Bone marrow-derived NCS-01 cells for ischemic stroke

Madeline Saft, Minako Koga¹, Cesario V. Borlongan²

Abstract:
Stroke stands as one of the most common causes of death among adults worldwide. Currently, tissue plasminogen activator serves as the only approved drug by the Food and Drug Administration for the treatment of acute ischemic stroke. Stem cell therapy serves as a viable treatment option and has been deemed as a safe and effective treatment for stroke patients. Adult human bone marrow-derived NCS-01 cells serve as a potential treatment for stroke given their ability to reduce stroke-induced pathological deficits by increasing cell viability and mitochondrial activity. Recently, we demonstrated the use of adult bone marrow-derived NCS-01 cells both on both in vitro and in vivo models. Using NCS-01 cells in rat stroke models subjected to middle cerebral artery occlusion, an effective dosage of $7.5 \times 10^6$ cells/ml, administered through the intracarotid artery within 3 days poststroke, was shown to display significant improvements in motor and neurological behaviors, reductions in infarct area, and peri-infarct cell loss. NCS-01 cells, in comparison with other lines of stem cells (Li cells), are shown to produce greater therapeutic effects, most likely due to the observed filopodia formation that allows the stem cells to extend and target the ischemic cells. Given these findings, NCS-01 stem cells serve as a potential treatment for stroke through the demonstration of profound efficacy and further research that favors their filopodia-mediated mechanism of action.

Keywords:
Filopodia formation, intracarotid artery, ischemia, NCS-01 cells, stroke

Introduction: Bone Marrow-derived NCS-01 Cells

The emergence of stem cell research provides evidence of exogenous and endogenous repair processes of the central nervous system.¹⁻⁴ Transplantation of adult bone marrow-derived stem cells, including mesenchymal stem cells (MSCs), demonstrates ease in isolation and amplification and has been further explored as donor cells for diseases such as Parkinson’s disease,⁶⁻⁸ amyotrophic lateral sclerosis,⁹⁻¹¹ Alzheimer’s disease,¹²,¹³ and stroke.¹⁴⁻¹⁶ The current mechanism of action for MSCs involves bystander repair processes through stem cell-secreted therapeutic factors.¹⁷⁻¹⁹ In addition, MSCs demonstrate immunomodulatory functions that are altered in response to host inflammatory molecules.²⁰ However, the use of MSCs for stem-cell therapy has been called into question as clinical trials have failed to reveal efficacy on a few occasions. Transplantation of bone marrow MSCs at 4 weeks poststroke demonstrated positive neurological outcomes, but improvements seemed to diminish 12 months after transplantation.²¹ This evidence suggests that the transplantation of MSCs follows a strict dose and therapeutic time window and may explain why clinical trials have failed to turn out reproducible results. Since MSCs have demonstrated neuroprotective effects, further testing should be warranted to show the potential of MSCs for stem cell therapy in stroke.

In order to identify transplantable bone marrow-derived MSCs, the cells must follow specific criteria including human origin, clinical grade, ample supply, and...
well-defined phenotypic markers. Adult bone marrow-derived NCS-01 cells satisfy these criteria and demonstrate phenotypic characteristics that will allow these cells to be compared to previous MSCs transplantation studies. In vitro oxygen glucose deprivation (OGD) models and in vivo middle cerebral artery occlusion (MCAO) models can be utilized to examine the mechanism of action of NCS-01 cells. Preclinical data have been accessed to proceed with clinical trials for the clinical application of intracarotid artery (ICA) transplantation of NCS-01 cells.

