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Exogenous stem cells pioneer a biobridge to the advantage of host brain cells following stroke: New insights for clinical applications

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Abstract:

Stroke continues to maintain its status as one of the top causes of mortality within the United States. Currently, the only Food and Drug Administration (FDA)-approved drug in place for stroke patients, tissue plasminogen activator (tPA), has a rigid therapeutic window, closing at approximately 4.5 h after stroke onset. Due to this short time frame and other restrictions, such as any condition that increases a patient's risk for hemorrhaging, it has been predicted that <5% of ischemic stroke patients benefit from tPA. Given that rehabilitation therapy remains the only other option for stroke victims, there is a clear unmet clinical need for treatment available for the remaining 95%. While still considered an experimental treatment, the utilization of stem cell therapies for stroke holds consistent promise. Copious preclinical studies report the capacity for transplanted stem cells to rescue the brain parenchyma surrounding the stroke-induced infarct core. At present, the exact mechanisms in which stem cells contribute a robust therapeutic benefit remains unclear. Following stem cell administration, researchers have observed cell replacement, an increase in growth factors, and a reduction in inflammation. With a deeper understanding of the precise mechanism of stem cells, these therapies can be optimized in the clinic to afford the greatest therapeutic benefit. Recent studies have depicted a unique method of endogenous stem cell activation as a result of stem cell therapy. In both traumatic brain injury and stroke models, transplanted mesenchymal stromal cells (MSCs) facilitated a pathway between the neurogenic niches of the brain and the damaged area through extracellular matrix remodeling. The biobridge pioneered by the MSCs was utilized by the endogenous stem cells, and these cells were able to travel to the damaged areas distal to the neurogenic niches, a feat unachievable without prior remodeling. These studies broaden our understanding of stem cell interactions within the injured brain and help to guide both researchers and clinicians in developing an effective stem cell treatment for stroke. This paper is a review article. Referred literature in this paper has been listed in the references section. The datasets supporting the conclusions of this article are available online by searching various databases, including PubMed. Some original points in this article come from the laboratory practice in our research center and the authors' experiences.

Keywords:

Biobridge, central nervous system disorders, mesenchymal stromal cells, neurogenesis, regenerative medicine, stem cell therapy, stroke, traumatic brain injury

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Introduction

Stem cells consistently demonstrate their therapeutic benefit both endogenously[1-4] and exogenously within injured tissues.[5-11] Stem cell research plays an integral role in regenerative medicine[1-13] and the demand for laboratory research in this field should not be understated, as a few clinical trials exist for stem cell treatment of neurological diseases.[14-17] Stem cells have displayed their capacity to facilitate organ repair through direct replacement of dead or

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dying cells and through the secretion of a wide range of trophic factors; these secreted factors contribute to the survival of damaged cells as well as healthy cells which become vulnerable to stress within the postinjury microenvironment.[18,19] While this understanding exists, much remains to be discovered regarding the precise mechanism in which stem cells can elicit therapeutic effects in an extensive range of pathologies. Additional findings on stem cell therapy for pathologies of the brain allow for the optimization of stem cell treatment protocols which may differ in cell type, delivery route, and dosage. Recent preclinical studies have revealed novel mechanism of stem cell therapy in which transplanted mesenchymal stromal cells (MSCs) recruit endogenous stem cells to the site of injury, specifically the impact area from traumatic brain injury (TBI) and infarct area of stroke. Two investigations from our laboratory demonstrated that MSCs were able to remodel the extracellular matrix (ECM) in a manner that facilitated migration of endogenous stem cells away from their origin within the neurogenic niches to distal brain regions such as the cortex.[20] After the initial TBI study, this phenomenon was termed the biobridge due to the resulting cellular matrix illustrating a biologic path of exogenous MSCs, and subsequently replaced by endogenous stem cells, from the neurogenic subventricular zone (SVZ) to the affected cortex.[20] Preliminary data from our ongoing study suggest that not only was a migration of endogenous stem cells enhanced after intracerebral injection (IC) but also with intravenous administration (IV) as well. Interestingly, migration patterns of the MSCs and endogenous stem cells appear to differ between the two delivery routes; understanding these differences will necessitate further investigations. These studies emphasize the importance of preclinical research as they give insight into the unique interactions of stem cells within the injured brain. This review aims to highlight the novel findings of these biobridge studies and how the knowledge gained from this research may be applied to stem cell treatment of stroke and potentially other neurological disorder such as Parkinson's disease (PD) and spinal cord injury (SCI).

General Stroke Pathology

Stroke is defined as a focal event in which decreased blood flow is delivered to a brain region as a consequence of hemorrhaging or blood vessel blockage. The latter form is termed ischemic stroke and is the most common type of stroke with a lower mortality rate than hemorrhagic stroke. The high metabolic activity of brain cells makes them sensitive to reduced oxygen and nutrients. Therefore, the cells directly downstream of the blocked blood vessel become vulnerable to cell death within minutes. These neuronal cells quickly undergo necrosis forming the infarct core. The surrounding tissue, termed the penumbra, is comprised of the cells still receiving some perfusion by adjacent nonischemic regions and is subjected to the harsh microenvironment formed by the emptied contents of the necrosed cells and the subsequent inflammation.[21] It is well beyond the capacity of current medical technologies to restore the dead cells within this infarct core. However, the cells on the periphery have the potential to be protected through therapeutic intervention.[22]

Currently, tPA, the only FDA-approved drug for stroke, must be administered within 4.5 h of onset. If delivered within this time frame, this thrombolytic drug assists in clot breakdown, aiming to recover the surviving penumbra before this area gradually succumbs to cell death. Due to risk of hemorrhaging and short time frame of eligibility, only 3%–8% of patients receive tPA unless admitted to a specialized stroke center within the appropriate time window. Researchers hope to find a therapeutic drug that is safe and effective at both acute and chronic stages. Intuitively, the sooner the treatment, the more room for recovery; however, secondary cell death persists chronically. Therefore, patients may benefit from a treatment able to terminate its progression. To develop a drug of this nature, it is pivotal to understand angiogenesis, neurogenesis, and neuroplasticity in detail, so that it does work effectively in reducing, halting and reversing the stroke-related damage.

Endogenous Repair and Neurogenesis Postischemia

The subgranular zone (SGZ) in the dentate gyrus of the hippocampus and subventricular zone (SVZ) of the lateral ventricles are well established neurogenic niches of the brain. These regions are the primary sources for neurogenesis in the adult mammalian.[23] Neural stem cells within this region do not divide as constantly as many other adult stem cell types throughout the body but may produce a range of actively proliferating neural precursor cells (NPCs).[21] Defining in detail distinguishing characteristics of each cell type as well as the process of newborn neuron synaptic integration is an ongoing pursuit.[23] It is atypical for neurogenesis outside of these designated neurogenic niches, though is hypothesized to be a possibility in the case of brain insult.[23,24]

As with any form of injury, ischemic stroke is followed by numerous molecular responses. These changes in the brain's molecular biology signal the neurogenic niches, thereby modulating NPC proliferation, differentiation, migration, and survival.[21] In rat stroke models, NPCs have been observed to migrate from the SVZ to the postischemic infarct within the striatum.[25,26] The

postischemic neurogenesis has been viewed in both the CA1 region of the hippocampus and the cerebral cortex. Regrettably, there has been no evidence in postmortem analysis of stroke patients supporting this same phenomenon. Significant formation of new cortical neurons within the range from 3 days to 13 years after stroke in humans has yet to be reported.[27] It is predicted that the uppermost conceivable rate of undetected neurogenesis following ischemia would not surpass >0.1%, or 1 out of every 1000 neurons.[27] On the cellular level, the gap between the cerebral cortex and source of endogenous neural stem cells in the adult brain is a considerably large distance for cell migration, therefore is a limitation of endogenous repair from ischemia-related neurogenesis.

Exogenous Stem Cells form a Biobridge

Stem cells of many types have been recognized repeatedly for their capacity for cell replacement and influence on microenvironment restoration through secretion of various trophic factors.[12,13] In recent years, the known therapeutic mechanisms of stem cell transplantation in stroke were expanded with the identification of stem cell-derived biobridges. As mentioned previously, this novel mechanism was first captured in a rat TBI model and later with a rat stroke model. Transplanted MSCs can remodel the ECM, permitting their migration to the neurogenic niches. In their wake, they leave a path that is accessible to activated endogenous stem cells, and as a result, they can migrate longer distances in far greater numbers. In the TBI animals, using laser capture assay and immunohistochemistry, MSCs were observed along a path from injured cortex to the lateral ventricles, particularly at earlier time points.[20] At later time points, endogenous stem cells were visualized alongside the exogenous stem cells and continued using this pathway through the ECM even when the presence of MSCs had largely diminished. To further understand the mechanism of stem cell-derived biobridge formation, levels of extracellular matrix metalloproteinases (MMPs) were evaluated, specifically MMP-9, chosen for its involvement in central nervous system (CNS) plasticity and neural progenitor cell migration, which is influenced by injury-induced chemokines.[28] Observed over the same trajectory as the biobridge foraged by the relocation of the grafted MSCs was elevated levels of MMP-9. Within stem cell treated animals, MMP-9 expression levels increased 9-fold along the biobridge.[20] This data suggest MMP-9 plays a critical role in the ECM remodeling required for biobridge formation. In addition, inhibition of MMPs hinders the effective migration of cells from SVZ to the injured cortex.[29] As with any tissue injury, the ECM takes part in an active role in stroke pathology and functional recovery[1,12] making it an important therapeutic target. There is evidence of alterations in MMP concentrations and function caused by stem cells of various sources such as umbilical cord and peripheral blood, and the adult brain, which subsequently influences changes in the ECM.[16,30,31] Additional research will be required to understand the effects of various concentration and type of MMPs on biobridge formation and endogenous promotion of stroke recovery.

Biobridge Role in Aiding the Endogenous Response

Researchers often attempt to enhance stem cell engraftment through various forms of combination treatments; however, the TBI study mentioned above (as well as many other reports) suggests that significant survival of the transplanted stem cells is not necessary to promote neuroprotection. Not only was this observed through improvement in motor and cognitive test performance but also with histological analysis as well. In regard to biobridge formation, the MSCs were gradually replaced by the host's stem cells which were seen to continue their spread from SVZ to the injured cortex. Through the action of MMP-9 secretion, transplanted MSCs are able to travel throughout the brain parenchyma. These MSC's provide the ECM remodeling that endogenous stem cells are able to take advantage of. Without the assistance of stem cells in "clearing a path" and promoting endogenous cell proliferation, there is not significant movement of host cells from SVC to distal damaged tissues.[20] This newly discovered mechanism of action by MSCs provides a potential solution to the shortcomings of the endogenous repair response within the brain, namely migration, differentiation and proliferation limitations. To the extent of 3 months post-TBI, and only in stem cell treated groups, cells double labeled with nestin and doublecortin were captured in a solid stream from the SVZ, across the corpus callosum and within the site of injury.[20]

In addition to providing evidence that negates the need for long-term stem cell survival, the pilot study regarding biobridge formation in stroke suggests invasive techniques may also be unnecessary for all stroke patients. While other studies have shown recovery with IV transplantation of stem cells, our recent unpublished results were able to capture the same biobridge behavior with this delivery method. Different patterns of migration were observed in IC versus IV groups, but the end product was comparable with the transplanted exogenous stem cells activating endogenous stem cells to migrate and eventually recruited to the injury site. This being a preliminary study, larger sample sizes and additional analysis will be required to solidify the observed migration patterns of exogenous and endogenous stem cells postischemia. Given the predictable location of infarct within the striatum when

middle cerebral artery occlusion is performed, it will also be important to analyze migration from the SGZ to the injured site. Much like microglia migration from posterior regions such as the hippocampus, MSCs and newly formed neural stem cells may be able to utilize the extensive white matter tracks to reach the striatum. These reports will further detail the role of biobridge formation in the stroke brain.

The Influence of Stroke Pathology Biobridge

As with any potential therapeutic drug for stroke, there is the issue of varying severity and location of insult. These factors influence the progression of stroke pathology in different ways and therefore, to optimize recovery in an individual patient, treatment strategies should differ between patients of dissimilar stroke presentations. It is likely that the extent of microenvironment alterations will affect the capacity for MSCs to facilitate biobridge formation. Particularly at the chronic stages, when the amount of dead and dying cells of the infarct core has increased and the penumbra has expanded to the previously healthy adjacent tissue, biobridge formation may be limited to its integrity and capacity to promote regeneration. Additional studies will be required to access biobridge formation across late stage stem cell injections, as well as the efficacy of MSC injections in humans across chronic time points.

Another important consideration is the degree of neuroinflammation within the stroke brain overtime. Neuroinflammation is pivotal to some degree in the acute phase for the process of brain injury recovery but becomes detrimental overtime. The protective acute neuroinflammation promotes removal of debris and dead cells and in the case of stem cell treatment may also serve to recruit stem cells to the sight of injury. The hypothesis stands that neuroinflammation provides the chemoattractants that cue the migration of stem cells. However, in the case of persistent inflammation within the injured brain, biobridge formation becomes a more difficult task for the MSCs to complete. With widespread pro-inflammatory signals, an increase in dead/dying cells, and an extended toxic microenvironment, MSCs may be limited in the capacity to overcome these hurdles propagated by chronic neuroinflammation.

Conclusion

According to the American Heart Association, approximately 795,000 Americans suffer from stroke annually contributing to an estimated 1 in every 20 deaths in the United States.[32] Between 2001 and 2011, the stroke mortality rate has dropped roughly 21.2%. However, the cost of healthcare has increased dramatically.[32] Together, the health-care costs related to moderate to the severe disability of stroke survivors is estimated at $17.5 billion, and if also accounting for lost productivity and premature mortality, this amount raises to a staggering $33.6 billion.[32]

With outrage expenses attributed to stroke and few treatment options available, the demand for effective therapies cannot be overstated. While stroke is the most common disease of the CNS, it should be mentioned that other disorders such as TBI, PD, and SCI with comparable disease progression are similarly lacking in available treatments. Stem cells, with their capacity to target many aspects of complex brain pathologies through cell replacement and bystander effects, make for a unique therapy in which biology fights biology, instead of traditional medicines which rely on a ligand-receptor paradigm.[33,34] Our previous and ongoing projects continue to emphasize the many novelties of stem cells treatments in the context of neurological disorders.[20] The newly discovered phenomenon of biobridge formation pioneered by MSCs has displayed its therapeutic relevance to both stroke and TBI and is predicted to extend to other disorders such as PD and SCI.

In summary, stem cells are a multipronged therapeutic agent capable of making the promise of regenerative medicine achievable. Cell replacement, by standard-effects such as secretion of growth factors and anti-inflammatory signals, and biobridge development are all mechanisms of action elicited by stem cell therapies which work together simultaneously, accounting for the robust recovery seen after these treatments.[20] The acknowledgment of biobridge formation further details the interactions between exogenous and endogenous stem cells, helping to explain the role of stem cell transplantation in the promotion of neurogenesis through the optimization of the endogenous injury response.

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Conflicts of interest
There are no conflicts of interest.

References

1. Borlongan CV. Bone marrow stem cell mobilization in stroke: A 'bonehead' may be good after all! Leukemia 2011;25:1674-86.
2. Barha CK, Ishrat T, Epp JR, Galea LA, Stein DG. Progesterone treatment normalizes the levels of cell proliferation and cell death in the dentate gyrus of the hippocampus after traumatic brain injury. Exp Neurol 2011;231:72-81.
3. Jaskelioff M, Muller FL, Paik JH, Thomas E, Jiang S, Adams AC, *et al*. Telomerase reactivation reverses tissue degeneration in aged telomerase-deficient mice. Nature 2011;469:102-6.
4. Wang L, Chopp M, Teng H, Bolz M, Francisco MA, Aluigi DM, *et al*. Tumor necrosis factor α primes cerebral endothelial cells for

erythropoietin-induced angiogenesis. J Cereb Blood Flow Metab 2011;31:640-7.
5. Yasuda A, Tsuji O, Shibata S, Nori S, Takano M, Kobayashi Y, *et al*. Significance of remyelination by neural stem/progenitor cells transplanted into the injured spinal cord. Stem Cells 2011;29:1983-94.
6. Mezey E. The therapeutic potential of bone marrow-derived stromal cells. J Cell Biochem 2011;112:2683-7.
7. Andres RH, Horie N, Slikker W, Keren-Gill H, Zhan K, Sun G, *et al*. Human neural stem cells enhance structural plasticity and axonal transport in the ischaemic brain. Brain 2011;134:1777-89.
8. Liu Z, Li Y, Zhang RL, Cui Y, Chopp M. Bone marrow stromal cells promote skilled motor recovery and enhance contralesional axonal connections after ischemic stroke in adult mice. Stroke 2011;42:740-4.
9. Hargus G, Cooper O, Deleidi M, Levy A, Lee K, Marlow E, *et al*. Differentiated parkinson patient-derived induced pluripotent stem cells grow in the adult rodent brain and reduce motor asymmetry in parkinsonian rats. Proc Natl Acad Sci U S A 2010;107:15921-6.
10. Mazzocchi-Jones D, Döbrössy M, Dunnett SB. Embryonic striatal grafts restore bi-directional synaptic plasticity in a rodent model of huntington's disease. Eur J Neurosci 2009;30:2134-42.
11. Lee HS, Bae EJ, Yi SH, Shim JW, Jo AY, Kang JS, *et al*. Foxa2 and nurr1 synergistically yield A9 nigral dopamine neurons exhibiting improved differentiation, function, and cell survival. Stem Cells 2010;28:501-12.
12. Yasuhara T, Hara K, Maki M, Mays RW, Deans RJ, Hess DC, *et al*. Intravenous grafts recapitulate the neurorestoration afforded by intracerebrally delivered multipotent adult progenitor cells in neonatal hypoxic-ischemic rats. J Cereb Blood Flow Metab 2008;28:1804-10.
13. Yasuhara T, Matsukawa N, Hara K, Yu G, Xu L, Maki M, *et al*. Transplantation of human neural stem cells exerts neuroprotection in a rat model of Parkinson's disease. J Neurosci 2006;26:12497-511.
14. Seol HJ, Jin J, Seong DH, Joo KM, Kang W, Yang H, *et al*. Genetically engineered human neural stem cells with rabbit carboxyl esterase can target brain metastasis from breast cancer. Cancer Lett 2011;311:152-9.
15. Yasuhara T, Matsukawa N, Hara K, Maki M, Ali MM, Yu SJ, *et al*. Notch-induced rat and human bone marrow stromal cell grafts reduce ischemic cell loss and ameliorate behavioral deficits in chronic stroke animals. Stem Cells Dev 2009;18:1501-14.
16. Pollock K, Stroemer P, Patel S, Stevanato L, Hope A, Miljan E, *et al*. A conditionally immortal clonal stem cell line from human cortical neuroepithelium for the treatment of ischemic stroke. Exp Neurol 2006;199:143-55.
17. Taguchi A, Sakai C, Soma T, Kasahara Y, Stern DM, Kajimoto K, *et al*. Intravenous autologous bone marrow mononuclear cell transplantation for stroke: Phase1/2a clinical trial in a homogeneous group of stroke patients. Stem Cells Dev 2015;24:2207-18.
18. Redmond DE Jr., Bjugstad KB, Teng YD, Ourednik V, Ourednik J, Wakeman DR, *et al*. Behavioral improvement in a primate parkinson's model is associated with multiple homeostatic effects of human neural stem cells. Proc Natl Acad Sci U S A 2007;104:12175-80.
19. Lee JP, Jeyakumar M, Gonzalez R, Takahashi H, Lee PJ, Baek RC, *et al*. Stem cells act through multiple mechanisms to benefit mice with neurodegenerative metabolic disease. Nat Med 2007;13:439-47.
20. Tajiri N, Kaneko Y, Shinozuka K, Ishikawa H, Yankee E, McGrogan M, *et al*. Stem cell recruitment of newly formed host cells via a successful seduction? Filling the gap between neurogenic niche and injured brain site. PLoS One 2013;8:e74857.
21. Merson TD, Bourne JA. Endogenous neurogenesis following ischaemic brain injury: Insights for therapeutic strategies. Int J Biochem Cell Biol 2014;56:4-19.
22. Broughton BR, Reutens DC, Sobey CG. Apoptotic mechanisms after cerebral ischemia. Stroke 2009;40:e331-9.
23. Bond AM, Ming GL, Song H. Adult mammalian neural stem cells and neurogenesis: Five decades later. Cell Stem Cell 2015;17:385-95.
24. Gould E. How widespread is adult neurogenesis in mammals? Nat Rev Neurosci 2007;8:481-8.
25. Zhang RL, Chopp M, Gregg SR, Toh Y, Roberts C, Letourneau Y, *et al*. Patterns and dynamics of subventricular zone neuroblast migration in the ischemic striatum of the adult mouse. J Cereb Blood Flow Metab 2009;29:1240-50.
26. Yamashita T, Ninomiya M, Hernández Acosta P, García-Verdugo JM, Sunabori T, Sakaguchi M, *et al*. Subventricular zone-derived neuroblasts migrate and differentiate into mature neurons in the post-stroke adult striatum. J Neurosci 2006;26:6627-36.
27. Huttner HB, Bergmann O, Salehpour M, Rácz A, Tatarishvili J, Lindgren E, *et al*. The age and genomic integrity of neurons after cortical stroke in humans. Nat Neurosci 2014;17:801-3.
28. Barkho BZ, Munoz AE, Li X, Li L, Cunningham LA, Zhao X, *et al*. Endogenous matrix metalloproteinase (MMP)-3 and MMP-9 promote the differentiation and migration of adult neural progenitor cells in response to chemokines. Stem Cells 2008;26:3139-49.
29. Zhao BQ, Wang S, Kim HY, Storrie H, Rosen BR, Mooney DJ, *et al*. Role of matrix metalloproteinases in delayed cortical responses after stroke. Nat Med 2006;12:441-5.
30. Lin CH, Lee HT, Lee SD, Lee W, Cho CW, Lin SZ, *et al*. Role of HIF-1α-activated epac1 on HSC-mediated neuroplasticity in stroke model. Neurobiol Dis 2013;58:76-91.
31. Sobrino T, Pérez-Mato M, Brea D, Rodríguez-Yáñez M, Blanco M, Castillo J, *et al*. Temporal profile of molecular signatures associated with circulating endothelial progenitor cells in human ischemic stroke. J Neurosci Res 2012;90:1788-93.
32. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, *et al*. Heart disease and stroke statistics–2015 update: A report from the american heart association. Circulation 2015;131:e29-322.
33. Acosta SA, Tajiri N, Shinozuka K, Ishikawa H, Sanberg PR, Sanchez-Ramos J, *et al*. Combination therapy of human umbilical cord blood cells and granulocyte colony stimulating factor reduces histopathological and motor impairments in an experimental model of chronic traumatic brain injury. PLoS One 2014;9:e90953.
34. Pastori C, Librizzi L, Breschi GL, Regondi C, Frassoni C, Panzica F, *et al*. Arterially perfused neurosphere-derived cells distribute outside the ischemic core in a model of transient focal ischemia and reperfusion *in vitro*. PLoS One 2008;3:e2754.