Review Article

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Bone marrow-derived NCS-01 cells for ischemic stroke

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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 7.5×10^6 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.[1-5] 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

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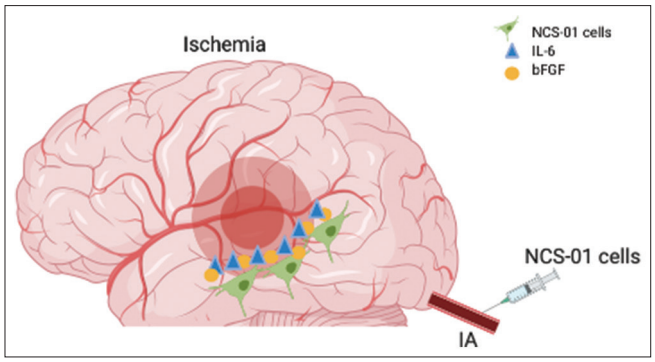

Figure 1: Brain circulation review 9

well-defined phenotypic markers.[1] 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.[1] 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 7.5×10^6 cells in 1 ml administered through ICA cell delivery.[1] 7.5×10^5 in 0.1 ml could also be pursued in future studies as a potential minimum dosage for stroke models.[1]

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 7.5×10^6 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.[1] 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.

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.[1] 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.[25] NCS-01 cells administered ICA improved brain infarction and neurological deficits, while Li cells did not.[1] 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. NSC-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.[22] The observed filopodia formation and transendothelial migration may be facilitated by adhesion molecules, such as Ninjurin 1,[23] and transcription factors, such as the serum response factor.[24] 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.

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 7.5×10^6 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*.

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