

Stem Cells Reduced Neuroinflammatory Response During the Process of Stroke

Mora Lee S*, Castro García P, Manzo Ríos M I

Laboratory Cell Therapy and Biotechnological Innovation, CUValles/CUCEI University of Guadalajara Blvd. Marcelino García Barragán #1421, C.P. 44430, Guadalajara, Jalisco, México.

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ABSTRACT

The stroke is among the main diseases that cause death behind cardiac diseases and cancer, in industrialized countries, causing 10% approximately of total deaths. One of the most incidence of strokes are ischemic type, while hemorrhagic type is decreased apparition. This blood flow interruption entails a lessening of oxygen input and nutrients that may cause irreversible neuronal damages. According as the search for new therapeutic approaches is increasing, nowadays there are multiple researchers using cell therapy for the stroke using various stem cells types. Thus also, the treatment of stroke is focused on several objectives and will be depending on the pathophysiologic state in which the disease is found. The most important are focused on neuroprotection after a stroke. Some types of stem cells such as the NSC, BMSC and ADSC have demonstrated therapeutic potential for stroke. These mechanisms include coronary thrombosis reduction, increased neurogenesis, inflammation reduction, cell differentiation, secretion of growth factors, cytokines and hormones, in addition to modulation of the immune response after transplantation, which makes them a potential therapy for neuroinflammation of the stroke.

Keywords: stem cells, cell therapy, neuroinflammation, stroke

INTRODUCTION

Stroke consists on the sudden blood flow interruption in a section or the whole brain either due the form of a clot (ischemic type) or a ruptured blood vessel that spreads blood on the brain's cells surrounding areas (hemorrhagic type). This blood flow interruption entails a lessening of oxygen input and nutrients that may cause irreversible neuronal damages. According the World Health Organization (WHO), stroke is, behind cardiac diseases and cancer, the third cause of death in industrialized countries, causing 10% approximately of total deaths; 85 % of these are on people older than 65 years old^{1,2}. Therefore, the life quality and expectancy have been reduced causing a significant increase on health expenses^{3,4}. Most of strokes are ischemic type (80%) while the remaining (20%) are hemorrhagic type⁵.

Hemorrhagic stroke occurs when a blood vessel weakens and get broken. The subsequent hemorrhage cannot be released to the exterior, spreading the blood around the brain cells causing lack of oxygenation in the affected area⁶. Ischemic stroke occurs when a blood vessel irrigating the brain gets obstructed by a blood clot. During focal ischemia, the blood vessel obstruction produces a gradient characterized by the appearance of two zones of ischemic lesion. The central zone is called Core or ischemic core, indicated by an absolute absence of blood flow; causing neuronal death in a short period of time, in this case, is the necrotic type. The second zone, at the Core's peripheral area, is called ischemic penumbra. This

area has a restricted blood flow where neurons show functional alterations but it still preserves a minimum metabolic activity⁷. If the energetic deficit is not restored, the neurons in the penumbra zone undergo a depolarization process, post-ischemic edema and cellular death due necrosis or apoptosis⁷⁻⁹. The increment on the cellular mortality rate in the penumbra zone causes an increase in size of the stroke. The penumbra zone is potentially viable and can be rescued from conversion into ischemic core, offering attractive therapy alternatives for strokes. Logically, the period of time for the penumbra zone to exist offers this opportunity window for therapeutic treatment.

PATHOPHYSIOLOGICAL PROCESS OF BRAIN ISCHEMIA

The pathophysiological processes and the molecular processes start showing immediately after the first start of ischemia and they are a solely dependent on the flow and time. The blood flow interruption causes a sequence of pathophysiological processes in space and time. Although, these processes follow an order, it has been proved that they present a degree of overlay. During time between the ischemia appearance and the neuronal death, a cascade of chemical reactions on the nervous cells develops that seem to be the cause of neuronal death. The main pathogenic mechanisms in this cascade include excitotoxicity, depolarization surrounding the infarct area, inflammation and apoptosis⁷⁻¹⁰. These processes trigger protective (anti-

excitatory) and repair (anti-inflammatory, anti-apoptotic) mechanisms, endogenous and, therefore it will be of great interest to know the strengthening mechanism to improve the post-ischemic treatment.

