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Therapeutics Targeting Nogo-A Hold Promise for Stroke Restoration

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Abstract: Neurorestorative therapies for stroke aim to reverse disability by reparative mechanisms (rather than to thrombolyse or to neuroprotect). A substantial and persuasive body of pre-clinical evidence has come from the evaluation of antibodies against Nogo-A (a myelin-associated inhibitor of plasticity) in rat models of stroke. Particularly impressive is the benefit of this therapy in models of permanent middle cerebral artery occlusion (MCAO) when given to elderly animals after a one week delay, in adult rats with co-morbidities, and in adult rats when treatment is delayed by up to 9 weeks after stroke (although antibodies against Nogo-A did not reverse disability in mice after proximal MCAO with reperfusion). We predict that antibodies against Nogo-A will improve outcome further when combined with suitable additional rehabilitation, and also that antibodies against Nogo-A will improve outcome in animal models of haemmorhagic stroke that affect the same brain regions as ischemic stroke caused by MCAO. Antibodies against Nogo-A have been shown to be safe in Phase I clinical trials for acute spinal cord injury, and this may eventually facilitate a trial in stroke.

Keywords: Neurorestoration, stroke, cerebral ischemia, Nogo, antibodies, plasticity.

INTRODUCTION

The World Health Organization defines stroke as "rapidly developing clinical signs of focal (at times global) disturbance of cerebral function, lasting more than 24 hours or leading to death with no apparent cause other than that of vascular origin," [1]; a definition which encompasses both ischaemic and haemorrhagic types of stroke. Stroke is one of the most common causes of death worldwide, accounting for approximately 9% of all deaths on earth [2]. In the western world 10-12% of deaths occur due to stroke. The vast majority of strokes occur in people aged more than 65 years old [3, 4]. As of 2002, stroke-related disability is considered as the sixth most common cause of reduced disability-adjusted life-years [5]. Due to the accumulating elderly population in western societies, stroke-related disability in the west is projected to become the fourth most important cause of reduced disability-adjusted life-years in 2030 [6]. Stroke also brings with it a massive financial burden. Globally, stroke is responsible for using 2–4% of total health-care costs, and for over 4% of direct health-care costs in industrialized countries: as at 2009 the total cost in England alone was estimated to be £8.9 billion per annum [7, 8]. Despite stroke being such a disabling and costly disease, the amount of money injected into stroke research is dishearteningly small relative to other diseases of similar burden [9].

Thrombolysis, secondary prevention, risk factor reduction and rehabilitation are the core principles of current ischemic stroke management and have improved the outcome of stroke. A particular challenge for implementation of thrombolytic therapies is the timeframe in which treatment needs to be initiated. The clot-busting drug tissue plasminogen activator (tPA) needs to be given to ischemic stroke patients within 3 hours [10] from the onset of symptoms, although there can be benefits up to 4.5 hours. Intraarterial thrombolysis is also used up to six hours after stroke. The time to admission for people with stroke is highly variable and is dependent on a large number of different factors. Studies have revealed average times to stroke admission and brain imaging ranging anywhere from 90 minutes to 680 minutes [11-15], and while these figures vary substantially, late arrival continues to be one of the most common reasons for failing to qualify for treatment with tPA. Public awareness, availability and efficiency of emergency medical services and distance to the nearest treatment centre are just a few variables of many that affect the time to stroke admission and subsequent treatment [16].

Development of novel therapeutic agents is difficult due to the nature of the pathology of stroke. Neurons lay claim to a large portion of the body's blood flow, making them extremely susceptible to hypoxia. An interrupted blood supply will lead to irreversible neuronal damage within the local area in minutes and the surrounding penumbral areas are compromised if normoxia is not restored. The mechanisms of cell death in stroke are extremely complex, involving numerous signalling molecules and pathways. Neuroprotection was previously seen as a potentially brilliant new strategy to counter neuronal damage in cerebral ischaemia but clinical trials of neuroprotective drugs such as glutamate antagonists, ion channel blockers, anti-inflammatories, and free-radical scavengers have not led to clinical therapies. This was a major setback both pharmacologically and financially as many millions of dollars had been invested in pushing these drugs as far as Phase III clinical trials [17-23].

One source of hope is that where translation of acute neuroprotective therapies has failed, translation of neurorestorative therapies may succeed, by targeting regenerative mechanisms *via* modulation of CNS plasticity

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in subacute or chronic phases of stroke [24]: these phases represent more stable baselines from which to assess recovery of function, a larger number of patients might be treated, rehabilitation time might be reduced, with potential benefits for patients, carers and the healthcare system as a whole. A neurorestorative therapy could be used in conjunction with current therapies to improve the overall outcome after stroke. The following is a critical review of the available literature involving Nogo-A in experimental stroke models and the potential for therapeutic benefit by modulating this inhibitory pathway.

