The role of sildenafil in the treatment of stroke
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The structural underpinning responsible for neuroplasticity and neurorestoration under physiological and pathophysiological conditions is only beginning to be elucidated. It is evident that life-long neurogenesis occurs in the human brain, with experimental data supporting its upregulation following an insult (e.g., stroke) and/or in response to pharmacological therapy. Sildenafil, a PDE5 inhibitor currently marketed for the treatment of erectile dysfunction, enhances neurorestoration in rat models of stroke, as measured by neurogenesis, synaptogenesis and angiogenesis. This neurorestorative effect is associated with improved outcome despite no observed effect on brain infarct size. This neurorestorative effect has also been observed in both young and old animals, and is demonstrable even if therapy is initiated 1 week post-stroke. The extended therapeutic window and novel mechanism of action of neurorestorative therapies, such as sildenafil, warrant further investigation for the treatment of stroke.

Keywords Cyclic GMP, neurogenesis, neuroplasticity, neurorestoration, sildenafil, stroke

Table 1. A comparison of neuroprotection versus neurorestoration.

<table>
<thead>
<tr>
<th></th>
<th>Treatment window</th>
<th>General features</th>
<th>Outcome</th>
<th>Central or peripheral effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuroprotection</td>
<td>Limited (~ 3 to 6 h)</td>
<td>Reduces neuronal death within the ischemic penumbra</td>
<td>Reduction of injury (e.g., infarct); limiting of neuronal death; stability of function</td>
<td>Central</td>
</tr>
<tr>
<td>Neurorestoration</td>
<td>Expanded (days, possibly weeks or longer)</td>
<td>Enhances angiogenesis, neurogenesis or synaptogenesis; improves regional cerebral blood flow</td>
<td>Infarct reduction is not necessary for benefit; restoration of structure and brain reorganization; improvement in function</td>
<td>Unclear (central, peripheral or both)</td>
</tr>
</tbody>
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Introduction
In the US, stroke remains the leading cause of disability and the third largest cause of death after heart disease and cancer. Post-stroke medical and rehabilitation care costs approximately US $140,000 per patient, with the resultant net socioeconomic burden in the US estimated to be between US $60 to 70 billion [1]. With increasing longevity and the rising incidence of risk factors for stroke, including metabolic syndrome, current projections estimate that the rate of stroke in the US will increase to over one million per year by 2050 [2]. Despite this alarming societal burden, few options are available for acute stroke therapy. Although tissue plasminogen activator was approved for stroke thrombolysis over a decade ago [3], treatment guidelines generally restrict its use to a 3-h window. Because the majority of patients do not reach the hospital until many hours or even days after their stroke, only a small fraction of patients qualify for thrombolysis, with even fewer (~ 1 to 2%) receiving thrombolytic therapy [4]. Moreover, stroke has proven to be quite intractable to treatment (clinical trial design flaws notwithstanding) despite a myriad of neuroprotective (NP) strategies targeting a diverse group of mechanisms of action (MOAs) that have demonstrated promise in animal models [5].

Thus, in recent years, the stroke field has begun to move beyond NP to consider a novel approach to disease modification – neurorestoration (NR) [6]. This review addresses the current status of NR therapies for the treatment of stroke, using the example of the PDE5 inhibitor (PDE5i) sildenafil to discuss the challenges and opportunities encountered with this strategy.

NR and stroke
NR may be defined as any therapeutic intervention that provides a durable improvement in structure and function, and where the observed benefit is not associated with limiting the cell death/structural damage associated with the primary insult. Rather, NR is the result of neuroplasticity in response to the underlying pathophysiological event. Neurorestorative activities identified in the post-stroke brain include neurogenesis, synaptogenesis and angiogenesis [7,8]. In humans, stroke recovery is associated with neurogenesis [9,10], and with cortical remodeling and reorganization [11]. Neurorestorative agents for acute neurodegenerative diseases, such as stroke, typically retain efficacy when administered on a delayed basis, generally considered to be ≥ 24 h post-stroke. This timeframe is beyond the therapeutic window of opportunity associated with NP therapy. Agents which act as NP or NR following stroke may be complementary therapeutic strategies and may involve overlapping pathways of disease (Table 1).
A number of therapeutic approaches that could be considered NR are currently in clinical trials for stroke following favorable effects in animal models, including statins [12], erythropoietin and analogs [13-16], β human chorionic gonadotropin [13-15], growth factors (eg, granulocyte colony-stimulating factor) [17] and agents that increase cyclic GMP (cGMP; nitric oxide [NO] donors and PDE5is) (Table 2) [18,19].