INFLAMMATION

The central nervous system (CNS) inflammatory response is characterized by microglia and astrocytes activation, as well as per the showing of some key inflammatory mediators with a limited invasion of surrounding inflammatory cells. This fact can be increased by the fast induction of inflammatory mediators such as cytokines, chemokine and prostaglandins that over-regulate adhesion molecules and increase permeability in the blood-brain barrier, facilitating the invasion of circulating inflammatory cells and the subsequent release of potentially toxic molecules for the brain neurons. As such, in a stroke the permeability of the blood-brain barrier is increased and the inflammatory cells get in touch with the central nervous system antigens at the brain and periphery. Excitotoxicity and oxidative stress caused by the initial ischemic event activate microglia and astrocytes, which react by secreting cytokines, chemokines and matrix metalloproteases (MMP). These inflammatory mediators lead to an upregulation of cell adhesion molecules on endothelial cells, allowing blood derived inflammatory cells, mainly neutrophils, to infiltrate the ischemic brain area. Neutrophils themselves also secrete cytokines, which cause a further activation of glial cells. All these processes result in neuronal cell death and enhance the damage to the ischemic brain. The evidence shows that inflammation contributes to increase the post-ischemic damage. Accordingly, neutrophils infiltration in the brain produce a receptors blockade to adhesion cells hence a inhibition of specific interleukins for decrease ischemic damage^{7,11}.

TREATMENT

Only one drug is approved for clinical use for the thrombolytic treatment of acute ischemic stroke and that is intravenous recombinant tissue plasminogen activator (rt-PA). When it delivered within three hours after symptom onset, rt-PA reduces neurological deficits and improves the functional outcome of stroke patients. However, this improvement in recovery is achieved at the expense of an increased incidence in symptomatic intracranial hemorrhage, which occurs in ~6% of patients. Furthermore, since the large majority of patients with acute ischemic stroke do not go to the hospital within three hours of stroke onset most do not receive rt-PA treatment.

Fibrinolysis is the current chosen treatment for stroke, but a combined therapy will be required in order to strengthen its action and avoid reperfusion deleterious effects. A diverse range of compounds are been tested whose main action is to block metabolic disturbances in the ischemic cascade and avoid or at least reduce the effect of cellular death and the reperfusion damage. Neuroprotection pharmacologically stops or limits the progression of ischemic cascade in the brain tissue once it is started. According its action mechanism, the neuroprotection strategies can be classified as: excitatory amino-acids

modulators, calcium flow modulators, anti-edema agents, leukocyte adhesion inhibitors, free radicals inhibitors, membrane reparation promoters (and degradation inhibitors) and compound with unknown effects. The pharmacological therapies currently used after cerebral ischemia are not satisfactory, thus a research of new therapies aimed to stimulate reparation through endogenous cell of damaged tissue is necessary.

Cell Therapy

Cell therapy is defined as pathology treatment though cells application, directly administered in an organ or tissue or in a systemic manner. Initially, in cerebral ischemia, cell therapy emerged as a therapy of cellular substitution to replace lost tissue after ischemia. Since different types of cells are lost (neurons, astrocytes and oligodendrocytes) as well as neuronal circuits, it can be expected that scientific evidence shows the inefficiency of transplanted cell, independently of the cellular type, to regenerate the lost tissue and functionality. Nevertheless, cellular transplant has shown a beneficial effect in ischemia evolution. It has been proposed that transplanted cells might act as biological bombs secreting growing factors, neurotrophins and cytokines which intervene at these beneficial effects¹²⁻¹⁴, even though the acting triggers and mechanisms remain unknown. As described next, there are several stem cell populations with diverse potential, and tests in the treatment of this pathology is coming.

Stem Cells Used in Inflammation Stroke

Stem cells can be generally defined by their two main properties: their auto-renovation ability and their differentiation ability (from other cellular types). It is very difficult, nonetheless, to establish a precise definition with no ambiguities, which encloses all types of known stem cell to the day. A stem cell is undifferentiated, immature cell, capable of symmetric or asymmetric division to produce several cells from which someone must be the same as the parent cell. A stem cell can, initially and under the proper conditions, divide itself indefinitely in time, keeping always a stable population of identical stem cells¹⁵. Under proper condition and having the right stimulation, stem cells can differentiate to several, many or even all types of specialized cells contained in a mature organism.

