

Review article: Paracrine effects of Transplanted Neural stem cells in Ischemic strokes

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Abstract

Ischemic stroke is one of the most common causes of death in the world. Pathophysiologically, ischemic stroke is associated with blood brain barrier disruption, cell loss, inflammation and intense immune reaction which worsen the condition. The Stroke Treatment Academic Industry Roundtable recommends the use of any treatment that has multiple mechanisms of action to restore the neurological function. Growing evidences from researchers to understand the biology of neural stem cells indicated that these cells have crucial role in brain homeostasis and have high therapeutic potential to modify and restore neurological function after neurovascular injury. Contemporarily, Paracrine hypothesis is growing and indicated that neural stem cells transplantation can release several neurotrophic factors which can regenerate, restore, and modulate the neurological diseases by mitigating the inflammatory process, reducing the immune reactions and also can stimulate the angiogenesis and neurogenesis. This, generate a new attitude toward the development of acellular therapy instead of using whole allogenic neural stem cells in the regenerative medicine. This review is to focus on the paracrine effects of transplanted neural stem cells in the brain ischemic injuries which can modulate and improve the neurological outcome after ischemic injury.

Key words: *Neural stem cells, paracrine factors, neurotrophic factors, angiogenesis, neurogenesis, ischemic stroke*

Introduction:

A large scale of literatures and researches has been focused on studying the biology of neural stem cells. These studies illustrated that neural stem cell therapy have wide varieties of beneficial effects in neurodegenerative diseases like Parkinsonism (1) and (2), Alzheimer's disease (3), strokes (4), spinal cord injuries, multiple sclerosis (5) and (6). Traditionally, it has been thought that the mechanism of action of neural stem cell therapy is restricted to stem cell proliferation to replace cellular loss, to cellular integration to the host neural circuit and to do synaptic remodeling (7) and (8).

Recently, it has been shown that the advantages of neural stem cell therapy may extend beyond the classical belief and may have neuroprotective, regenerative and anti inflammatory activities and more effects which enable the host tissues to repair after injury and can resist the degeneration process

(8). NSCs have the potential to send wide varieties of signals, to migrate to specific micro-environment and can accomplish more complex behaviors. It has been thoroughly investigated that NSCs transplantation have tropic effects on host cells and modulatory effect on adaptive and innate immune responses which ultimately promote healing process of the injured tissues (9).

Pathophysiology in Ischemic stroke

In order to understand the paracrine effects of NSCs in ischemic strokes, it is mandatory to know the pathophysiology after ischemic insult. In addition to the initial ischemic injury there are other accumulative damages occurs due to reperfusion, disruption of BBB, and inflammation which make the condition worse (8).

Reperfusion injury and BBB disruption

BBB is a special complex structure formed by the tight junctions of the endothelial cells and the astrocyte processes. Intact BBB is crucial in maintaining brain homeostasis and regulate neuronal signals by controlling the trafficking of molecules to and from the CNS (10). BBB control white blood cell entrance to the CNS for the regular immune surveillance and after infec-

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tion and tissue damage where debris need to be removed (11).

Ischemia causes deprivation of glucose and oxygen which result in metabolic distress. Also, Ischemia causes ATP store exhaustion which leads to ion transport dysregulation and accumulation of glutamate, all of the mentioned metabolic changes cause disruption of BBB (12).

Blood flow restoration is important after stroke to reduce the size of infarcted area. However, blood flow reperfusion cause oxidative stress and challenge the BBB by increasing the inflammatory mediators, metalloproteinases (MMPs), oxidative damage to cellular molecules and by changing in tight junction proteins (13). The inflammatory cytokines increase the infiltration of the CNS by leukocytes while MMPs cause the BBB to be leaky and enhance the entry of toxic molecules to the ischemic tissue (14).

Inflammatory responses

Disrupted and leaky BBB allows the white blood cells like the neutrophils, macrophages and the lymphocytes (which represent the innate and the adaptive immune responses) to enter the CNS. Inflammation and immune cells play important roles in wound healing while in ischemic strokes they can worsen the condition and increase the damaged area (15). After ischemia the dying cells release pro-inflammatory cytokine and the loss of contact with living neurons stimulate the microglia which is normally reside in the CNS to secrete tissue necrosis factor- α (TNF- α), interleukin-1 beta (IL-1 β), and reactive oxygen species (16). 24 hours after ischemic stroke and perfusion injury, the innate immune cells start to attach to the disrupted endothelium of the BBB and begin to invade the CNS. Macrophages are the most cells which infiltrate the area. Macrophages and the microglia start secretion of TNF- α , IL-8, and IL-12 which attract more neutrophils to the ischemic area and worsen the condition by secreting nitric oxide, MMPs, and cathepsins (17).