Although the first report that neurogenesis is operant in the adult mammalian brain was presented over 40 years ago [20••], it is only during the last decade that it has been firmly established that new neurons are generated continuously from stem cells in certain regions of the adult brain in all studied mammals, including humans [21••]. The most active regions for neurogenesis are the subgranular zone of the hippocampal dentate gyrus, the subventricular zone (SVZ) and the olfactory bulb; these sites are also responsible for active neurogenesis during the embryologic period [22••]. Although it appears that neurogenesis wanes with increasing age [23], neurogenesis is enhanced in elderly stroke patients, with immature neurons identified within the ischemic penumbra [9•,10]. This is consistent with preclinical research that also demonstrated the capacity for enhanced neurogenesis, even in aged animals [8•,24].

The NO cascade and NR

Augmenting the NO cascade is one NR approach that is currently under examination. NO first began receiving attention when it was discovered that ‘endothelial-derived relaxing factor’ was NO [25•], an integral molecule involved with maintaining endothelial cell integrity, as well as participating in hemodynamic homeostasis (ie, blood pressure [BP] and flow). It is now understood that a variety of cells, including vascular smooth muscle cells and neurons, produce NO either constitutively or inducibly following perturbation (eg, inflammation) [26]. The increased expression of neuronal NO synthase within the SVZ during embryogenesis suggests a role for the NO pathway in neurogenesis [27]. Moreover, the administration of NO donors (iv) increases neurogenesis in the adult rat SVZ and dentate gyrus, suggesting an expanded role for the NO cascade beyond embryogenesis [28]. Treatment with NO donors beginning 24 h post-stroke in rat models (when the stroke is pathologically matured) is associated with increased neurogenesis and an improvement in functional outcome, despite no change in the infarct volume [28]. NO is also a potent activator of soluble guanylate cyclase, the enzyme that converts GTP to cGMP. Thus, the delivery of an NO donor increases cGMP levels within both ischemic and non-ischemic rat brains, suggesting a permissive role for NO in neurogenesis and that cGMP may serve, at least in part, as a downstream mediator of the NO effects [28].

In addition to enhancing cGMP levels by augmenting NO availability, cGMP levels may also be increased by inhibiting its metabolism by the PDE5 enzyme. The strategy of increasing the downstream mediator cGMP without affecting NO levels may be preferred due to the mixed outcomes in stroke reported in animal models following alterations in NO levels [29].

Sildenafil and stroke

The most studied of the PDE5i, both preclinically and clinically, is sildenafil [30,31••]. Thus, although there are preclinical reports of post-stroke administration of other PDE5i, this review focuses primarily on sildenafil. In rat models of middle cerebral artery (MCA) stroke, delayed sildenafil treatment initiated up to 1 week post-stroke stimulated neuronal stem cell proliferation within the SVZ and dentate gyrus, enhanced neovascularization and synaptic density, and improved functional and behavioral endpoints, despite no effect on brain infarct size [7,8•,24,32]. The delayed timing of drug administration, neuronal proliferation and other structural changes – all consistent with neuroplasticity – coupled with the lack of effect on

<table>
<thead>
<tr>
<th>Therapeutic(s)</th>
<th>Potential mechanism(s) of action</th>
<th>Clinical trial design</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-CSF (filgrastim)</td>
<td>Mobilizes peripheral blood stem cells</td>
<td>Window for initiating therapy: 3 to 30 days</td>
<td>[17]</td>
</tr>
<tr>
<td>Lovastatin</td>
<td>HMG-CoA reductase inhibitor; increased eNOS, VEGF, PI3K-AKT and growth factors</td>
<td>Duration of therapy and dosing: &gt; 24 h</td>
<td>[12]</td>
</tr>
<tr>
<td>NTG patch</td>
<td>Effects on NO-cGMP, PKG and/or PI3K-AKT</td>
<td>Window for initiating therapy: 48 h</td>
<td>[18]</td>
</tr>
<tr>
<td>NTx-265 (Stem Cell Therapeutics Inc)</td>
<td>β-hCG is thought to increase the number of NSCs. EPO is the second drug administered in the regimen, and aims to promote the differentiation of these newly formed NSCs into new neurons.</td>
<td>Window for initiating therapy: 24 to 48 h</td>
<td>[13-15]</td>
</tr>
<tr>
<td>Sildenafil</td>
<td>PDE5i</td>
<td>Window for initiating therapy: 4 to 7 days</td>
<td>[19]</td>
</tr>
</tbody>
</table>

β-hCG β human chorionic gonadotropin, cGMP cyclic GMP, eNOS endothelial nitric oxide synthase, EPO erythropoietin, G-CSF granulocyte colony-stimulating factor, NO nitric oxide, NSC neural stem cell, NTG nitroglycerin, PDE5i PDE5 inhibitor, PI3K phosphotyidylinositol-3-kinase, PKG phosphokinase G.
brain infarct size, even when administered within 2 h of the ischemic event [7], have provided support for an NR effect of sildenafil. Moreover, the benefit with sildenafil is seen in both young and aged animals [8,24], an important finding considering that the aged are the population most at risk for stroke. The PDE5i tadalafil also demonstrated enhanced recovery in a rat model of stroke following the initiation of therapy 24 h after stroke induction [33].

