Mechanisms Underlying Improved Recovery of Neurological Function After Stroke in the Rodent After Treatment With Neurorestorative Cell-Based Therapies

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Abstract—We discuss the mechanisms of action underlying the beneficial effects of treating ischemic stroke in the rodent with exogenously administered cells. The essential hypothesis proposed is that the administered cells enhance recovery of neurological function by stimulating the production of restorative factors by parenchymal cells. These activated endogenous brain cells evoke white matter remodeling in the brain and the spinal cord and generate microenvironments within the injured brain that amplify brain plasticity and lead to improvement in neurological function poststroke. (Stroke. 2009;40[part 2]:000-000.)

Key Words: stroke ■ cell-based therapies ■ mechanisms ■ neurological function

Cell based therapies have shown remarkable ability to improve neurological function when administered after stroke onset.1 An outstanding question, however, is what are the mechanisms that contribute to this functional improvement? Given that many of the cells used in the treatment of neural injury and stroke are stem or progenitor cells, the early assumption was that these cells, as stem-like, replace dead neural tissue. However, the data on the treatment of stroke in the adult with cells do not support this hypothesis. Far too few cells actually enter brain to replace tissue; there is little or no evidence of true differentiation and integration and functional replacement of neural cells by stem cells in the injured brain. Marked functional improvement is present often within days after onset of treatment in the absence of any indication of replacement of cells. We therefore propose that cells stimulate the injured brain and evoke restorative events which remodel brain and lead to improvement of neurological function. Data presented will primarily focus on bone marrow stromal or mesenchymal cells (MSCs), as prototypical adult cells that may be employed to treat stroke.

MSC Induction of White Matter Changes

There is a significant increase in both progenitor and mature oligodendrocytes in the ipsilateral hemisphere of the ischemic brain after treatment with MSCs.2 Oligodendrocytes generate myelin and contribute to the integrity of white matter tracks in the brain. Stimulation and amplification of these cells may lead to restructuring of axons and myelin. White matter architecture in the vicinity of the ischemic lesion was altered by the cell treatment, and axonal density in the periinfarct area was significantly increased in the treated animals. Thus, MSC treatment appears to restructure white matter in the ipsilateral hemisphere.3

Axonal remodeling was evident in the contralateral hemisphere. Pseudorabies virus labeled with green fluorescent protein (PRV-GFP) and red fluorescent protein (PRV-RFP) were injected into the left and right extensor forelimb muscles4 four days before sacrifice, respectively. In normal rats, without stroke, the left pyramidal neurons show few green labeled cells. After stroke, there is a significant increase in green and yellow pyramidal neurons, indicating ipsilateral or transcallosal rewiring. Treatment with MSCs brought a significant increase in left hemisphere green and yellow pyramidal cells. Comparable cross rewiring is also present in the ipsilateral hemisphere. These data indicate that treatment of stroke with MSCs creates new circuitry in both the ipsilateral and contralateral hemispheres.

Response to treatment of stroke with MSCs extends beyond the brain to the spinal cord.5 Induction of stroke increases neurite extension from the intact to the denervated cord. This axonal rewiring is significantly enhanced by MSCs. Functional recovery is highly and significantly correlated with the extent of neurite extension from the intact to the affected spinal cord. These data suggest that functional recovery induced by treatment of stroke with a cell-based therapy is mediated by a broad array of changes...
in both the brain and the spinal cord. Using laser capture techniques, we found that MSCs significantly reduce the expression of inhibitory proteins within astrocytes, including Neurocan, and an array of inhibitory glycoproteins. These data suggest a pivotal role of the astrocyte in mediating brain and spinal cord plasticity after MSC treatment. In addition to the reduction of inhibitory glycoproteins that facilitate and make permissive neurite extension, the presence of MSCs stimulate production of an array of neurotrophic factors by the astrocytes that actively promote new circuitry and white matter remodeling. 

MSCs within the ischemic brain induce many growth factors within the parenchymal tissue, particularly within astrocytes. Signal transduction cascades are altered in cocultures of astrocytes with supernatant from MSCs. Human MSCs injected intravenously induce a significant increase in rat VEGF. Astrocytes also express angiopoietin 1 and its receptor Tie2, agents that contribute to both angiogenesis and the maturation of newly formed vessels.

The expression of these agents promotes vascular remodeling and induces angiogenesis and arteriogenesis, primarily in the boundary zone of the ischemic lesion. These newly formed vessels are important for tissue perfusion, but most likely provide their benefit from the factors expressed by angiogenic and newly formed vessels, such as BDNF, VEGF, VEGFR2 and matrix metalloproteinases, such as MMP-2 and -9. Thus, the astrocytes by expressing angiogenic factors may initiate a vascular niche, around which tissue is remodeled, forming new synapses and attracting endogenous neuroblasts originating in the subventricular zone.

Ipsilateral to the ischemic lesion, VEGF and VEGFR2, eNOS, as well as MMPs attract neuroblasts, and BDNF nurtures their survival. Coculture of SVZ cells with ischemic endothelial cells significantly increases SVZ proliferation and differentiation into neuronal phenotype, concomitantly with a reduction of differentiation of SVZ into astrocytes. Neuroblasts selectively migrate to blood vessels in the peri-lesion area. Using DCX-GFP mice, we have demonstrated that these SVZ cells persist and adhere to blood vessels 6 months poststroke (Zhang et al, unpublished data). Both VEGF and MMPs attract SVZ cells, and these factors likely mediate the attraction and localization of neuroblasts to vasculature.

Laser capture of cells from frozen tissue extracted from the SVZ demonstrates that the SVZ cells also express an array of angiogenic factors, including Angiopoietin 2, VEGFR2, and FGF. The SVZ cells by expressing these factors along with other factors, such as transforming growth factor β, induce angiogenic and local synaptic rewiring. Experiments using supernatant from SVZ cells with primary endothelial cells promote proliferation of these endothelial cells, suggesting that the vascular attraction of SVZ cells establishes a feedback loop for angiogenesis.

These data point to a strong coupling between the administered cells, in this case MSCs and parenchymal cells. MSCs stimulate parenchymal cells, likely by releasing cytokines, trophic factors, as well as connexins and bone morphogenic proteins. These factors upregulate the expression of oligodendrocytes, which may stimulate white matter remodeling. MSCs activate the production of trophic and vascular factors within the astrocyte. Astrocytes are highly responsive to the presence of exogenously administered cells and produce factors that promote brain plasticity, and, as we have shown here, also have a significantly reduced expression of inhibitory proteins for axonal and neurite outgrowth. This reduction of inhibitory factors along with a reduction of glial scar creates a permissive environment within the injured brain and spinal cord to promote neurite extension and axonal remodeling. These data suggest that new circuitry in the brain and spinal cord stimulated by exogenously administered cells likely contributes to functional recovery. Of note is that motor function recovery may also be coupled to remodeling of the spinal cord. Therefore, increased focus and attention should be paid to the response of the spinal cord to injury and the plasticity within the spinal cord and the cortical spinal track as mediating motor function recovery.

MSCs also induce within endothelial cells additional proangiogenic factors and trophic factors. The new and activated vessels then likely orchestrate further angiogenesis, attract neuroblasts to their locale, and enhance white matter and neurite plasticity. The neuroblasts which are attracted to vessels also subsequently further amplify the angiogenic response, leading to recovery.

This work describes series of interlocking neurorestorative events that are activated by the presence of exogenously administered cells. Essentially, the cells act as catalysts, with their potency to enhance recovery deriving from their action on parenchymal cells.

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References
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