**Determining Correct Dosage and Route of Administration for NCS-01 Cells**

NCS-01 cells serve as a potential cell-based therapy for stroke patients and the mechanism of action can be evaluated in both in vitro and in vivo studies. In the OGD in vitro model, NCS-01 cells can be used to repair ischemic cells in a dose-dependent manner. In in vivo studies, delivery of NCS-01 cells administered through the ICA demonstrated a reduction in infarct size and was dose-dependent as well. In addition, cultured NCS-01 cells demonstrated therapeutic molecule secretion as a mechanism of action that mediates the cells. ICA cell delivery was shown to be more effective in reducing infarct size in comparison to cells administered intravenously (IV). This may be due to the fact that ICA cell delivery can target more cells and therapeutic molecules into the brain and damaged site. Studies have demonstrated an effective dosage of $7.5 \times 10^6$ cells in 1 ml administered through ICA cell delivery. $7.5 \times 10^5$ in 0.1 ml could also be pursued in future studies as a potential minimum dosage for stroke models.

**Neuroprotective Properties of NCS-01 Cells in Comparison with Other Mesenchymal Stem Cells**

In comparison with other MSCs, such as Li cells, in vitro studies show that both cell types can rescue OGD-induced host cell death. NCS-01 increased cytokine (interleukin-6 and basic fibroblast growth factor [IL-6 and bFGF]) release more than Li cells. Even though both types of cells are characterized as MSCs, NCS-01 cells differ slightly. In addition, NCS-01 cells improved mitochondrial activity of EPCS, astrocytes, and neurons and support the modes of action to rescue host cells in vitro. This is a crucial finding as mitochondrial dysfunction has been recognized as a significant feature that contributes to neural damage following an ischemic stroke. NCS-01 cells administered ICA improved brain infarction and neurological deficits, while Li cells did not. Therefore, given the same doses of MSCs, NCS-01 cells produced greater therapeutic effects than Li cells.

**Filopodia Formation and Interleukin-6 and Basic Fibroblast Growth Factor Treatment**

NCS-01 cells demonstrate a mechanism of action involving filopodia formation under stroke conditions. As depicted in Figure 1, these stem cells exhibit the ability to travel long distances and reach their targeted site and suggest the potential use of NCS-01 cells to be administered in an environment remote from the initial brain insult, but still reach the injured cells. NCS-01 also demonstrates the overexpression of transmembrane glycoprotein CD44 in vitro that promotes the elongation and spread of filopodia and in vivo accelerates the migration and invasion of perivascular sites. The observed filopodia formation and transendothelial migration may be facilitated by adhesion molecules, such as Ninjurin 1, and transcription factors, such as the serum response factor. The role of transmembrane glycoproteins, adhesion molecules, and transcription factors can be further analyzed to produce the strong outcomes of NCS-01 cells in stroke.

**Usage of NCS-01 for Transient or Permanent Middle Cerebral Artery Occlusion**

ICA delivery of NCS-01 displays the ability to improve stroke-induced impairments for both transient and permanent MCAO. However, improvements in stroke animals were observed to be greater for those subjected to transient MCAO. Given the effective dosage of $7.5 \times 10^6$ cells in 1 ml, delivery of cells 3 days post-MCAO produced strong therapeutic effects, but delayed treatment beyond 3 days did not provide as strong results. It is important to note that cells delivered up to 1 week after MCAO did demonstrate a significant recovery and provide insight on the capabilities of NCS-01 cells and their wide therapeutic window. Therefore, administration of NCS-01 cells early in transient MCAO yields the most effective treatment. These findings suggest that treatment is most beneficial in patients within <3 days of initial ischemic stroke onset.
As stroke is still one of the most prevalent disabilities worldwide, there is a significant need for discovering a plausible treatment. Since stem cell therapy has emerged as a promising method of treatment, NCS-01 demonstrates astounding therapeutic properties, such as cell secretion of bFGF and IL-6 and the ability of filopodia to extend and reach the ischemic cells.[1] Therefore, NSC-01 cells serve as a potential treatment option for stroke.