On the other hand, the adaptive immune cells, specifically T lymphocytes (the helper and the cytotoxic) and the antigen presenting cells begin to invade the ischemic area through the disrupted BBB and exacerbate the damaging process (18) and (19). T helper cells start secretion of interferon $\text{INF}\lambda$ which attract more immune cells to enter the CNS while the cytotoxic cells trigger cell apoptosis through Fas ligand pathway (20).

The neutrophils are other immune cells which also infiltrate the ischemic area. The infiltration of these cells is usually preceded by the infiltration of other cells like macrophage, microglia, and lymphocytes. Neutrophil infiltration causes release of proteolytic enzymes and their accumulation in human brain is usually associated with poor prognosis (21).

Immunomodulation of transplanted NSCs:

The transplanted NSCs have what is called (bystander effect), by which the NSCs can release neurotrophic factors like brain derived neurotrophic factor (BDNF), glial derived neurotrophic factor (GDNF), and also nerve growth factor (NGF). These factors can reduce the inflammatory reactions which happen with the early stage of strokes. Like reduction in the inflammatory signals of activated microglia which inhibit the extravasation of immune cells and also reduce blood brain barrier

leakage (9). Several studies have shown that secondary injury of stroke can be modified by the intraparenchymal transplantation of NSCs (fetal and iPSCs) since they have neuro-protective effect on the injured brain, and the reduction of inflammatory process is correlated with reduction in the infarct size and functional recovery (22).

It has been illustrated that transplantation of fetal NSCs and iPSCs- NSCs result in reduction in expression of pro-inflammatory cytokines like interleukins (IL-1 β and IL-6) and tissue necrosis factor (TNF- α). The reduction in the cytokines was partially due to increase in the expression of BDNF (23) and (22).

Angiogenesis

The area which surround the ischemic core is called penumbra, this area is hypoperfused but salvageable due to the growth of collateral circulation and increased density of microvascularization. Penumbra could be the target to survive the tissue surrounding the infarct area, to reduce the size of infarcted area, and can improve the functional outcome. It has been shown by previous studies that after strokes, neurotrophic and regenerative growth factors released from the local NSCs and endothelial compartment, and they migrate to the ischemic region. These factors will stimulate the angiogenesis (24). Interestingly, it has been shown that NSCs transplantation in rodents can increase the density of micro-vascularature and increase the proliferation of endothelial cells. The angiogenic behavior of transplanted NSCs is mediated by Vascular Endothelial Growth Factor (VEGF) which is either secreted by the NSCs or by the host cells (25).

Effective therapy for stroked patient required protection and recovery of both neuronal and vascular compartments, the neurovascular unit. This is because that when cerebral ischemia occurs this will affect both the neuronal and the vascular compartments as if they are acting as one unit. Therefore, creation of vessel network in the penumbra requires formation of functional neurovascular unit which maintain cerebral perfusion, homeostasis and selectively permeable. Horie et al (2011) demonstrated that NSCs transplantation result in high expression of tight junction proteins (occluding, claudin, and ZO1) and reduce BBB leakage (26).

Neurogenesis

It has been demonstrated that adult brain contains NSCs in the subventricular zone (SVZ) (27) and dentate gyrus (DG) (28). These stem cells have the potential to proliferate and replace the cell loss. However, It has been demonstrated that neurogenesis in these area increased after brain injury and strokes but still at low level to replace all cell loss. In spite of the evidences that the nascent neurons integrate with the host cells and make synapses with them, about 80% of the nascent neurons die 2 weeks after transplantation (29). It has been shown that the endogenous neurogenesis can be stimulated and increased by NSCs transplantation in preclinical stroke models. NSCs transplantation can augment the proliferation of endogenous NSCs in the SVZ and DG and migration of the neuroblasts and neurons to the affected area to be differentiated into neurons (25). The mechanism is not clear till now, but it is believed

that transplanted NSCs release regenerative and neurotrophic factors.

In the first month after stroke, the patients who receive NSCs show several signs of recovery in motor function of the limb, language, cognitive and other functions. These might be due to reconnection in the neuronal circuits. It has been shown that the motor and sensory circuits increased in areas proximal to the infarcted zone (30). The modulation in neural circuit occurs by changes in the dendrites, axons and synapses. Previous studies documented that intra-parenchymal NSC transplantation in stroke models can reorganize the synapses by increasing the expression of synaptophysin protein and increased the expression of GAP-43 which is axonal growth protein (31). Additionally, transplantation of fetal and ESC-NSCs stimulated dendritic growth and branching (32). The increased expression of synaptophysin is associated with increased expression of genes which are associated with neurite growth like thrombospondin (TSPs) 1 and 2, VEGF, and GDGF. These factors are also secreted by transplanted NSCs which are responsible for NSCs-induced axonal and dendritic plasticity (32). Moreover, NSCs transplantation has been documented in increase myelination process for the new circuits by increasing the proliferation of oligodendrocytes (33).

