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Combination Treatment of Experimental Stroke with Niaspan and Simvastatin, Reduces Axonal Damage and Improves Functional Outcome

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Abstract

In this study we examined the effect of combination treatment of experimental stroke with Niaspan, a prolonged-release formulation of Niacin (vitamin B3), and Simvastatin, a cholesterol-lowering drug, on functional outcome, axonal damage, axonal density and the of Iba-1 immunoreactive microglia expression in the ischemic brain of rats. Adult male rats were subjected to 2 hours middle cerebral artery occlusion (MCAo) and treated with or without Niaspan alone, Simvastatin alone and combination Niaspan and Simvastatin starting 24 hours after MCAo and daily for 14 days. Neurological functional tests were performed. Axonal damage and density were evaluated by Amyloid Precursor Protein (APP), Bielschowsky silver, respectively. Nogo66 Receptor (NgR) expression and immunoreactive microglia (Iba-1) were also measured in the ischemic brain. Niaspan and Simvastatin monotherapy and combination treatment significantly promotes functional outcome after stroke ($p < 0.05$) compared to MCAo control animals. While combination treatment with Niaspan and Simvastatin induces additive but not synergetic effects when compared to Niaspan or Simvastatin monotherapy groups. Combination treatment significantly decreased APP expression and increased Bielschowsky silver expression. NGR and Iba-1 expression were significantly decreased in the ischemic brain. These data suggest that treatment of experimental stroke with combination of Niaspan and Simvastatin significantly improves functional outcome, reduces axonal damage and increases axonal density. Decreased expression of the NGR and reduced activated microglia may contribute to functional recovery after stroke.

Keywords

Niaspan; simvastatin; axonal damage; microglia; Nogo Receptor; stroke

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Introduction

Niacin is a lipid-altering agent that favorably affects multiple lipid parameters. Niacin is the most effective medication in current clinical use for increasing high density lipoprotein (HDL) cholesterol [1]. Niaspan, a prolonged-release formulation of Niacin (vitamin B3), reduces the Niacin-induced major side effects of flush and hepatotoxicity [2]. Niacin has been evaluated in combination with simvastatin in several clinical trials and has demonstrated efficacy, with clinical improvement in multiple lipid parameters [3,4]. Simvastatin like other statins is a lipid altering drug with proven lipid and coronary artery disease (CAD) outcome efficacy [5]. Apart from their lipid-lowering activities, statins have also been shown to mediate pleiotropic effects by enhancement of endothelial function, angiogenesis and reduction of inflammatory responses [6,7]. Several recent clinical trials have demonstrated the efficacy and safety of combined Niaspan and Simvastatin. [3,8-10]. The randomized, double - blind ARBITER 2 study [11] demonstrated the benefits of combination of Niaspan and Statin for inhibition of atherosclerosis. Previous studies have shown that Statin or Niaspan mono-therapy of stroke animals induces angiogenesis and improves functional outcome [12-15]. Whether combination treatment of stroke with Niaspan and Statin regulates functional outcome has not been investigated.

In the central nervous system (CNS), injured axons not only degenerate but are unable to regenerate and have a limited capacity to sprout and re-establish lost connections [16,17]. Therefore, axonal damage often results in long term disability [16]. Following the immediate primary CNS injury, there is a cascade of down stream events termed “secondary injury” which culminate in progressive axonal degeneration [18-20].

Experimental studies in rodents revealed a rapid, progressive activation of microglia cells in the ischemic brain after stroke [21]. Microglia are thought to orchestrate damage in the penumbra [22,23]. In vitro studies showed that injury from various ischemia-like insults was increased in the presence of microglia [24-26].

In this study, we examined the effect of combination treatment of stroke with Niaspan and Simvastatin on functional outcome, axonal damage and axonal density, as well as the expression of Nogo66 Receptor (NgR) and Iba-1 immunoreactive microglia in the ischemic brain in a rat model of middle cerebral artery occlusion (MCAo).

Material and Methods

Animal middle cerebral artery occlusion (MCAo) model and experimental groups

Adult male Wistar rats weighing 270-300 g were employed in all experiments. Transient right middle cerebral artery occlusion (MCAo) was induced for 2 hours by advancing a 4-0 surgical nylon suture (18.5-19.5 mm), determined by the animal weight, with its tip rounded by heating near a flame, to block the origin of the MCA, using a method of intraluminal vascular occlusion modified in our laboratory [27]. Two hours after MCAo, reperfusion was performed by withdrawal of the suture. Experimental groups consist of rats subjected to 2 hours of MCAo. Twenty-four hours after MCAo rats were randomly separated into 4 groups:

1. Control group: rats were gavaged with water daily for 14 days (N=8 rats).
2. Niaspan alone treatment group: rats were gavaged with Niaspan (40mg/kg, Kos Pharmaceuticals, Canburry, NJ) alone daily for 14 days (N= 6 rats).
3. Simvastatin alone treatment group: rats were gavaged with Simvastatin (1mg/kg, Merck, Somerset, NJ) alone daily for 14 days (N= 7 rats).