Stroke preclinical studies have shown the therapeutic potential of a myriad of stem cells. NCS-01 cells, derived from the bone marrow with minimal cell culture manipulations, rescue cell death, decrease infarct size, and improve neurological outcomes. The optimal minimal dosage for NCS-01 cells is 7.5 × 10⁶ cells in 1 ml and is best administered through the ICA route.[1] NCS-01 is most effective in reducing infarct volume and neurological deficits a few hours poststroke, but are still effective up to a few days poststroke. Given this wide time range for administration, NCS-01 cells could be used to treat a larger number of patients. In addition, NCS-01 is effective for both transient and permanent MCAO occlusions and could be used on patients that were unable to receive revascularization procedures such as tissue plasminogen activator or endovascular interventions.[1] Finally, NSC-01 cells release high levels of cytokines bFGF and IL-6 and differ from other harvested forms of MSCs. The observed filopodia formation of NCS-01 cells warrants further research on cell processes between normal and ischemic tissues and could demonstrate that tissues closest to the infarct area may be optimal for stem cell survival. The ability of NCS-01 cells to form filopodia over long distances serves as a ground-breaking repair mechanism that allows remote regeneration of ischemic cells. Thus, NCS-01 cells serve as a novel therapeutic option for stroke that can provide strong neuroprotective properties.

**Conclusion**

In conclusion, NCS-01 cells demonstrate the potential to improve neurological and motor behaviors poststroke by reducing the infarct area and cell loss in neighboring regions as well. This mechanism of action involves filopodia formation and secretion of therapeutic molecules. NCS-01 cells secrete bFGF and IL-6 under OGD in vitro conditions and also might secrete other cytokines and cell-surviving factors.[1] In addition, ICA delivery of NCS-01 cells demonstrates the ability to reach the highest number of damaged cells. Both in vitro and in vivo models follow a dose-dependent mechanism and the best time to deliver the cells is within 3 days post-MCAO.[1] Finally, NCS-01 cells can be used to improve neurological deficits through a broad therapeutic window and sheds light on the potential use of these cells as therapy for stroke patients.

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**Conflicts of interest**

Prof. Cesario V. Borlongan is Associate Editor of *Brain Circulation*.

**References**

1. Kaneko Y, Lee JY, Tajiri N, Tuazon JP, Lippert T, Russo E, et al. Translating intracarotid artery transplantation of bone marrow-derived NCS-01 cells for ischemic stroke: Behavioral and histological readouts and mechanistic insights into stem cell therapy. Stem Cells Transl Med 2020;9:203-20.
2. Benjamin EJ, Blaha MJ, Chiuve SE, Cushman M, Das SR, Deo R, et al. Heart disease and stroke statistics-2017 update: A report from the American Heart Association. Circulation 2017;135:e146-603.
3. Go AS, Mozafarian D, Roger V, Benjamin EJ, Berry JD, Blaha MJ, et al. Executive summary: Heart disease and stroke statistics-2014 update: A report from the American Heart Association. Circulation 2014;129:399-410.
4. Kim JS, iP A helpers in the treatment of acute ischemic stroke: Are they ready for clinical use? J Stroke 2019;21:160-74.
5. Hess DC, Borlongan CV. Stem cells and neurological diseases. Cell Prolif 2008;41 Suppl 1:94-114.
6. Chen D, Fu W, Zhuang W, Lv C, Li F, Wang X. Therapeutic effects of intranarial transplantation of mesenchymal stem cells in rat models of Parkinson's disease. J Neurosci Res 2017;95:907-17.
7. Levy YS, Bahat-Stroomza M, Barzilay R, Burshtein A, Bulvik S, Barhum Y, et al. Regenerative effect of neural-induced human mesenchymal stromal cells in rat models of Parkinson's disease. Cytotherapy 2008;10:159-172.
8. Sadan O, Bahat-Stromza M, Barhum Y, Levy YS, Pinskensky A, Peretz H, et al. Protective effects of neurotrophic factor-secreting cells in a 6-OHDA rat model of Parkinson disease. Stem Cells Dev 2009;18:1179-1190.
9. Boido M, Piras A, Valsecchi V, Spigolon G, Mareschi K, Ferrero I, et al. Human mesenchymal stromal cell transplantation modulates neuroinflammatory milieu in a mouse model of amyotrophic lateral sclerosis. Cytotherapy 2014;16:1059-72.
10. Forostyak S, Homola A, Turnovcova K, Svitil P, Jendelova P, Sykova E. Intrathecal delivery of mesenchymal stromal cells protects the structure of altered perineuronal nets in SOD1 rats and amends the course of ALS. Stem Cells 2014;32:3163-72.
11. Marconi S, Bonaconsa M, Scandi I, Squintani GM, Rui W, Turano E, et al. Systemic treatment with adipose-derived mesenchymal stem cells ameliorates clinical and pathological features in the amyotrophic lateral sclerosis murine model. Neuroscience 2013;248:333-43.
12. Bae JS, Jin HK, Lee JK, Richardson IC, Carter JE. Bone marrow-derived mesenchymal stem cells contribute to the reduction of amyloid-β deposits and the improvement of synaptic transmission in a mouse model of pre-dementia Alzheimer's disease. Curr Alzheimer Res 2013;10:524-31.
13. Lee HJ, Lee JK, Carter JE, Chang JW, Oh W, et al. Human umbilical cord blood-derived mesenchymal stem cells improve neuropathology and cognitive impairment in an Alzheimer's disease mouse model through modulation of neuroinflammation. Neurobiol Aging. 2012;33:588-602.
14. Acoza SA, Tajiri N, Hoover J, Kaneko Y, Borlongan CV. Intravenous bone marrow stem cell grafts preferentially migrate to spleen and abrogate chronic inflammation in stroke. Stroke 2015;46:2616-27.
15. Borlongan CV. Concise review: Stem cell therapy for stroke patients: Are we there yet? Stem Cells Transl Med 2019;8:983-8.
16. Eckert MA, Vu Q, Xie K, Yu J, Liao W, Cramer SC, et al. Evidence for high translational potential of mesenchymal stromal cell therapy to improve recovery from ischemic stroke. J Cereb Blood Flow Metab 2013;33:1322-34.

17. Bliss T, Guzman R, Daadi M, Steinberg GK. Cell transplantation therapy for stroke. Stroke 2007;38:817-26.

18. Chen X, Li Y, Wang L, Katakowski M, Zhang L, Chen J, et al. Ischemic rat brain extracts induce human marrow stromal cell growth factor production. Neuropathology 2002;22:275-9.

19. Liu X, Ye R, Yan T, Yu SP, Wei L, Xu G, et al. Cell based therapies for ischemic stroke: From basic science to bedside. Prog Neurobiol 2014;115:92-115.

20. Lyden J, Grant S, Ma T. Altered metabolism for neuroprotection provided by mesenchymal stem cells. Brain Circ 2019;5:140-4.

21. Bang OY, Lee JS, Lee PH, Lee G. Autologous mesenchymal stem cell transplantation in stroke patients. Ann Neurol 2005;57:874-82.

22. Deboux C, Ladraa S, Cazaubon S, Ghribi-Mallah S, Weiss N, Chaverot N, et al. Overexpression of CD44 in neural precursor cells improves trans-endothelial migration and facilitates their invasion of perivascular tissues in vivo. PLoS One. 2013;8:e57430.

23. Ahn BJ, Le H, Shin MW, Bae SJ, Lee EJ, Lee SY, et al. Ninjurin1 enhances the basal motility and transendothelial migration of immune cells by inducing protrusive membrane dynamics. J Biol Chem 2014;289:21926-36.

24. Scandaglia M, Benito E, Morenilla-Palao C, Fiorenza A, Del Blanco B, Coca Y, et al. Fine-tuned SRF activity controls asymmetrical neuronal outgrowth: Implications for cortical migration, neural tissue lamination and circuit assembly. Sci Rep. 2015;5:17470.

25. Russo E, Nguyen H, Lippert T, Tuazon J, Borlongan CV, Napoli E. Mitochondrial targeting as a novel therapy for stroke. Brain Circ 2018;4:84-94.