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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 Wallace 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, mitochondrial damage and ATP depletion. Metabolic collapse induce formation of reactive oxygen species (ROS) leading to protein and lipid oxidation and cell death. Control of excitatory neurotransmitter levels is lost leading to increased calcium intracellular 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 characterized 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 Ca^{2+} 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 $\text{TNF-}\alpha$, and inhibited by the necroptosis-inhibiting factor-1 (Nec-1) [35]. Pyroptosis is triggered 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 correlated 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 areas, playing a beneficial, harmful or dubious role. Thus, the results of the interaction between these mechanisms are directly related 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 astrogliosis 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, intra-vascular 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 easily cross 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 minocycline 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 μ 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.

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