Literature on the clinical use of sildenafil post-stroke includes a case report outlining the recovery of a patient administered sildenafil (150 mg/day maintenance dose, initiated 10 days post-stroke) who had been ‘locked-in’ following a near total pontine infarction [34]. This report highlights the unanswered questions regarding the therapeutic window for treatment, as well as the optimal duration of therapy. Currently, there are ongoing clinical trials examining both nitrates [18] and sildenafil [19], where therapy is initiated 48 h and 4 to 7 days post-stroke, and continued for a limited duration (7 and 14 days, respectively; Table 2). Because NO is a vasodilator, the monitoring of BP is of considerable importance because BP lowering in the acute stroke period is usually contraindicated [35]. The clinical experience with sildenafil suggests that it is a modest vasodilator with a mean BP lowering effect typically < 10 Torr that is not dose-related (ie, maximal effect observed with a 50-mg dose) [36]. The use of sildenafil in combination with nitrates or α-adrenergic blockers is contraindicated [31].

Both the site (central versus peripheral nervous system) and the specific mechanism(s) responsible for the structural and functional improvements observed with sildenafil therapy following stroke are unclear. The effects of sildenafil may be centrally or peripherally mediated, or both. The PDE5 enzyme has been identified in many locations, with high levels in cerebellar Purkinje neurons (but otherwise much more modest brain expression), platelets and smooth muscle cells of blood vessels, including the cerebrovasculature [37,38]. Also, sildenafil crosses the blood-brain barrier (BBB) [39]. Moreover, the BBB at and around the site of injury is compromised following stroke in a biphasic manner; in the first few hours post-stroke and then again 24 to 48 h post-stroke [40]. This second, delayed, re-opening of the BBB may provide a window of opportunity for several days to allow even brain impenetrant molecules to gain CNS access and exert a central effect.

The effect of sildenafil may be peripheral, possibly mediated by an increase in cerebral blood flow (CBF). In healthy young rats, transient increases in local CBF (measured by laser doppler) after the administration of sildenafil were observed at a dose (2 mg/kg) associated with improved stroke outcome and, in a satellite cohort, increased brain cGMP levels [7]. In another study examining CBF post-stroke, treatment with sildenafil (10 mg/kg for 1 week beginning 24 h post-stroke) was associated with a significantly increased CBF within the ischemic boundary area of treated animals compared with the control group when studied for up to 6 weeks [41]. The effect of sildenafil on CBF was associated with increased angiogenesis, which was quantified histologically. Another PDE5i zaprinast (10 mg/kg) also increased penumbral CBF and brain cGMP in a rat model of MCA embolic stroke [42]. However, unlike sildenafil or tadalafil, the acute administration of zaprinast reduces brain infarct size, perhaps suggesting that not all PDE5is exert their beneficial effects through the same MOA.

In clinical trials, varying results have been reported on the effect of sildenafil on CBF. MCA blood flow and velocity were unchanged following the administration of sildenafil (100 mg) in healthy male volunteers [43,44]; however, other studies in men with erectile dysfunction [45] or patients with severe pulmonary hypertension [46] reported an improvement in cerebrovascular reactivity to varying CO2 levels following the administration of sildenafil (50 mg), supporting an effect on CBF in individuals with vascular disease. Nonetheless, the benefit of increasing CBF to the stroke penumbra on a delayed basis is unclear. Alternatively, an increase in CBF may improve post-stroke recovery primarily through an NR effect (eg, angiogenesis), rather than via NP.

The role of angiogenesis in NR is currently under investigation. Sildenafil upregulates the phosphoinositol-3-phosphate kinase (PI3K)/AKT pathway and also increases VEGF levels, with both mechanisms associated with an increase in angiogenesis (Figure 1). Angiogenesis may enrich the local environment by facilitating the delivery

Figure 1. Effects of sildenafil on neurorestoration.

[Diagram showing the effects of sildenafil on neurorestoration, with pathways involving NO → GC → cGMP → PKG → downstream effectors such as PDE5, cAMP, CREB, VEGF, and CBF.]

CBF, cerebral blood flow; cGMP, cyclic GMP; CREB, cAMP response element binding protein; GC, guanylate cyclase; NO, nitric oxide; PDE5i, PDE5 inhibitor; PI3K, phosphatidylinositol-3-kinase; PKG, phosphokinase G.
of chemotactic and growth factors that orchestrate neurogenesis and other NR activities, and otherwise provide support for local brain reorganization, rather than simply serving as alternative conduits for improving CBF to underperfused brain regions where reperfusion or collateral blood flow is insufficient.

The ability of sildenafil to positively influence the reparative/restorative capacity of the brain through effects on neurogenesis and neuronal architecture (eg, synaptogenesis) has been demonstrated in animal models of stroke [7]. The elevations in brain cGMP levels that are associated with enhanced neurogenesis are also associated with improved functional outcome. While Nogo-A and myelin associated glycoprotein (MAG) are thought to suppress neural regeneration following injury, elevated levels of cyclic nucleotides (eg, cGMP) block Nogo-A or MAG-induced inhibition of neurite outgrowth [47]. This NR activity appears to also be mediated through mechanisms further downstream from cGMP. For example, the PI3K/AKT pathway, which is associated with increased angiogenesis, also upregulates neurogenesis [48]. The cGMP-phosphokinase G (PKG) cascade also enhances the levels of cAMP response element binding protein (CREB), which regulates the expression and survival of immature neurons in the adult hippocampus [49].

Future studies are needed to further elucidate the role of neurogenesis in stroke outcome. These studies will need to assess not only the extent of increased neurogenesis (and/or neuronal survival) and their subsequent migration to the site of the lesion, but also their functional integration into the pre-existing neurocircuitry and the interaction of local neurons with the supporting glia [50••]. Neuronal migration to the site of the lesion may improve outcome by either directly replacing the lost neuronal pool or by otherwise supporting the ongoing re-organization and plasticity in the non-injured tissue. Finally, there are also emerging data that sildenafil may facilitate the evoked release of peptides from the neurohypophysis through activation of large conductance Ca2+-activated potassium channels [51]. Confirmation of these in vitro results in in vivo models awaits further investigation, but it is of interest that the neurohypophysis (a circumventricular organ) lies outside the BBB, thus facilitating drug delivery to the site of action. The role of the neural-hormonal system in brain repair remains unclear, but highlights that there may be additional variables to consider in understanding NR under both physiological and pathophysiological conditions. An improved understanding of the natural history of NR [52•, 53••] will facilitate the drug development of NR therapeutics seeking to optimize stroke outcome.

Conclusions

When considering disease-modifying therapies for stroke, NR is seen as an emerging and viable alternative or complementary therapeutic strategy to NP. Whereas NP therapeutics operate within a limited time frame, neurorestorative therapies may allow for a much wider window of opportunity, measured not in hours, but in days and perhaps weeks. This feature would allow for a far greater percentage of the patient population suffering from stroke to be considered for treatment. Currently, there are marketed agents being studied for NR that appear to have more pleotropic actions than were first envisaged. One example is sildenafil, which demonstrated improved CBF, neurogenesis, angiogenesis and synaptogenesis following experimental stroke, even when therapy is delayed for up to 1 week and aged animals are examined. In these studies, the improvements in functional outcome that occur despite no change in infarct volume are intriguing. Ultimately, functional improvements that are robust and durable will need to be demonstrated in clinical trials. Surrogate markers that may assist in answering this question include functional MRI or diffusion tensor imaging, which can demonstrate improvements in structure, organization and functional connectivity. The potential for a neurorestorative therapy to significantly impact stroke outcome, either as monotherapy or as a complement to thrombolysis (and perhaps a future NP therapy) warrants further investigation.

Acknowledgement

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References

- **of outstanding interest**
- **of special interest**


4. A landmark trial demonstrating the efficacy of tissue plasminogen activator in acute stroke.


10. An important translational study documenting the capacity for aged animals to experience similar degrees of post-stroke improvement to that observed in young animals.


12. This study builds upon earlier research in animals and humans by demonstrating the existence of post-stroke neurogenesis in humans.
Stem Cell Therapeutics announces that embryologic CNS development and brain plasticity after stroke and the implications of this finding for understanding the extent of brain being studied.


The role of sildenafil in the treatment of stroke


• A focused review of the role and inter-relationships of statins, erythropoietin, sildenafil/NO pathway and cell-based strategies as therapeutics in a new paradigm for stroke treatment.


• A contemporary overview of the natural history of NR following stroke.

• An overview of adult neurogenesis, limitations for self repair, and physiological and pharmacological strategies for upregulation.