Neurorestorative therapy post stroke[1]

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Summary

There are two approaches to treat stroke; a neuroprotective approach which focuses on the lesion and a neurorestorative one, directed to the entire CNS. The primary purpose of therapy for stroke is to improve neurological function. Neurorestorative therapy of stroke whether by cell-based or pharmacological agents is a viable and potentially important means to improve neurological function post stroke.

Key words: neurorestorative; stroke; cell-based; astocytes; recovery; white matter; pharmacological therapy

Introduction

Historically, treatment of stroke and brain injury has focused on neuroprotective approaches in which the objective was to reduce the volume of cerebral infarction. This approach, after decades of work, has yielded limited success and has provided us with thrombolytic therapy for stroke using tissue plasminogen activator (tPA), a treatment with a 4.5 hour therapeutic window and with a substantial risk of haemorrhagic transformation [1, 2]. In the United States, fewer than 5% of patients with ischemic stroke receive tPA, and those receiving this agent have only modest benefit. There is therefore a compelling need to develop novel therapies for stroke and neural injury based on neurorestoration, that is treating the intact and compromised cerebral tissue, and not neuroprotection, in which the lesion is treated. By developing agents which can be employed days and weeks after ictus, that essentially remodel the intact brain so as to compensate for the infarction, many more patients can be treated.

There are two complementary approaches to promote recovery of function after stroke; cell-based therapy and pharmacological therapy. This article briefly overviews some of the authors´ work in cell-based therapy for stroke in the adult which promotes brain remodelling and details how this therapy primarily stimulates parenchymal cells to produce factors that induce brain plasticity. This will then be followed by a brief overview of some of our efforts to stimulate recovery of function using pharmacological agents.

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Astrocytes mediate recovery of function in cell-based therapies

Exogenously administered cells to treat stroke primarily act as catalysts to stimulate parenchymal cells to induce remodelling of the brain [3, 4]. The key parenchymal cell is the astrocyte, which orchestrates recovery via the expression of important trophic factors, such as vascular endothelial growth factor (VEGF). Treatment of stroke in a rodent with human mesenchymal stromal cells (MSCs) induces an increase in rodent VEGF, which is readily distinguished from human VEGF. The expression of VEGF is primarily localised to astrocytes. VEGF has pleiotropic effects, including stimulating angiogenesis. There is a robust increase in angiogenesis in the boundary peri-infarct zone of animals treated with MSCs. These activated and angiogenic vessels subsequently produce other trophic factors including brain derived neurotrophic factor (BDNF), VEGF receptor 2 (VEGFR2), and nitric oxide synthase (NOS) which in combination with VEGF promote neurogenesis in the subventricular zone (SVZ). After stroke, there is a significant increase in neurogenesis in the SVZ. This increase is significantly amplified after treatment with cell-based therapies, such as with MSCs [5]. In the normal brain, newly formed cells in the SVZ migrate down the rostral migratory stream to the olfactory bulb. However, after stroke, cells, primarily neuroblasts from the SVZ, migrate to the boundary of the lesion and treatment with MSCs evokes a significant increase in the number of neuroblasts migrating to the boundary zone. These neuroblasts are selectively attracted to the microvasculature in the peri-infarct area. There is an exquisite coupling between the vascular cells and the neuroblasts. The vascular cells, via the VEGFR2, promote proliferation and neuronal differentiation of the migrating neuroblasts. Concomitantly, the neuroblasts promote additional angiogenesis. Thus, we have a tapestry of recovery where cell-based therapy stimulates parenchymal cells to produce factors, for example VEGF, which amplify angiogenesis. Angiogenesis couples with neurogenesis, leading to the migration and interaction of neuroblasts with endothelial cells to produce restorative microenvironments within the injured brain [6].