EXPRESSION OF NOGO-A AND NOGO RECEPTORS IN ANIMAL MODELS OF STROKE

Nogo-A is a membrane protein that is predominantly expressed in oligodendrocytes of the adult mammalian CNS [25-28] and has a central role in constraining axonal regeneration and collateral sprouting when the mammalian CNS is damaged [29]. More than two decades of work show that Nogo-A restricts spontaneous fibre regeneration and repair in the adult CNS [29] by retarding neurite outgrowth and triggering collapse of neuronal growth cones by binding to the Nogo receptors (NgR1 and 2) [30] and another, elusive receptor [31, 32] (Fig. **1**). Signaling *via* Rho to the cytoskeleton is an important mechanism occurring further along the NgR activation pathway [33].

A role for Nogo-A's anti-regenerative properties have been seen in experiments involving either antibody-mediated targeting of Nogo-A, Nogo-A gene deletions, NgR blockade or blockade of the downstream messengers Rho-A and Rho-kinase in primate, rat and mouse models of spinal cord injury, and although in some of these paradigms conflicting data has been obtained, there is now a large and persuasive body of evidence from multiple laboratories showing that antibodies against Nogo-A improve outcome after spinal cord injury and enhance collateral sprouting and long-distance axon regeneration (e.g., of the corticospinal tract).

Nogo-A and NgR expression has been investigated in neonatal rats with hypoxia-ischaemia. These rats displayed up-regulation of both Nogo-A and NgR in the ischaemic cortex after hypoxia-ischaemia [34]. Expression of Nogo-A mRNA was enhanced at 6 hours and reached a peak at 12 hours; Nogo-A protein expression was increased at 12 hours, peaking at around 24 hours. The authors also found a similar change in mRNA and protein levels of NgR. They also reported that Nogo-A and NgR colocalised to neurons 24 hours after injury.

A larger number of groups has investigated the expression of Nogo-A and Nogo receptors in the adult ischaemic brain to evaluate their potential roles in the pathophysiology of stroke. In an adult rat model of transient global ischaemia, expression of Nogo-A, NgR and RhoA proteins increased transiently over a 48-hour period, but this returned to normal levels by 7 days. Nogo-A was highly expressed in the myelin sheath whereas NgR expression was increased in axons and dendrites [35]. In a rat model of transient focal ischemia, Nogo-A, NgR, and RhoA protein levels were all increased by 24 hours but returned to normal levels by 96 hours. Again, Nogo-A was mainly located in the myelin sheath, whereas RhoA was expressed predominantly in the cytoplasm and NgR was mainly axonally and dendritically expressed [36]. In contrast, another group has reported that Nogo-A is expressed in cortical neurons in the brains of normal rats and rats after focal cortical ischemia: it was transiently enhanced in the external capsule after stroke and expression eventually returned to baseline levels. However, Nogo-A expression was neither increased nor decreased in any other white matter area that was studied up to 28 days after stroke [37].

With respect to the ageing brain, one study has indicated that normal, elderly rats did not exhibit changes in levels of mRNA for inhibitory ligands such as myelin associated glycoprotein (MAG) or oligodendrocyte myelin glycoprotein (Omgp), or NgR or other members of the Nogo-A receptor complex (Lingo-1, TROY; see Fig. **1**) in the cortex or hippocampus [38], whereas Nogo-A mRNA was shown to be reduced in the hippocampus [38]. In contrast, a different study showed 6-fold higher expression of Nogo-A mRNA in aged rats relative to young adult rats [39] although the level of Nogo-A protein was the same in young and aged adult rats showing that there may be a mismatch between gene expression and protein expression [39]. More work remains to be done to reconcile these two papers. In one of these studies, upon inducing stroke by distal middle cerebral artery occlusion (MCAO), Nogo-A mRNA levels in young adult rats increased by more than 4 fold and peaked at 2 weeks post stroke [39]. However in aged rats, Nogo-A mRNA levels declined post stroke, reaching a low of 0.14 fold 7 days after stroke. Surprisingly, Nogo-A- protein was reduced in both old and young adult rats one and two weeks after stroke.