Extracellular vesicles (secretomes)

Previous studies documented that purified cultured media of NSC can reduce cell death, stimulate neurogenesis and functional recovery in preclinical studies (34) and (35). Studies on rat demonstrated that utilization NSC cultured media in stroked rats has protective effect and also can improve the functional outcome in several behavioral tests. These effects were due to neurotrophic factors and due to micro or nano particles (Extracellular Vesicles-EV) which are secreted into the cultured media by NSCs. EVs have been found to carry cargos of proteins, RNA and DNA which reduce the immune reactions, reduce the size of infarcted area, improve the integrity of white matter and improve functional recovery in preclinical models of ischemic strokes in mice and pigs. Moreover, EVs can harbor CD29 and CD41 which have crucial roles in maintaining the integrity of BBB (34&36).

Micro-RNAs (small RNAs) have been found in the cultured media and have been shown to play an important role in enhancing neurogenesis and angiogenesis. For example, miR-9 is highly expressed in the NSCs and has been documented to regulate signaling of VEGF which stimulate the neurogenesis, angiogenesis and NSCs migration (37). Other micro-RNA is the let-7 family, which are also highly expressed in NSCs and have neuro-protective effect through regulation of expression of caspase3, TNF- α , and IT-12 (38). Additionally, miR-210 expression increased in an area which exposed to ischemia and this micro-RNA improve the neurogenesis and angiogenesis (39).

All together, these indicate the cultured media of NSCs contain active biomolecules and regenerative factors which are packaged together in EVs or swim in the media in free form. These factors may make the NSC therapy no longer important (4).

The routes of NSCs transplantation

Generally, there are two main ways for the administration of NSCs to the stroked individuals, intraparenchymal (IP) and intravenous (IV) ways. The most popular way is the transcranial IP injection (40). This method of administration has several advantages being the direct transplantation of the cells to the injured area eliminating the off target side effects, avoiding the cells to pass through the BBB, and secured delivery of large number of NSCs to the affected area (41). However, the most disadvantage of this method is the invasive approach which required craniotomy that might result in additional complications for the ischemic individuals who already compromised. Kallakde, et al. (2016) illustrated that transcranial IP transplantation of NSCs is well tolerated, safe, and has few side effects. Also, they demonstrated that IP administration of NSCs is worthy for more clinical studies (40).

On the other hand, IV administration is another common approach which is the least invasive than IP route. Moreover, IV route can be used in acute stage where the individuals are unfit for craniotomy or to be administered by health care staff who are unable to do craniotomy. Additionally, IV transplantation of NSCs can be given by paramedics during ambulance way to the hospital or in far away areas in which the availability of neurosurgeon is limited (42). The hurdle of IV route is few number of cells to be engrafted in the brain due to their accumulation in off target like lung and liver (43). IV route of NSCs administration has been documented to has neuroprotective and anti-inflammatory effects and also has the potential to improve the clinical outcome in stroked patients without the evidence of cellular engrafting, this is due to the releasing of regenerative trophic factors by the NSCs (44).

Other method of NSCs delivery is intranasal application (INA). Since BBB is the most obstacle point against drug to reach the CNS, INA has been proposed to deliver small molecules and proteins to the CNS. Frey (1990) open a new era by discovering the route to the brain from the nose (45). In INA, the cells can reach the brain either directly through migration with olfactory or trigeminal nerves or through cerebrospinal circulation (46). INA is less invasive and can be performed several times. Danielyan was the pioneer in delivering cells to the CNS through INA, he used labeled mesenchymal stem cells (MSCs) of rat and human glioma cells which administered through the nasal cavity and he found some of the cells in the olfactory bulb, cerebellum and subarachnoid (47). After that several studies have documented that INA of stem cells from different tissues can posses therapeutic effects and can be found in different locations in the CNS (48), (49), and (50).

Neural stem cell lines

Several protocols have been developed to generate NSC lines which have the capability to proliferate and produce neural cell lineage like neural cells, astrocytes, oligodendrocytes, and other glial cells. These cells are immortal and have the ability to be expanded in culture. These NSCs can be derived from fetal tissues, embryonic stem cells, induced pluripotent stem cells, and Mesenchymal Stem Cells

1. Fetal NSCs

This line is generated from fetal tissue like mesencephalon, spinal cord, or fetal cortex from the 7th days to 21st days post-conceptional. These cells have the capability to expand in several passages when culturing them in serum free media containing mitogens like epidermal growth factor and basic fibroblast growth factor and maintaining their neurogenic and gliogenic potential even without genetic manipulation (51). It has been shown that fetal-NSCs have therapeutic effect in several neurological diseases like strokes, brain and spinal cord injuries (40). However, these cells have been found to be aged (senesced) earlier than other NSC line, therefore, these cells must undergo genetic manipulation to immortalize them and enhance their expandability. CTX0E03 is one example of immortalized fetal-NSCs which demonstrate their therapeutic activities like angiogenic, neurogenic and immunomodulatory activity by releasing several paracrine factors not by the cell engraftment (52), (53) and (40). However, these cells have limited long term cellular engraftment, the cause of limited engraftment is seems to be multifactorial like the hostility of the stroked area (high level of reactive oxygen species and high amount of inflammatory mediators) and the lack of cellular integration (53).

2. Embryonic stem cells (ESCs)

After the first development of ESCs line from the inner cell mass of the embryo, several research groups established ESC-NSCs from different species like human, mouse, and primate. These cells have long term expansion and have the advantages over fetal-NSCs line in which they have immortal nature to produce large number of cells to meet the clinical demand (33).

3. induced pluripotent stem cells (iPSCs).

It has been confirmed that adult somatic cells can be reprogrammed into pluripotent cells by over-expression of certain transcription factors. The progeny cells (iPSCs) have the plasticity and the ability into neuronal differentiation like the ESCs (54). The iPSC-NSCs have been illustrated to have therapeutic potential in several neurological diseases. Since these cells are derived from the patient's somatic cells the risk of immune rejection is avoided (55). Several studies have shown that transplantation of iPSC-NSCs into stroked pigs is associated with functional recovery by improving neuronal circuit through increasing the expression of VEGF in the brain (56) and (57). Polentes et al. (2012) pointed out that transplanted iPSC-NSCs can be differentiated into site specific neurons and can integrate into the host circuit which might prevent stroke associated neuronal deficit (57). Lau et al., (2018) have shown that iPSC-NSCs transplantation can reduce the immune reactions in pig stroke model and also can improve the integrity of white matter (42). Additionally, iPSC-NSCs treated stroked pig showed faster recovery than the non treated control stroke pigs. Further studies are needed to shorten the time of reprogramming which still as one of the disadvantages of iPSC technology (41) and (40)

4. Mesenchymal stem cells (MSCs)

Mesenchymal stem cells can be derived from several adult tissues like bone marrow, fat, and umbilical cord. Some Bone Marrow-derived MSCs (BM-MSCs) can express nestin, neurofilament light chain and microtubule associated protein 2

(MAP-2) which are the markers for the NSCs that have the capability to renew themselves and to differentiate into neurons to promote neuronal regeneration (58) and (59). Recently, BM-MSCs can be introduced intranasally to several conditions like brain injury, neurodegenerative diseases, and also tumors (48)

Timing of Neural Stem cell transplantation

1. Acute phase (24hr after stroke)

As we mentioned before, in the early phase of stroke, the reperfusion injury, BBB disruption and inflammatory reaction increase the damage to the CNS. The most common drug treatment to minimize acute damage of stroke is minocycline, this drug has neuroprotective effect through its anti-apoptotic and anti-inflammatory activities and also has the ability to reduce BBB damage (60). Therefore, NSCs transplantation during acute phase of strokes can act in several mechanisms to reduce the damage of ischemic insult.

Huang et al. (2014) demonstrated that human NSCs transplantation 24hr after into the hippocampus of mice after Middle Cerebral Artery occlusion (MCAO) resulted in increase migration of NSCs to the lesion area, can reduce the volume of infarct, minimize BBB injury and also reduce the activity of microglia and decrease macrophage inflammatory protein-1 α (23). Eckert et al. (2015) illustrated that transplantation of human iPSC-NSC into stroke rodent models can reduce MMP activity which was associated with BBB dysfunction and can decrease IgG level which infiltrate the brain due to the leakage of BBB (22).

Moreover, NSCs transplantation during the acute phase can mitigate the inflammatory reactions by down regulating the inflammatory molecules (61), (62), and (63). Additionally, NSCs during the acute phase can improve the functional outcome by stimulating the angiogenesis and neurogenesis (25) and (64).

2. Chronic phase (more than 7 days after stroke)

Several clinical studies documented that NSCs transplantation during the chronic phase has promising restorative action because of the reduction of the inflammatory signals. NSCs transplantation 7 days after stroke can avoid the hostile niche in the acute phase of stroke and might enhance neurogenesis and angiogenesis (65) and (66).

Conclusion

NSCs can provide promising effects in the treatment of strokes by different mechanism like their neuroprotective effects, stimulating the angiogenesis, augmenting the endogenous neurogenesis, and by neuronal modification through the resynaptogenesis of the neuronal circuits. Also they can exert their action through their anti-inflammatory effects by limiting the immune responses after strokes. To improve the beneficial effects of NSCs transplantation more studies are required to understand the other factors which are critical for succeeding the NSC transplantation like NSCs number, the timing of the transplant, the route of transplantation

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