:

[101] Liu Z, Fan Y, Won SJ, Neumann M, Hu D, Zhou L, Weinstein PR, Liu J: Chronic treatment with minocycline preserves adult new neurons and reduces functional impairment after focal cerebral ischemia. Stroke 2007; 38: 146-52. 10.1161/01.STR.0000251791.64910.cd:

[102] Guimaraes JS, Freire MA, Lima RR, Picanco-Diniz CW, Pereira A, Gomes-Leal W: Minocycline treatment reduces white matter damage after excitotoxic striatal injury. Brain Res 2010; 1329: 182-93. 10.1016/j.brainres.2010.03.007:

[103] Bahrami F, Morris DL, Pourgholami MH: Tetracyclines: drugs with huge therapeutic potential. Mini Rev Med Chem 2012; 12: 44-52. 10.2174/138955712798868977:

[104] Jiang Z, Alamuri TT, Muir ER, Choi DW, Duong TQ: Longitudinal multiparametric MRI study of hydrogen-enriched water with minocycline combination therapy in experimental ischemic stroke in rats. Brain Res 2020; 1748: 147122. 10.1016/j.brainres.2020.147122:

[105] Stirling DP, Koochesfahani KM, Steeves JD, Tetzlaff W: Minocycline as a neuroprotective agent. Neuroscientist 2005; 11: 308-22. 10.1177/1073858405275175:

[106] Hayakawa K, Mishima K, Nozako M, Hazekawa M, Mishima S, Fujioka M, Orito K, Egashira N, Iwasaki K, Fujiwara M: Delayed treatment with minocycline ameliorates neurologic impairment through activated microglia expressing a high-mobility group box1-inhibiting mechanism. Stroke 2008; 39: 951-8. 10.1161/STROKEAHA.107.495820:

[107] Zhu S, Stavrovskaya IG, Drozda M, Kim BY, Ona V, Li M, Sarang S, Liu AS, Hartley DM, Wu DC, Gullans S, Ferrante RJ, Przedborski S, Kristal BS, Friedlander RM: Minocycline inhibits cytochrome c release and delays progression of amyotrophic lateral sclerosis in mice. Nature 2002; 417: 74-8. 10.1038/417074a:

[108] Lu Y, Huang Z, Hua Y, Xiao G: Minocycline Promotes BDNF Expression of N2a Cells via Inhibition of miR-155-Mediated Repression After Oxygen-Glucose Deprivation and Reoxygenation. Cell Mol Neurobiol 2018; 38: 1305-13. 10.1007/s10571-018-0599-0:

[109] Domercq M, Matute C: Neuroprotection by tetracyclines. Trends Pharmacol Sci 2004; 25: 609-12. 10.1016/j.tips.2004.10.001:

[110] Jordan J, de Groot PW, Galindo MF: Mitochondria: the headquarters in ischemia-induced neuronal death. Cent Nerv Syst Agents Med Chem 2011; 11: 98-106. 10.2174/187152411796011358:

[111] Buja LM, Vela D: Immunologic and inflammatory reactions to exogenous stem cells implications for experimental studies and clinical trials for myocardial repair. J Am Coll Cardiol 2010; 56: 1693-700. 10.1016/j.jacc.2010.06.041:

[112] Lu P, Wang Y, Graham L, McHale K, Gao M, Wu D, Brock J, Blesch A, Rosenzweig ES, Havton LA, Zheng B, Conner JM, Marsala M, Tuszynski MH: Long-distance growth and connectivity of neural stem cells after severe spinal cord injury. Cell 2012; 150: 1264-73. 10.1016/j.cell.2012.08.020:

[113] Lu P, Kadoya K, Tuszynski MH: Axonal growth and connectivity from neural stem cell grafts in models of spinal cord injury. Curr Opin Neurobiol 2014; 27: 103-9. 10.1016/j.conb.2014.03.010:

[114] Rosenzweig ES, Brock JH, Lu P, Kumamaru H, Salegio EA, Kadoya K, Weber JL, Liang JJ, Moseanko R, Hawbecker S, Huie JR, Havton LA, Nout-Lomas YS, Ferguson AR, Beattie MS, Bresnahan JC, Tuszynski MH: Restorative effects of human neural stem cell grafts on the primate spinal cord. Nat Med 2018; 24: 484-90. 10.1038/nm.4502:

[115] Kadoya K, Lu P, Nguyen K, Lee-Kubli C, Kumamaru H, Yao L, Knackert J, Poplawski G, Dulin JN, Strobl H, Takashima Y, Biane J, Conner J, Zhang SC, Tuszynski MH: Spinal cord reconstitution with homologous neural grafts enables robust corticospinal regeneration. Nat Med 2016; 22: 479-87. 10.1038/nm.4066:

[116] Kumamaru H, Lu P, Rosenzweig ES, Kadoya K, Tuszynski MH: Regenerating Corticospinal Axons Innervate Phenotypically Appropriate Neurons within Neural Stem Cell Grafts. Cell Rep 2019; 26: 2329-39 e4. 10.1016/j.celrep.2019.01.099:

[117] Kumamaru H, Lu P, Rosenzweig ES, Tuszynski MH: Activation of Intrinsic Growth State Enhances Host Axonal Regeneration into Neural Progenitor Cell Grafts. Stem Cell Reports 2018; 11: 861-8. 10.1016/j.stemcr.2018.08.009:

[118] Lu P, Gomes-Leal W, Anil S, Dobkins G, Huie JR, Ferguson AR, Graham L, Tuszynski M: Origins of Neural Progenitor Cell-Derived Axons Projecting Caudally after Spinal Cord Injury. Stem Cell Reports 2019; 13: 105-14. 10.1016/j.stemcr.2019.05.011: