Promoting Neurological Recovery in the Post-Acute Stroke Phase: Benefits and Challenges

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Abstract

Background: Profound cellular and biochemical remodeling processes occur in the brain following an ischemic stroke, raising the possibility that we may be able to promote neurological recovery by harnessing the brain's endogenous recovery processes with pharmacological or cell-based therapies. There is a compelling body of evidence that cerebral plasticity and neurological recovery can be stimulated in the post-acute ischemic brain in this manner. Summary: This overview of neurorestorative therapies highlights the main challenges in their development, and summarizes the implications of these findings to stroke patients. Key Message: Neurorestorative therapies are a highly promising avenue of treatment for the restitution of neuronal networks damaged by stroke. Clinical Implications: The key supporting data have so far been obtained using in vivo models in animals, underscoring the need to validate these findings in humans. For human studies, several potentially confounding variables should be kept in mind, including age, and the presence of risk factors and comorbidities (such as hyperlipidemia and diabetes). Stroke patients vary considerably in age and genetic background, as well as in the etiology, localization and size of brain infarcts. The choice of patient population is a critical factor in the success of clinical trials, as patient heterogeneity could mask any potential therapeutic benefits.

Following the failure of neuroprotection therapies [1, 2], efforts in stroke research have shifted from the acute to the post-acute stroke phase. Studies in rodents indicate that profound remodeling processes take place in the brain following an ischemic stroke [3, 4]. This has invigorated hopes that we may be able to promote neurological recovery by means of pharmacological or cell-based therapies. Remodeling of ischemic brain tissue involves interactions between neurons, glial and microvascular cells that create a microenvironment in which neurological recovery may ensue. Neurons and brain capillaries sprout. Neuronal outgrowth enables the formation of functional axons and synapses in the brain both over long (e.g., along pyramidal tract; [5, 6]) and short (e.g., within motor cortex; [7, 8]) distances, thus allowing for the recruitment and formation of neuronal networks and/or restitution of neuronal networks that were damaged by the stroke event. In the process of brain remodeling, proliferating microvascular cells play a supportive role, enabling the migration of neural precursor cells and promoting the remodeling of neurons and glial cells via secretion of growth factors [3]. This rearrangement of cell–cell interactions is followed by the resti-
tution of a functional blood-brain barrier, leading to the recuperation of brain homeostasis [9, 10].

Based on promising observations in animal models of ischemic stroke, clinical studies in human patients are currently in progress, the results of which are eagerly awaited. In these studies, plasticity-promoting growth factors (e.g., granulocyte colony-stimulating factor [G-CSF]), cell-based therapies (e.g., multipotent mesenchymal stromal cells [MSC]), neutralizing antibodies directed against axonal growth inhibitors (e.g., NogoA), and pharmacological compounds (e.g., the phosphodiesterase-5 inhibitor sildenafil) are analyzed (reviewed in [4]). While cell-based therapies, that is, based on stem and precursor cells of different origin, have initially been used with the aim of cell replacement, it was more recently recognized that the beneficial effects of stem cells depend on paracrine bystander effects, rather than on neuronal differentiation and integration [6, 11].

The interactions between neurons, glial cells and microvascular cells are finely tuned. They involve mutual cell communication via the release of growth factors and physical cell–cell interactions across the extracellular matrix that is itself subjected to remodeling processes after stroke [10]. In view of the complexity of these systems, taking into consideration both the structural and functional heterogeneities of brain structures and the heterogeneities of ischemic strokes with respect to their size, etiology, and localization [4], the development of neurorestorative therapies is a formidable challenge. Importantly, it was noted that neurorestorative processes in the adult brain may differ substantially depending on the presence of associated risk factors and comorbidities, and they are also influenced by ageing processes.

In this minireview, we highlight the benefits and challenges of neurorestorative therapies, pointing out ways how future study failures may be circumvented in an ischemic stroke. Minireviews, however, cannot replace the need for thorough literature assessments. For this purpose, the reader is referred to more detailed works of the authors, in which the concepts outlined here are presented in more detail (see references [3, 4, 9]).

Benefits of Neurorestorative Therapies

Neurorestorative therapies can be instituted over extended time windows in the stroke recovery phase. In rodents, beneficial effects on neurological recovery were reported even when treatments were initiated days, weeks, or months after stroke [5, 11–16]. In sharp contrast to neuroprotection, the efficacy of neurorestorative therapies does not depend on the successful reperfusion of tissue. Thus, neurorestorative therapies are efficacious even under conditions of permanent focal cerebral ischemia [12, 14, 17–19]. In view of the extended therapeutic window, and considering that tissue reperfusion is not a requirement, neurorestorative therapies can potentially be of benefit to all stroke patients.

Effects on Neuronal Plasticity

Subsequent to the anterograde Wallerian degeneration of injured axons, the surviving proximal axon segments (i.e., the axon stumps) reorganize along the infarct rim [19, 20] and grow out behind the site of injury [5, 21]. In the pyramidal tracts, the stroke event per se promotes axonal sprouting both ipsilateral [5, 21] and contralateral [8, 18, 21, 22] to the stroke. The endogenous responses are enhanced by neurorestorative treatments, which promote the sprouting of pyramidal tract axons originating from the contrallesional motor cortex that traverse the midline in order to reach neurons denervated by the stroke [5, 12, 14, 17, 18, 21, 23].

Importantly, this pattern of contrallesional long-distance axonal sprouting is relatively uniform for different types of treatment, namely growth factors (e.g., erythropoietin, vascular endothelial growth factor [VEGF]) [5, 21, 22], neutralizing antibodies directed against the axonal growth inhibitor NogoA [14, 17], neurostimulants (e.g., amphetamine) [24], and cell-based therapeutics, such as neural precursor cells (NPC) [12] and multipotent MSC [18]. Contrallesional plasticity as a basis for neurological recovery is observed in rats [12, 14, 17], mice [5, 18, 21], and macaque monkeys [25]. Stroke-related neuronal plasticity is evident not only in young, but also in aged animals [14, 26, 27]. Thus, in the experimental setting, there is solid evidence for axonal remodeling as a correlate of restorative therapies.

Effects on Angiogenesis

Following a stroke, angiogenic growth factors are released by neurons and glial cells that induce endothelial proliferation and sprouting [3, 28–30]. Acting on VEGF receptor-2 (VEGFR2) that is highly abundant at the tip of new-formed capillaries, VEGF induces endothelial proliferation, and concurrently upregulates the transmembrane ligand Delta-like (DII)-4, which signals to microvascular cells in the capillary stalk, downregulating VEGFR2 on the stalk cells via interaction with its receptor Notch-1, thus preventing uncontrolled capillary growth [31, 32]. This lateral inhibition, which is a key principle
underlying structural development during ontogeny, is recapitulated following ischemia [33–35].

In adolescent mice, VEGF promotes angiogenesis in the ischemic brain [36], elevating regional cerebral blood flow (CBF) during subsequent ischemic episodes, thus stabilizing energy metabolism and preventing secondary brain infarction [37]. Interestingly, whereas VEGF-induced angiogenesis is most pronounced in the vicinity of evolving brain infarcts [36, 37], long-distance pyramidal tract plasticity is mainly induced by VEGF contralateral to the stroke [21, 23]. Hence, neurorestorative treatments induce complex changes within both the vasculature and neuronal networks, setting the stage for neurological recovery processes.

**Effects on Neurogenesis**

Neurogenesis is stimulated in response to stroke and is significantly amplified by therapeutic interventions that promote functional recovery [38–41]. Neurogenesis is sustained in the germinial niches in the subventricular zone (SVZ) of the lateral ventricle and the subgranular layer (SGL) of the dentate gyrus that host NPC even during adulthood [39, 40]. In the event of a stroke, NPC proliferate and migrate in the direction of the ischemic lesion, propagating in chains along blood vessels [42, 43]. That NPC promote neurological recovery could be attributed to bystander effects related to the interaction of neuroblasts with the microvasculature in the vicinity of the ischemic lesion, which creates an environment that nurtures and fosters brain remodeling [11, 12, 44]. By the release of trophic factors, microvessels and neuroblasts mutually support each other, resulting in a tight interplay resembling dancers in a tango (fig. 1). In the brain parenchyma, the release of trophic factors reduces delayed neuronal degeneration [11, 43], promotes neuronal plasticity [12], modulates glial responses [11, 45], and attenuates inflammatory responses [11, 45].

**Effects on Glial Cells**

In addition to controlling water shifts within the brain, astrocytes remove excitatory neurotransmitters (e.g., glutamate and electrolytes (e.g., potassium) from the extracellular space, thus controlling neuronal excitability and plasticity [46]. Subsequent to ischemia, astrocytes secrete lipoproteins, namely apolipoprotein-E, into the perivascular space, thus inducing the ATP-binding cassette transporters, namely the drug efflux transporter ABCB1 [47, 48], contributing to the maintenance of brain homeostasis.

Chondroitin sulfate proteoglycans released by astrocytes and oligodendrocytes create a growth-repulsive microenvironment for axons and dendrites under physiological conditions [49–51]. Therapeutic interventions promoting axonal plasticity, such as VEGF, and bone marrow stromal cells (BMSC) further decrease the proteoglycan levels [21, 52]. Post stroke, astrocytes play a major role in mediating brain plasticity and subsequent neurological recovery [53–55]. White matter remodeling in response to BMSC treatment is fostered by astrocytes, which increase tissue-plasminogen activator (tPA) activity [56, 57]. The sonic hedgehog (Shh) pathway, a major morphogen in brain development [58], which is stimulated after BMSC treatment in astrocytes, affects multiple processes and molecular pathways [59]. Increasing Shh levels permits the expression of Gli transcription factors, which leads to the activation of tPA and likely the generation of other proteins [57, 60–62]. tPA promotes brain plasticity via proteolytic and non-proteolytic pathways [63, 64].

**Challenges for Neurorestorative Therapies**

While the requirements for brain recovery have been assessed in recent years, the overwhelming majority of studies published were conducted using healthy adolescent rats and mice, which potentially increases the problems in a typical age-related disease such as stroke, in
Compromised Brain Remodeling Associated with Age

Stroke primarily affects humans in advanced stages of their life. Aged individuals recover less well from stroke than young individuals [65]. This may be due to the fact that infarct development and neuronal degeneration are accelerated, as was shown in aged rats when compared with young rats [66]. On the other hand, attenuated brain tissue remodeling may compromise recovery processes. Studies conducted in aged rats have demonstrated that neurological impairment is more severe and functional recovery less successful than in young rats [67–69]. When interpreting these data, it should be kept in mind that aged rats already exhibit reduced performance prior to their stroke, at least in some parameters such as spontaneous activity [70] and Morris water-maze [71] tests, which may explain in part the age-related recovery impairment. Aged rats respond to plasticity-promoting therapies. Improved neurological recovery associated with the preservation of pyramidal tract axons ipsilateral to the stroke and enhanced pyramidal tract sprouting contralateral to the stroke were found in 25-month-old or 12-month-old ischemic rats treated with neutralizing NogoA antibodies [14], pharmacological compounds [72], or BMSC [26]. In the case of BMSC delivery, neurological improvements persisted in middle-aged rats (12 months) for at least one year [26]. Although neurological recovery was successful, dendritic and synaptic plasticity of hippocampal CA3 and CA1 pyramidal and dentate gyrus granule cells were not influenced by NogoA antibodies in aged (24 months) rats; yet improvements in spatial memory were present [73]. Indeed, the expression of plasticity-related proteins in neurons differs between young and old animals. Thus, IGF-1 was primarily localized to astrocytes in the peri-infarct cortex of two-month-old rats, but exhibited predominantly as neuronal labeling in two-year-old rats [74]. Lingo-1 immunostaining, in contrast, was stronger in the neurons of older (two years) than young (two months) rats [74].

Specific aspects related to aging were not only observed for neuronal sprouting, but also for neurogenesis and angiogenesis. The number of proliferating NPC in the SVZ and SGL were lower in the brain tissue of 15-month-old than 3-month-old rats, both under physiological and ischemic conditions [75]. Although the de novo generation of neurons in the ischemic striatum was very similar in both groups, neurogenesis was decreased in the dentate gyrus of 15-month-old rats when exposed to focal cerebral ischemia [75]. It has been proposed that the decline in neurogenesis in old animals is related to a reduced expression of VEGF receptor-2 present on the surface of NPC of 2-year-old, but not in 3-month-old or 12-month-old mice [76]. After delivery of an adeno-associated viral VEGF vector, both NPC proliferation and brain capillary formation were compromised in very old mice [76]. The treatment of 18-month-old rats post stroke with sildenafil significantly increased neurogenesis and angiogenesis and also led to the improvement of neurological function [77]. So also, the treatment of 19–20-month-old rats with G-CSF enhanced neurological recovery, cellular proliferation and neurogenesis in ischemic tissue [78].

Astrogial responses to stroke injuries were also influenced by ageing. Shortly after stroke, nestin- and GFAP-positive cells demarcated the infarct core significantly earlier in aged rats than in young rats [79]. Proliferating astrocytes led to the premature glial scar formation in aged rats that presumably limits neurological recovery. It should be noted that there are at least three cell types contributing to the formation of the astrogial scar. Nestin-positive cells are the first to delineate the glial scar in aged rats, becoming visible in the brain on day 3 after focal cerebral ischemia, before GFAP-positive astrocytes (found on day 7) and astrocytes expressing the N-terminal fragment of amyloid precursor protein (APP) (found on day 14) are detectable [80–82]. The precipitous build-up of the astrogial scar is considered to impede axonal growth.

Although existing evidence is limited to a rather small number of studies, the preserved neurological recovery in old animals argues against specific age limits for neurorestorative treatments. Despite this, age aspects need to be controlled in clinical proof-of-concept studies.

Subtle Alterations of Growth Factors, but Intact Brain Remodeling Associated with Arterial Hypertension

Stroke patients exhibit a high prevalence of vascular risk factors. Three out of four stroke patients suffer from arterial hypertension, half of all stroke patients exhibit hypercholesterolemia, and one out of four stroke patients is dia-
Hyperlipidemia reduces angiogenesis [86] and promotes blood-brain barrier permeability [87]. These vascular changes involve multiple players, namely reduced endothelial NO synthase (eNOS) activity, excessive lipid peroxidation, and overactivation of matrix metalloproteinases (MMP)-2 and -9, calpain-1/2 and the small GTPase RhoA. These alterations were noted in the dentate gyrus [85]. In focal cerebral ischemia, these alterations did not result in major disturbances of contralateral axonal sprouting in response to neutralizing NogoA antibody treatment, enabling neurological recovery in a way that is very similar to that in non-hypertensive rats [17]. This preserved response to NogoA antibodies suggests that growth factor abnormalities may not be clinically relevant. Whether this conclusion is true for conditions of prolonged arterial hypertension, which in humans causes cerebral microangiopathy, remains to be shown.

**Compromised Brain Remodeling Associated with Hyperlipidemia**

Hyperlipidemia reduces angiogenesis [86] and promotes blood-brain barrier permeability [87]. These vascular changes involve multiple players, namely reduced endothelial NO synthase (eNOS) activity, excessive lipid peroxidation, and overactivation of matrix metalloproteinases (MMP)-2 and -9, calpain-1/2 and the small GTPase RhoA [86, 87]. In rats subjected to focal cerebral ischemia, vitamin B3 administration, which elevates high-density lipoprotein (HDL) and thereby reduces serum cholesterol, increased angiogenesis, the expression of VEGF and angiopoietin-1, and enhanced the phosphorylation (i.e., activation) of eNOS and the angiopoietin-1 receptor Tie2, thus improving neurological recovery [88]. After focal cerebral ischemia in rats, vitamin B3 supplementation enhanced white matter remodeling in the peri-infarct tissue, increased BDNF and TrKB levels and downregulated Nogo receptor levels [89]. These observations suggest that the pathophysiological sequelae associated with hyperlipidemia are amenable to therapeutic interventions, and that lipid-modulating strategies may particularly be promising to achieve this purpose.

As long as hyperlipidemia persists, brain responses to restorative therapies are nonetheless compromised, as suggested by a recent study, in which the effects of a cholesterol-rich diet-induced hyperlipidemia on VEGF-induced cerebral angiogenesis, post-ischemic regional CBF and recovery of the cerebral energy state were analyzed [90]. In this study, impaired angiogenesis was noticed following VEGF treatment in hyperlipidemic mice. The impaired angiogenesis was associated with blunted regional CBF responses and secondary breakdown of the energy state, once animals were subsequently exposed to focal cerebral ischemia [90]. Subsequent in vitro studies showed that upon exposure to low density lipoproteins (LDL), which mediate most of the detrimental effects of hyperlipidemia, VEGF’s receptor VEGFR2 was internalized by endothelial cells and degraded via the late endosome in a syntaxin-16-dependent manner [91]. As a consequence of the LDL exposure, VEGF receptor phosphorylation and downstream signaling were compromised. These data questioned the concept of therapeutic angiogenesis, which aims at restoring blood flow in advanced stages of atherosclerosis. Patients with advanced intracranial atherosclerosis particularly often exhibit hyperlipidemia [92, 93].

**Compromised Brain Remodeling Associated with Diabetes**

That impaired glucose control has detrimental effects for plasticity-promoting therapies was recently shown in rats suffering from streptozotocin-induced type I diabetes. Paradoxically, delivery of BMSC did not improve neurological recovery in diabetic rats, but increased mortality, blood-brain barrier leakage, and brain hemorrhage [94]. Besides, excessive angiogenesis was noted in diabetic rats receiving BMSC; this was associated with cerebral arteriole narrowing and neointima formation inside the internal carotid artery [94]. In histochemical studies, increased macrophage accumulation was noted in blood vessels of diabetic rats that had been treated with BMSC [94]. These abnormalities were attributed to an increased angiogenin expression in the brain and brain-supplying arteries of diabetic rats. The authors suggest that early BMSC treatment within 24 h after stroke onset should not be considered in diabetic patients. Further studies identifying the conditions of efficacy and safety of cell-based therapy for diabetic patients are warranted.

In contrast to BMSC, delivery of vitamin B3 was shown to enhance neurological recovery in streptozotocin-induced type I diabetes [95]. In line with the improved recovery, enhanced sprouting of motor cortical axons across the midline in the direction of the lesion-sided motor cortex was observed. When combined with BMSC, vitamin B3 delivery reversed the increased brain hemorrhage, and decreased blood-brain barrier leakage and arteriosclerosis-like changes that were induced by BMSC [96]. These results suggest that stroke patients suffering from diabetes might benefit from vitamin B3, whereas BMSC treatment should, if at all, be considered with great caution in diabetic patients.
Consequences for Translation to Human Patients

The benefits of neurorestorative therapies in experimental studies on ischemic stroke are evident. With restorative therapies, we can essentially treat all patients, and we are not hampered by reduced tissue perfusion, which restricts the delivery of neuroprotective agents, nor the necessity of rapid intervention, within hours after stroke. Yet, restorative therapies require stringent proof-of-concept studies, translating in a one-to-one manner experimental findings to the patient. If not properly translated, this promising approach, for which compelling experimental data have been accrued, will meet a premature end within a myriad of failed neuroprotective trials.

Over a long period of time, the lack of potential confounders in young, otherwise healthy laboratory animals was strongly appreciated in the stroke field. Reproducible models inducing uniform stroke lesions were developed in genetically inbred animal strains (mostly rodents), allowing insights into post-ischemic remodeling and neurological recovery processes even in small numbers of animals. With these models, the therapeutic potential of neurorestorative treatments was identified. The recent insights that responses to neurorestorative therapies may differ depending on age, risk factors and co-morbidities undoubtedly raise the need for evaluating recovery processes under conditions more closely resembling those in human patients. This knowledge increases the complexity of research on neurorestorative therapies.

Although post-stroke neurorestoration identified in animals appear relevant to humans, testing restorative therapy in the human is even more complex than in the laboratory animal [4]. While experimental studies make use of defined pathophysiological states, the clinic is even much more diverse. Stroke patients vary considerably in age and genetic background, as well as in the etiology, localization, and size of brain infarcts. Thus, the question arises whether clinical neurorestoration studies should enroll all stroke patients independent of genetic backgrounds, stroke localization and size, or whether studies should focus on patients within more defined subgroups. Heterogeneous patient populations may be a reason for the failure of therapeutic interventions as they may mask the therapeutic benefits seen in certain subgroups.

A number of conditions have been identified in animal studies, which potentially undermine neurorestorative therapies, among which hyperlipidemia and diabetes are particularly noteworthy. Future studies should carefully mimic such conditions in preparation for clinical trials. Thus, patients with certain risk factor profiles, for example, poorly controlled diabetes, should possibly be excluded from clinical trials. Risk factors should rigorously be treated based on existing therapeutic guidelines. The overriding message of this minireview is a strong credo in the potential of neurorestorative therapies. A robust substrate for restorative processes is present in the majority of stroke patients, even the elderly. This endogenous recovery potential offers itself for therapeutic use.

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None.

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