A study making use of archival fixed tissue from marmosets that had received permanent occlusion of the middle cerebral artery revealed a transient increase in the number of Nogo-A positive cells within some areas of white matter surrounding the lesion at 2 months post stroke; this returned to baseline by 3 and 4 months. White matter located distally from the lesion, and peri-lesional grey matter expressed normal levels of Nogo-A [40]. This study suggests that Nogo-A levels may be raised for longer periods after ischaemic injury in marmosets than in rodents [40].

A number of papers have examined Nogo-A and NgR expression in developing and adult humans [41-43]. In the adult, Nogo-A mRNA expression was observed in oligodendrocytes and motor neurons (e.g., in the spinal cord) and in sensory ganglia neurons. In adult human tissues Nogo-R gene activity was found in neocortex, hippocampus and a subset of large and medium-sized neurons of the dorsal root ganglia. Nogo-R mRNA was not expressed in the adult human spinal cord at detectable levels. Nogo-A and NgR have also been examined acutely or chronically after cerebral ischemia in adult humans [44, 45]: Nogo-A protein was upregulated in oligodendrocytes surrounding ischemic lesions whereas NgR protein was increased in reactive astrocytes and microglia/macrophages [44]. Moreover, after cerebral infarction in humans, Nogo-A protein (and other myelin inhibitors) persisted in the spinal cords (in the degenerating corticospinal tract) for up to three years [45]: this is particularly important information given that several therapies deliver therapies against Nogo-A to the spinal cord after stroke (see below).

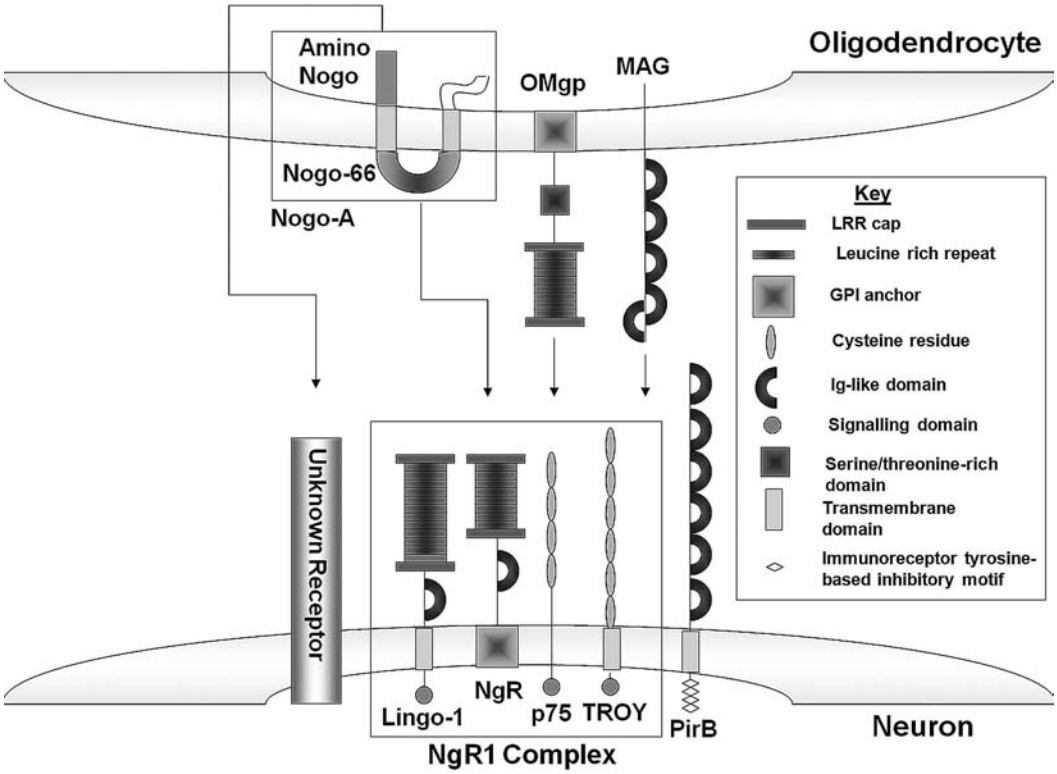

Fig. (1). Structure of the principle myelin associated inhibitors and their receptors. Schematic showing the structure of Nogo-A, MAG and OMgp, the three major myelin associated inhibitors of neurite outgrowth expressed by oligodendrocytes. Nogo-66, OMgp and MAG can bind to PirB or the NgR1 complex expressed by neurons. The structure of PirB and the NgR1 complex and its components: NgR1, Lingo-1 and P75 or TROY is shown. The amino terminal of Nogo-A may exert its inhibitory action through binding an undetermined neuronal receptor.

In summary, a reasonable number of studies reveal the presence of Nogo-A (and other myelin-associated inhibitors), NgR (and other receptor components), and their downstream effectors (e.g., RhoA) after stroke, although the time-window for any increase is yet to be delineated and may vary according to the method of inducing stroke and the age and species of the subject. This increase is often reported as being localized to peri-lesional areas. Nogo-A expression persists in debris, in surviving oligodendrocytes, and in neurons. These data strongly support a conclusion that Nogo-A has a role to play in the pathology of stroke. Somewhat controversially, there is evidence to contradict an up-regulation of the Nogo-A pathway in stroke in a model that is clinically relevant; Nogo-A protein expression was decreased in aged rats subjected to distal MCAO. One reasonable conclusion is that residual Nogo-A in elderly rats, humans and other primates after stroke is still substantial enough to prevent complete spontaneous repair.

ANTAGONISM OF NOGO-A PROMOTES FUNCTIONAL RECOVERY AFTER STROKE

Many reports from spinal cord injury models have confirmed that interference with Nogo-A signaling induces axonal sprouting, dendritic sprouting and functional recovery [46]. These promising findings led to research into whether neutralization of Nogo-A can induce axonal sprouting and functional recovery in stroke. To explore the consequence of Nogo-A inhibition on the plasticity of cortical efferents and functional recovery in focal ischaemic stroke, IN-1, an anti-Nogo-A monoclonal IgM antibody, was administered to adult rats following MCAO [47]. Functional outcome was measured with a skilled pellet-reaching task for which forelimb dexterity is essential, mediated by corticospinal tracts (CST), rubrospinal tracts and reticulospinal tracts [48-51]. Control rats (MCAO only) that received no treatment or a control antibody (IgG, anti-horseradish peroxidase) were permanently impaired, as shown by their forelimb test, and they improved modestly until plateauing at around 50% of the baseline function, whereas IN-1 treated rats progressively recovered to a mean of 80% of their baseline level. Previous reports from spinal cord models support these findings; IN-1 has induced recovery of skilled forelimb movements after a unilateral CST lesion in the medullary pyramids [52, 53].

It is impressive that the effects of antibodies against Nogo-A have also been examined in animals with co-morbidities typically seen in human stroke victims. For example, the Nogo-A–specific antibody, 7B12 (IgG), has been demonstrated to improve long-term neurological outcome when given intraventricularly to normotensive and Spontaneously Hypertensive rats (SHR), when administered after 24 hours after stroke [54]. (SHRs are prone to vascular abnormalities, have reduced collateral cerebral blood flow, suffer spontaneous strokes throughout the brain and often perish prematurely [55]). The authors induced cerebral ischaemia by permanent MCAO or using photothrombotic methods and used Montoya's staircase test to evaluate

grasping ability of the forepaw contralateral to the infarct. Magnetic Resonance Imaging showed that there were no differences in mean lesion volumes between groups at 24 hours or 9 weeks after stroke. Grasping performance in controls returned to a mean of ~55% of the baseline where it plateaued, until the end of the study. In rats treated with 7B12, grasping ability greatly improved to reach ~70% of baseline levels [54]. 7B12 improved outcome in the both healthy normotensive rats and SHRs. These results indicate that Anti-Nogo-A antibodies could be an effective treatment for stroke at least up until a 24-hour time frame.

In a subsequent experiment, IN-1 treatment was delayed until 1 week after stroke in adult rats [56]. This resulted in recovery of skilled forelimb function, which was again quantified with a forelimb test that required dexterity. In this study, rats treated with IN-1, 1-week post stroke, managed to recover up to 75% of their baseline function in a skilled forelimb task [56]. Encouragingly, animals given IN-1 a week after stroke showed significant recovery at 5 weeks from the time of administration, while animals administered IN-1 after the stroke [47], took only 6 weeks to show significant improvement (a week longer). There was no difference between the level of functionality recovered and the delay in treatment, suggesting that there may be no advantage to early administration of antibodies against Nogo-A and that there may be no deleterious role for Nogo-A in the early pathology of stroke.

Most impressively, antibodies against Nogo-A (11C7, IgG) were infused intracerebroventricularly 9 weeks after ischemic stroke in adult rat [57], when spontaneous behavioural recovery had reached plateau. Encouragingly, they displayed improvement in skilled forelimb movements in this model of chronic stroke. Even when given 9 weeks after stroke, the treated animals showed significant improvement 5 weeks after commencing treatment and achieved a mean of 78% of their baseline ability, when assessed at the end of the study [57]. These results are supported by previous studies mentioned above [47, 54, 56] where administration of Anti-Nogo-A antibodies immediately, after 24 hours or after 1 week resulted in similar levels of functional recovery. This study has confirmed that even when delayed for long periods, anti-Nogo-A treatment could rehabilitate sensorimotor function to at least 75% in rats with experimental stroke [57]. This study is of particular importance because the data give hope that anti-Nogo-A therapy can be used not just in early stages of treatment for stroke, but also in the chronic stages of such diseases, making it available in principle to a larger proportion of stroke survivors.

This body of work is also distinguished by its demonstration that anti-Nogo-A therapy (7B12, IgG) causes improvements in skilled paw function when initiated in very elderly rats (25 months old) one week after permanent distal MCAO [58].

Another major outcome of stroke is cognitive impairment. An investigation into the effect of anti-Nogo-A antibodies on stroke in aged rats revealed marginally quicker recovery of performance in a spatial memory task (the Morris water maze nagivational task), in rats that were administered anti-Nogo-A therapy 1 week after stroke [59]. Gross motor deficits after stroke have the potential to distort the results of the Morris water maze test, but the authors measured swim velocity in the test subjects and noted no difference between stroke groups. Their observations of the rats also confirmed the lack of differences between stroke groups in gross motor defects as they were seen to ambulate, feed, groom, and swim comparably [59]. However, in this animal model, only a small deficit persisted on the cognitive task one week after stroke, so there was little scope for a substantial benefit of the anti-Nogo therapy: in the future, this therapy should be evaluated in a more severe model of cognitive impairment.

Neglect is another common and debilitating consequence of stroke in humans. It is a multifaceted spatial-attentional disorder where the individual fails to respond to a stimulus on the side of the body contralateral to the brain injury [60]. In one study, rats underwent frontal (medial agranular cortex) lesions which induce neglect and then were immediately administered anti-Nogo-A antibodies (IN-1, 7B12 or 11C7) by implanting hybridoma cells intraventricularly. The authors used an orientation test in which they measured each rat's response towards or away from a transient stimulus to assess the level of neglect and recovery from neglect. They found that all three anti-Nogo groups recovered to a significantly greater extent than the control antibody group; this difference was present when considering total neglect or individual modalities of neglect separately [60]. Although the model of injury was aspirative rather than ischemic stroke, this study is important because neglect is a major factor in stroke. For example, patients with neglect do not do benefit maximally from rehabilitation due to disregard of their affected limb: accordingly, a treatment that enhanced recovery from neglect could improve rehabilitation and thus the overall patient outcome.

It should be noted that in many of these studies, the antibodies were given intraventricularly through the cortex on the intact side (by infusion or by implanting hybridoma cells), which is not a clinically straightforward route, and involves additional disturbance of the blood-brain barrier. However, the authors have also evaluated the intrathecal route after a one week delay in adult rats using 11C7 antibodies against Nogo-A with good CNS penetrance [61]; recovery was also obtained using this clinically-feasible route of administration. In total, this body of work is impressive in its scope.

To our knowledge, there is only one paper which reports a negative outcome when using antibodies against Nogo-A after stroke [62], and the fact that it is in the public domain is a credit to the authors, given a widespread failure of stroke researchers to report negative outcomes in the literature [63]. This study used mice (not rats) and different model of stroke, involving 30 minutes of transient, proximal MCAO and subsequent reperfusion, rather than permanent MCAO using a ligature [57, 61] or MCAO by electrocoagulation [47, 54] or by photothrombosis of the cortex [64] in rats. Treatment of mice (intracerebroventricularly or intrastriatally) 30 minutes after transient, proximal MCAO (i.e., during the period of reperfusion) with 11C7 antibodies against Nogo-A led to no measurable improvements in grip strength measured three days after stroke [62]: however, treatment of mice prior to proximal MCAO (and reperfusion) with antibodies against Nogo-A led to a worsening of outcomes,

measured using the rotarod and grip strength tests at seven (but not three) days after stroke [62]. Nogo-A knockout mice also exhibited worsening of grip strength after transient proximal MCAO. In this small study there was also some evidence for increased mortality in Nogo-A knockout mice. Accordingly, additional work is required (using models of stroke that involve transient occlusion and reperfusion) to determine whether antibodies against Nogo-A can be used safely and effectively, because in humans reperfusion may be occurring either spontaneously or after thrombolysis.

Another study in rats targeted NgR, which is a common receptor for the Nogo-66 inhibitory domain of Nogo-A, Omgp, and MAG [64, 65] (Fig. **1**) and reported functional recovery and corticofugal plasticity when an NgR antagonist was given 7 days post stroke for a duration of 2 months. Another group has confirmed that a function-blocking NgR fragment causes forelimb sensorimotor recovery, even when given 1 week after stroke [64]. Knockout mice devoid of NgR or Nogo-A and Nogo B also show enhanced sensorimotor function relative to heterozygous controls [64] after photothrombotic cortical ischemia. However, in another study, after proximal MCAO and reperfusion, Nogo-A knockout mice showed a slight worsening of grip strength 7 days after stroke (p. 7 in [62]).

Researchers have explored whether adenovirus-mediated gene knockdown of NgR (AdNgR) in rats decreased cortical and hippocampal expression of NgR mRNA and protein after cerebral ischemia and subsequent reperfusion [66]. It was found that intracerebral infusion of AdNgR prevented MCAO induced enhancement of NgR and resulted in functional recovery in skilled forelimb performance, which was assessed by a Montoya staircase test [66]. The AdNgR rats showed significantly improved performance from 3-9 weeks when compared to controls. Adenovirus was shown to transfect the ischaemic brain, including hippocampal neurons. After 24 hours of AdNgR treatment cortical and hippocampal levels of NgR mRNA and protein in AdNgR rats were reduced by 60% when compared to control MCAO rats; this inhibition dropped to 40% after 2 weeks [66]. They also saw a reduction in NgR mRNA and protein levels 9 weeks after stroke, which was also accompanied by functional recovery. NgR knockdown did not have any effect on infarct volume. This observation is consistent with a previous study [64] in which antagonists that blocked Nogo-A/NgR signaling did not induce neuroprotection. Thus gene knockdown of NgR may overcome inhibition of axon growth signaling and allow for greater axon regeneration, which may aid in sensorimotor recovery in stroke (see below).

In summary, antibodies against Nogo-A induce significant functional recovery in many experimental stroke models. Sensorimotor function after treatment with anti-Nogo-A antibodies has reached as much as 78% of the baseline ability in rodents with MCAO. The time window for initiating treatment that promotes successful recovery of function is currently up to 9 weeks from the time of stroke but future experiments may show this extends even further. Moreover, comparing across experiments, treatment even as late as 9 weeks is as effective as treatment given immediately after stroke. As well as sensorimotor function, anti-Nogo-A therapy has shown some efficacy in improving neglect in rats with medial agranular cortex lesions. These results are important as stroke affects a wide range of brain functions, which makes a drug that targets multiple aspects of stroke symptoms an exciting prospect. Anti-Nogo-A treatment has been effective in experimental models of MCAO in aged rats and hypertensive rats. Collectively, this data is very promising due to the clinical relevance of many of the models.

NOGO-A INDUCES NEUROANATOMICAL PLASTICITY

There are several mechanisms behind the observed functional improvements. Blockade of Nogo-A reduces growth cone collapse and overcomes inhibition of neurite outgrowth [25, 26, 67], thereby providing a more suitable environment for new neurite growth [47]. Functional recovery in stroke is accompanied by plasticity of cortical efferents from the unlesioned hemisphere to the striatum, red nucleus, pons and cervical spinal cord. *In vivo* and *in vitro* studies have shown enhanced expression of growth promoting genes including c-Jun and GAP-43 in presence of IN-1 [68, 69] and these probably occur in sprouting neurons in response to released inhibition of growth.

Normally, the corticorubral tract descends mainly ipsilaterally with only a small contralateral component [52, 70-72]. Anti-Nogo-A antibodies enhance corticorubral sprouting, originating from the undamaged hemisphere and extending over the midbrain's midline to the parvocellular region of the red nucleus whether treatment was started immediately after stroke [47] or one or nine weeks after stroke [56, 57]. This results in increased bilateral supply to the denervated red nucleus [47] and then, if appropriate synapses are made, rubrospinal axons may relay cortical sensorimotor commands to the spinal cord. The authors compared the corticorubral crossing indices in these studies and found similarities in the crossing index between their 1 week-delayed treatment and that of the immediate treatment study [57]. This data suggests that treatment with anti-Nogo-A therapy is just as efficacious at inducing corticorubral axonal plasticity when given after 9 weeks as it is when given immediately. IN-1 treatment has also shown increased plasticity of corticostriatal [73] corticopontine, and corticorubral fibres [74] following unilateral aspiration lesions to the cortex. Corticostriatal fibre outgrowth has also been reported after cortical ablation [73] or cortical thermocoagulation injury [75].

The 7B12 anti-Nogo antibody was shown to induce midline crossing in the cervical spinal cord of the CST fibres from the less-affected sensorimotor cortex when administered to normotensive and spontaneously hypertensive rats following MCAO [54]. Some spontaneous CST crossing in the spinal cord occurs in rats after stroke, but CST crossing was significantly higher in 7B12-treated animals. However, 7B12 did not induce sprouting of all pathways indiscriminately: corticopontine midline crossing in control antibody treated rats was similar to that in 7B12 treated rats. Regardless of treatment, there was a correlation between fibre outgrowth and improvement in forepaw function in both normotensive and SHRs. This implies that after photothrombotic cortical injury and MCAO in SHRs, behavioural outcome can be improved by lesion-induced fibre outgrowth in the cervical spinal cord. Although there was a correlation between corticospinal midline crossing and

behavioural improvement, other brain regions and systems are also likely to be involved in this sensorimotor task and that they may be influenced by 7B12. Indeed, in elderly rats after stroke and anti-Nogo antibody treatment, functional brain imaging indicated increased probability of activation in the thalamus during electrical stimulation of the affected paw, 8 weeks after stroke [58].

The hippocampus has a central role in spatial memory tasks [76], spurring investigation into the effect of anti-Nogo-A treatment on neuroanatomical plasticity in the hippocampus after MCAO [59]. Functional improvements were seen after administering anti-Nogo-A antibodies to aged rats after MCAO: however, although there was a decrease in dendritic complexity in CA3, CA1 and the dentate gyrus neurons of the ipsilesional hippocampus after stroke, there were no differences between rats treated with anti-Nogo-A antibodies and rats treated with control antibodies. To explain their results the authors proposed that structural plastic changes might be occurring in other areas such as the medial entorhinal cortex, lateral septum, striatum and subiculum [77]. Indeed, septohippocampal axonal growth has previously been seen with anti-Nogo-A treatment [78]. Another possible explanation is that biochemical and electrophysiological changes might be occurring in the hippocampus or other areas that are important for spatial memory tasks [59].

In the study investigating recovery from neglect the authors chose animals that had recovered after anti-Nogo-A antibody treatment, and they made a knife cut in the corpus callosum on the ipsilesional side either medial to the dorsocentral striatum (DCS) or lateral to the DCS (because the DCS is implicated in recovery from neglect [79]). Only the rats subjected to medial cuts re-exhibited neglect suggesting that the mechanism behind recovery involves transcallosal inputs from the less-affected hemisphere to areas medial to the cingulum bundle, such as the ipsilesional DCS [60].

Adenoviral-mediated NgR knockdown after MCAO in rats resulted in greater axon sprouting of corticorubral and corticostriatal pathways from the unlesioned hemisphere [66]. Knockout mice devoid of NgR or Nogo-A and Nogo B also show enhanced sensorimotor function when compared to heterozygous controls. The authors of this study also found that the axonal sprouting of intact pathways to the denervated red nucleus and spinal cord was responsible for the functional recovery [64].

A large number of studies have hypothesized that sprouting of cortical efferent pathways is responsible for functional motor improvement after interference with Nogo-A signalling [47, 52, 73, 80]. This is in line with several studies of motor corticofugal pathways in newborn rats with unilateral sensorimotor cortical lesions, where the less-affected, contralesional hemisphere sprouted bilateral projections to the striatum [73], thalamus [81], red nucleus [70], tectum [82], basilar pontine gray [83], and spinal cord [66, 84].

In summary, anti-Nogo-A treatment induces axonal sprouting in rats that received treatment after stroke, axonal sprouting that has more often than not [56] been correlated with functional sensorimotor recovery. Sprouting of axons from contralesional cortex regions into subcortical structures of the damaged hemisphere is seen in rats treated with anti-Nogo-A antibodies. This new sprouting of corticoefferent axons has been observed to originate from cortical areas contralateral to the lesion and has extended to the red nucleus, pontine nuclei, and spinal cord of rats that were subjected to stroke and were subsequently treated with anti-Nogo-A antibodies. These changes have occurred regardless of whether there was a delay in administration of anti-Nogo-A antibodies. We look forward to additional experiments examining whether these collateral sprouts form functional synapses after stroke [85]: advances are being made in this area in models of spinal cord injury [86, 87]. Brain imaging indicates that Nogo-A antibodies also alter activity in the thalamus. Altogether, these changes likely cause the improvement of sensorimotor movement, although formal proof of this relationship remains to be shown for most types of plasticity, for example by re-lesioning [60].

FUTURE DIRECTIONS AND TRANSLATION

It will be important to determine whether, after stroke, treatment with Nogo-A antibodies delivers improvements which are not seen with rehabilitation alone. This is important because other groups have shown that a similar magnitude of recovery of forelimb grasping occurs on the Montoya's staircase pellet-reaching task with rehabilitation alone, even when rehabilitation is only started 15 days after stroke [88]. In the case of chronic stroke, antibodies against Nogo-A do appear to provide a more beneficial outcome than delayed rehabilitation, because antibodies against Nogo-A can provide a benefit even when treatment is initiated after 9 weeks [57] whereas rehabilitation declines in efficacy considerably by 4 weeks [88]. However, those rehabilitation studies were done with a different model of (smaller) focal stroke and it will be important to compare rehabilitation plus antibodies to Nogo-A in a single study. One careful study in a model of spinal cord injury show that this issue does need to be examined empirically because in some circumstances, rehabilitation does not combine additively or synergistically with treatment with antibodies against Nogo-A but can actually worsen outcome [86]. In mitigation, a large number of studies now show that the efficacy of rehabilitation depends on the type, specificity, intensity, and timing of rehabilitation, and it seems likely that an effective combination of rehabilitation and antibodies against Nogo-A can be found.

It will also be important to ensure the highest methodological quality of preclinical studies going forward [89, 90], to avoid bias and to estimate effect sizes correctly [63]. The vast majority of papers relating to anti-Nogo therapeutics do not mention whether or not treatment allocations were concealed, how randomization was performed (e.g., randomized block design) and at what stages blinding was in effect. In stroke studies (especially those with elderly rodents), drop-out due to mortality or treatment side effects are a particular concern, and it will be important to report these incidences as they have signal importance (and have been reported in a small number of Nogo-A knockouts [62]). Inclusion and exclusion criteria, and numbers of animals omitted from analyses should also be reported. These and other criteria are laid out in the

ARRIVE guidelines (Animal Research: Reporting of *In Vivo* Experiments), which have been endorsed by many journals [1], including stroke specialty journals [89, 91].

It will also be important to determine whether antibodies against Nogo-A improve outcome in haemmorhagic stroke because this might increase the number of stroke patients that can be included in any clinical trial. It seems reasonable to predict that antibodies against Nogo-A will improve outcome in haemmorhagic strokes that affect similar areas of the brain as MCAO ischemic strokes (with all other things being equal) because the mechanism of repair induced by Nogo-A antibodies does not appear to involve neuroprotection and, accordingly, the cause whereby stroke originally occurs may well be irrelevant.

To date, we do not know whether the human anti-human Nogo-A antibodies have yet been evaluated in animal models of stroke, and we know of no studies in non-human primates after stroke using antibodies against Nogo-A, although several studies have reported benefits of antibodies against Nogo-A in monkey models of spinal cord injury [92-97]. However, we do know of two Phase I clinical trials using antibodies against Nogo-A: Novartis' ATI355 human anti-human Nogo-A antibody [46] and GlaxoSmithKline's 1223249 antibody (NCT00406016 and NCT01435993, respectively; www.clinicaltrials.gov). The latter Phase I study is recruiting patients with multiple sclerosis (MS). The Novartis Phase I study was carried out in SCI patients and so far results have been positive with regards to safety and adverse effects [46]. Any resulting Phase II trials should test the efficacy of the drug and if results are also positive, it will be a major breakthrough in the treatment of CNS injury, particularly against backdrop of such an alarming failure rate of the pharmaceutical industry to produce successful therapeutic agents for stroke.

CONCLUSION

In recent times experimental research has greatly enhanced the understanding of functional recovery and reorganization after injury to the CNS. Neuroplastic changes such as axonal sprouting, synapse formation and reorganization of activity patterns occurs at several different levels in the CNS after a lesion. These mechanisms help to restore function after cortical and spinal injuries. Interference with pathways that restrict neuronal regeneration and plasticity have been successful in aiding recovery after SCI and stroke, with one major pre-clinical success being the inhibition of Nogo-A or NgR signaling by antibodies, receptor blockers and genetic knockdown/knockout techniques [46]. Successful clinical trials in SCI or MS may follow through to future clinical trials in stroke, which could potentially revolutionise the treatment of this debilitating disorder [46].

ABBREVIATIONS

ARRIVE guidelines	=	Animal Research: Reporting of *In Vivo* Experiments guidelines
MAG	=	Myelin-associated glycoprotein
MCAO	=	Middle cerebral artery occlusion
NgR	=	Nogo receptor
Omgp	=	Oligodendrocyte myelin glycoprotein
SHR	=	Spontaneously hypertensive rat
tPA	=	Tissue type plasminogen activator

[1] http://www.nc3rs.org.uk/page.asp?id=1796

CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest.

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