Neural stem cells (NSC)

The neural stem cells are those cells of neuronal origin with somewhat limited capacity for self-renewal and expansion, with a potential differentiation of a few neural types in unipotent occasions. Neuronal and glial progenitors their aim is differentiation into neuron and glia respectively. Thus, the neural progenitors give rise to a particular type of neuron, which would be a tool to repair the damage in the CNS has been injured. Neural stem cells can be obtained from various regions of the fetal development and adult. The NSC are able to differentiate into cell types such as cortical neurons, interneurons, hippocampal pyramidal neurons, which are affected after a stroke occurs¹⁶. The NSC also differentiate into oligodendrocytes^{17,18} astrocytes¹⁹⁻²¹ and possibly endothelial cells²². The majority of cases can not be distinguished to dopaminergic neurons, motor neurons or

Purkinje cells¹⁶. When transplanted NSCs tend to migrate to infarcted areas where they can generate functional neurons that make connections with host cells. They have shown neuroprotective effects²³⁻²⁵ and immunomodulators^{26,27} in various models of neurodegenerative diseases and brain damage. Research is showing that the NSC can differentiate mainly to glia²⁸. The results obtained with transplantation of NSC after cerebral ischemia has shown no improvement in infarct size^{29,30}. With this background, the investigators have studied the NSC, in order to determine whether age has effect to treatment with NSC after suffering cerebral ischemia. By managing the NSC and the observed time that neurobehavioral damage and stroke decreased. Histologically, it was observed that NSC differentiates into glial cells. It is noted likewise that angiogenesis and neurogenesis improves, and an increased expression of vascular endothelial growth factor (VEGF) in young and elderly subjects was perceived, which it leads to aging is not limited to a beneficial treatment with NSC after cerebral ischemia³¹. In the case of other types of chronic neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS) and Alzheimer's disease, NSC even have been useful in therapy. For the treatment of ALS, the motor neuron has been transplanted from NSC giving a positive outcome delaying clinical signs for 7 days and extending life up to 20 days. This indicates that treatment with cell-derived motor neurons grafted from NSC could be helpful in the treatment of ALS patients without significant adverse effect³². In the case of Alzheimer's, after a transplant of NSC, contemplated that levels of synaptic proteins like Synaptophysin (SYN) and the protein 43 associated with growth (GAP-43) were induced by transplantation of NSC and were observed that were morphologically normal synapses increasing when they are given to the NSC. Concluding that induced NSC neurons have increased the number of synapses in the upregulation of synaptic proteins and GAP-43 SYN, synaptogenesis therefore may be an important agent in improving the symptoms of Alzheimer³³. Another great use that it had been proposed the NSC is to restore gastrointestinal function in people who have suffered damage at the enteric nervous system or they were born with it. Briefly, glial cells and neurons were obtained *in vitro* from enteric nervous system stem cells of post-natal mouse, they were implanted and it was observed that these cells were able to migrate into the intestinal wall and differentiate into neurons and glial cells. So that, these isolated progenitor cells postnatal enteric nervous system, could serve as a source for neurogastrointestinal motility disorders therapy³⁴. Recently, there are reports that stem cell therapy is an effective treatment *in vivo* after suffering stroke; it is giving a neuroprotective effect. In 2008 was investigated the effect that it had on brain and peripheral inflammation after intracerebral hemorrhage have been submitted. They observed that spleen activations of alpha tumor necrosis factor (TNF- α), interleukin 6 (IL-6), and nuclear factor kappa B (NF- κ B) reduced, also have less neurological damage, as well as a decrease in cerebral edema, inflammatory infiltration and apoptosis reduced

too. Also was observed that the neural stem cells also inhibit *in vitro* activation of macrophages, finally it concluded that after intravenous injection of NSC originates an antiinflammatory activity which leads to neuroprotection by disrupting inflammatory responses splenic injury after cerebral hemorrhage³⁵. Similarly, they are not only useful therapies for hemorrhagic strokes NSC but also those are generated by ischemia, Herman et al. observed that NSC can be large antagonists of inflammatory processes, giving a significant neuroprotection when cerebral ischemia that occurs. The brain tissue protection is associated with low expression of inflammatory markers, glial scar formation, and death by apoptosis. NSC accumulated in brain, focusing on the main adjacent infarction zone, and where the majority of neural stem cells remain without being differentiated up to 30 days after transplantation²³. It has also been investigated the immunomodulatory effects of NSC that have beneficial effect on the stroke originated by ischemia or hemorrhage, suppressing mitogenically or allogeneically the T cells generation; NSC can override the activation and proliferation of human peripheral T cells³⁶. Also the NSC effects, when co-graft olfactory ensheathing cells (OEC), are explored in rats that have suffered a traumatic brain injury (TBI). After transplanting the NSC they can survive and migrate into brain. It was observed that the number of neurons in the cortex from the combined implementation was more abundant than to the other groups; apoptotic cells showed a decrease. At the molecular level we found that the expression of IL-6 and BAD gene in the group co-graft were regulated significantly compared with either alone groups (NSC or OECs). With the above, it is shown that the administration in conjunction with OECs NSC, is a new tool for the TBI treatment through anti-inflammation mechanism³⁷. However, they are not always effective treatments with stem cells, such as Reeves et al. reported; last year, where attribute that the hypertrophic inflammatory *cauda equina* syndrome was acquired for a patient that received a therapy with neural stem cells. This patient has suffered a stroke after other diseases, such as macular degeneration, depression and osteoarthritis own age, which led her to previous treatment with neural stem cells, after one year indicated that women had progressive pain leg numbness and difficulty walking. When undergoing studies and imaging was observed that the lumbosacral roots of the *cauda equina* showed great enlargement, which was not observed before treatment with stem cells, also using electrodiagnostic studies were confirmed a multiple lumbosacral radiculopathies chronic in abundance³⁸. A biopsy was performed in lumbar dorsal sensory where the results showed lumbar degeneration and loss of myelin fibers with endoneurial inflammation, so this was attributed to the stem cells injection which had received the patient before.

Bone marrow-derived stem cells (BMSC)

The bone marrow is a niche of bone-marrow stromal cells (BMSC) with self-renewal and asymmetric division abilities, which are the main characteristics of stem cells. The BMSC progenitors, also known as MSC, which are

potentially inducible to differentiation in specialized cells such as chondrocytes, osteoblasts, and adipocytes,³⁹⁻⁴⁰; its easy autologous tissue isolation is reflected in its application on clinical and preclinical studies, being addressed mainly to the nervous systems, where they are differentiated towards glial cells or neurons for neurodegenerative diseases therapy^{41,42}. Also applied in animal stroke models favoring the angiogenesis such as VEGF (Vascular Endothelial Growth Factor)⁴³, and neurogenesis endogenous. The expression of these factors is increased in the hypoxic tissues supporting the hypothesis that such factors might represent the guide signals for the circulating progenitor cell enlistment in assisting the endogen reparation mechanism on the stroked tissues⁴⁴. These multipotent cells, through the appropriate stimulation with auto system, with the related factors will allow an alternative as reparation or surrogate therapy and/or cellular stimulation for the damaged tissue. The BMSC use as cell sheet engineering in patients with ischemic stroke is considered as cell stimulator and protector in the acute phase of vascular brain disease. A side of reducing the size of the injury^{45,46}, they are capable of multiply and migrate towards to the damaged zone without requiring a previous immunosuppression⁴⁷. Nonetheless, one of the first actions occurring during ischemia development and the immune system activation as a consequence, activates the inflammatory signals causing tissue damaging and retarding reparation mechanism. In consequence, the control of the risk factors intervening during neurodegeneration of damaged brain, are decisive for the life expectancy in the patient^{48,49}. *In vitro* studies have shown that BMSC can suppress immunoregulatory effects; baboon,⁵⁰ human⁵¹, and rodent⁵², BMSC can effectively suppress T lymphocyte proliferation when added to a mixed-lymphocyte culture. The effects are independent of MHC, and of T lymphocyte proliferation induced by allogeneic antigens derived from recipients, donors, or even a third party⁵³. BMSC have been shown to decrease proinflammatory cytokine gene expression in experimental acute lung injury^{54,55} myocardial infarction⁵⁶, and acute renal failure and to upregulate IL-10 expression in rat models of myocardial infarction and cerebral infarction^{57,58}. BMSC treatment reduced the presence of microglia in the damaged brain parenchyma and decreased the density of peripheral infiltrating leukocytes at the injured site, as well as reducing proinflammatory cytokines and increasing anti-inflammatory cytokines, possibly through enhanced expression of TSG-6. TSG-6 may, in turn, act by suppressing activation of the NF- κ B signaling pathway and decreasing the production of proinflammatory cytokines to initiate a proinflammatory cytokine cascade⁵⁹. The immunomodulation provoked by BMSC, when transplanted by intravenous via, causes an alteration of the apoptosis-related proteins expression such as Bcl-2 promoting neuronal survival balancing as well as the modulation in the inflammatory response and conducting a sensorial function recovery; in rats increases and at the same time, creates an suitable environment for the implanted cell survival.

ADSC (adipose tissue-derived stromal cells)

The adipose tissue (TA) is an alternative source for progenitor cells in cellular therapy since they shelter a population known as ADSC (adipose tissue-derived stromal cells) that can be obtained by a less invasive method and in higher amount than another sources. It has been proved in several studies that ADSC share stem cells characteristics similar to MSCs of bone marrow. These cells are obtained through a lipoaspirate process (PLA), regularly at cosmetic liposuctions, achieving the recollection of immense amounts of cells and they are easy to cultivate under normal conditions⁶⁰. The adipose tissue is a very complex tissue formed by mature adipocytes, preadipocytes, fibroblasts, muscular and vascular cells, resident macrophages and lymphocytes⁶¹. The stroma-vascular cells (SVF), of adipose tissue, are the focus in stem cells studies⁶². As the BMSC, the ADSC can be also subject of differentiation of osteocytes, chondrocytes and even, other cells from mesodermal heritage such as cardiomyocytes, hepatocytes, etc. after induction *in vitro*. In addition, ADSC are also known to be able to differentiate in epithelial cells and neurons⁶³. Due its cellular plasticity to multiples heritages allows the ADSC conversion to specialized cells, this will be useful for tissue and cell surrogate therapy⁶⁴. In nervous system pathologies, the importance of the ADSC has been proved in the cellular therapy future, due the easiness of obtaining and differentiation. Since other MSC derivative from BMSC have the ability to decrease the inflammatory response and the size of the ischemic stroke injury⁶⁵. ADSC isolation from adipose tissue and that have not been inducted to differentiation are managed through systemic circulation but also have been directly injected in the damaged tissue to initiate reparation processes. The chemokine receptors expression in the ADSC produces that these intermediaries can be conducted to the specific sites of the injury, where it will initiate the inflammatory mechanism, which stimulates the transendothelial migration and the leukocytes diapedesis⁶⁶. ADSC are also MSC type with a minimum immunological reaction in the host in autologous transplants and with a immunomodulation effect when the inflammatory mechanisms are initiated in pathological processes such as the stroke⁶⁷. These immune regulations effects consist of indirect inhibition of T cell activation during recognition, though the inhibition of TNF- α and INF- γ production an increase in levels of IL-10 cytokine anti-inflammatory is produced⁶⁸. Available data clearly support the concept that alogenical, the MSC can be used as therapeutic agents. The secretion and stimulation of trophic factors in the ADSC allow the regulation of the inflammatory harmful effect providing a broader window for neuronal survival. Currently, there are not many studies about the influence of these cells has over regulatory transcription factors of inflammation such as NF κ B when they are activated en response to pro-inflammatory cytokines. When ADSC are in culture, express high levels of adipocytes markers because they shall keep a profile of gens for adipocytokines such as Leptin, adiponectine, PAI-1, Interleukin-10 or inclusive IL-6, cytokines with an

important role in the inflammatory processes. As it has been demonstrated in experimental models, the ADSC action could down-regulate the expression of TNF-alpha allowing an increase in IL-10 levels, which would explain their protective effect in cerebral ischemia⁵⁷. In the other hand, adipokines such as adiponectine in this case, it acts suppressing the formation of leucocytes colonies, reducing the phagocyte activity and decreasing the tumour necrosis factor alpha-receptor secretion (TNF-alpha) in the inflammation macrophages. On its side, the Leptine is an endogenous arbitrator of neuroprotection in brain ischemia. Due before it mentioned, the influence of these adipose-cytokines over the ADSC response during a stroke event must be a great relevance. The neuronal and glial cells that occur during ischemia in the CNS degeneration, have not been proven to be irreversible to the use of both BMSC and ADSC. However, to maximize the optimum effect should be transplanted cells in the injury zone, signaling pathways, trophic factors, cytokines, should be better studied.

CONCLUSION

It is known that the study subjects after suffering a stroke, when older, the stroke results are worst, that is to say, infarct size is larger and they have worse neurological complications compared to younger study subjects. Stem cells have become attractive candidates for cellular therapy in stroke treatment of which so far no ideal therapeutic measures are available. The beneficial effects of stem cells might include neuroprotection, angiogenesis, inflammatory, and immune response. The inflammatory response after stroke is essential to initiate the machinery that is responsible for repairing the damage and also to disposing dead cells, these activated immune cells cause a lot of short and long-term damage to the brain. Stem cell therapy after stroke could improve cerebral function, most likely by the production of paracrine factors. One of the systems influenced by these paracrine factors is the immune system. Although, most animal studies demonstrated that impaired neural function has been significantly improved after administration of various stem cells. These results suggest that NSC, BMSC and ADSC have the ability to modulate inflammation associated cytokine release and immune cells in stroke induced cerebral inflammatory responses. This study serves as the basis for future studies and offers new insights into the mechanisms responsible for the beneficial immunomodulatory effect of stem cells transplantation in terms of functional neurological recovery after stroke. In future, stem cell combined with other type of therapy, as biomaterials, nanoparticles, gene therapy, etc., will play important roles in experimental and clinical application.

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