White matter changes induced by cell-based therapy

The ultimate therapeutic benefit and enhanced functional recovery evoked by cell-based therapy derives from den-

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dritic and axonal plasticity. The authors have demonstrated that treatment of neural injury with cell-based therapy amplifies neurite outgrowth that significantly correlates with, and likely evokes, functional recovery. The astrocyte also plays a major role in mediating neurite outgrowth. Using single cell laser capture technology, we have demonstrated that MSCs significantly reduce the mRNA for inhibitory glycoproteins [7]. A major reason for the failure of neurons in the central nervous system to grow is the presence of inhibitory molecules. Downregulation of these inhibitory proteins creates a permissive environment which promotes neurite outgrowth. The authors have demonstrated that Neurocan at the mRNA and protein levels, as well as other inhibitory proteins, are significantly downregulated after treatment of stroke with a cell-based therapy, such as MSCs. This reduction in inhibitory proteins leads to a significant increase in axonal density in the ischemic boundary zone compared to control animals. The increase in axonal density and changes in neurite architecture are not restricted to the immediate area of the lesion. In addition, there is a significant increase in neurite outgrowth in the contralateral hemisphere of the ischemic lesion. Using fibre tracing techniques, neurite outgrowth is shown to be significantly increased in the contralateral hemisphere. These newly remodelled neurites originated from the ipsilateral hemisphere, but transcallosally extended processes to the contralateral hemisphere. This neurite remodelling is highly and significantly correlated with functional recovery post stroke [8]. The focus of plasticity induced by cell-based therapies and the recovery of function in general has been in the brain. Few investigators have measured the response of the spinal cord architecture to stroke and particularly, cell-based therapies. Using fibre labelling methods, we have demonstrated that the spinal cord responds to cell-based treatment by significantly increasing neurite extension from the intact cord to the denervated cord, with a highly significant correlation between motor and somatosensory function and axonal extension into the affected cord [9]. In composite, these data indicate that brain remodelling, particularly neurite and axonal outgrowth, play a pivotal role in neurological recovery, and that remodelling occurs throughout the central nervous system (CNS) and is not only relegated to the site of injury. Cell-based therapies substantially enhance this white matter remodelling. Given the important role of white matter change on functional recovery, we sought ways to monitor white matter structural changes non-invasively. Magnetic resonance imaging (MRI), using variations of diffusion tensor imaging, fractional anisotropy, and Q-Ball, are highly sensitive to these changes in white matter and can be employed to identify altered white matter in cerebral tissue [10]. These techniques may prove very valuable for the management of patients.

Pharmacological agents for neurorestorative therapies

There are a number of agents which can be used to enhance brain plasticity similarly to cell-based therapies. Nitric oxide syntheses (NOS) are present in the developing brain and promote brain development. Taking this cue from the developing brain, we employed nitric oxide (NO) donor molecules to treat stroke, with treatment initiated no less than 1 day post stroke. The animals showed a significant improvement in neurological outcome. To delve deeper into the mechanisms of action of NOS, we proposed that NO increases cyclic guanosine monophosphate (cGMP) and that cGMP, which is a major signalling molecule, induces recovery of function. To test this hypothesis, we increased cGMP in the brain using an inhibitor of phosphodiesterase 5 (PDE5). PDE5 is the enzyme that degrades cGMP, and by inhibiting PDE5 we therefore effectively increase cGMP. A major PDE5 inhibitor is sildenafil (Viagra). We therefore treated animals post stroke with sildenafil and achieved significant and substantial increase in neurological functional recovery [11]. The authors have also employed sildenafil to treat locked-in patients and are completing a Phase 1 stroke study in the treatment of the acute stroke patients with sildenafil. There are other agents, which also increase cGMP, such as statins, which we have shown also greatly enhance neurological recovery post stroke and neural injury. Presently, we have a Phase I clinical trial for the treatment of patients suffering intracerebral haemorrhage. Agents which increase high density lipoproteins (HDL), such as slow release niacin (i.e. Niaspan), have been employed to treat stroke and have shown substantial neurological benefit when treatment is initiated days after stroke. Other neurorestorative agents under consideration and presently studied in our laboratory include, erythropoietin (EPO), carbamylated EPO (CEPO) and Thymosin B4.

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