4. Combination treatment group: rats were gavaged with Niaspan (40mg/kg, Kos Pharmaceuticals, Canburry, NJ) in combination with Simvastatin (1mg/kg, Merck, Somerset, NJ) daily for 14 days (N= 8 rats).

Functional tests

A modified neurological severity score (mNSS) evaluation and Foot-fault tests were performed [28,29] before MCAo, and at 1, 7, 14 days after MCAo by an investigator who was blinded to the experimental groups.

Histological and immunohistochemical assessment

At fourteen days after MCAo, animals were sacrificed and brains were fixed by transcardial perfusion with saline, followed by perfusion and immersion in 4% paraformaldehyde before being embedded in paraffin. Seven coronal sections of tissue were processed and stained with hematoxylin and eosin (H&E) for calculation of volume of cerebral infarction [30]. The indirect lesion area, in which the intact area of the ipsilateral hemisphere was subtracted from the area of the contralateral hemisphere, was calculated using the Global Lab Image analysis system (Data Translation, Malboro, MA) [30]. Lesion volume is presented as a volume percentage of the lesion compared with the contralateral hemisphere.

Immunohistochemical staining

A standard paraffin block was obtained from the center of the lesion (bregma -1 mm to +1 mm). A series of 6 µm thick sections were cut from the block. Every 10th coronal section for a total 5 sections was used for immunohistochemical staining. Antibodies against Amyloid Precursor Protein (APP; dilution 1:50, Cell Signaling Technology), Nogo66 Receptor (NgR; dilution 1:50, Santa Cruz Biotech Inc, Santa Cruz, Calif), and Ionizing calcium-Binding Adaptor molecule 1 (Iba-1; dilution 1:1000, Wako) were employed. Control experiments consisted of staining brain coronal tissue sections as outlined above, but the primary antibodies were omitted, as previously described [31]. Bielschowsky silver immunostaining was used to demonstrate axons. In brief, for Bielschowsky silver staining, slides were placed in 20% AgNO₃ in the dark, and then NaOH and sodium thiosulfate were added to the slides in turn. The immunostaining analysis was performed by an investigator blinded to the experimental groups.

Quantitation

For quantitative measurements of APP, five slides from each brain, with each slide containing 6 fields from the ischemic border area (IBZ) which is adjacent to the ischemic core were digitized under a 20× objective (Olympus BX40) using a 3-CCD color video camera (Sony DXC-970MD) interfaced with an MCID image analysis system (Imaging Research, St. Catharines, Canada) [12,32,33]. For quantitative measurements of Bielschowsky silver staining and NGR, positive area of Bielschowsky silver immunoreactive cells and NGR were measured in the striatal white matter bundles in the ischemic border. For quantitative measurements of Iba-1, positive area of Iba-1 immunoreactive cells was measured in the ipsilateral hemisphere. Data were analyzed in a blinded manner and presented as percentage of positive area for, APP, NGR, Iba-1 and Bielschowsky silver immunoreactive cells, respectively.

Statistical Analysis

Behavior tests (mNSS and foot-fault test) were performed at day 1 after MCAo before treatment (baseline), day 7 and 14 after MCAo. The Global Test using Generalize Estimating Equation (GEE) [34] was implemented to test the group differences on functional recovery measured from the two behavioral tests. The analysis was started by testing the treatment (Niaspan and

Simvastatin) interaction, followed by testing the subgroup analysis, if the interaction or main effect was detected at the 0.05 level. The Global Test on multiple outcomes was considered more efficient than a single outcome, when the group effects were consistent on all the outcomes (e.g. positive correlation). The significant treatment interaction may indicate synergy effects of the two treatments and no interaction indicates the additive effects of the two treatments. Comparisons between treatment groups of data measured by independent t-test on day 14 were made using analysis of variance models. Variables included the lesion volume, APP, Bielschowsky silver, NGR and Iba-1. All data are presented as mean $\pm$ standard error.

Results

Neurological outcome (Figure 1)

To test the effect of the combination treatment of stroke rats on functional outcome, GEE statistical analysis was performed to detect the differences in functional recovery among the 4 groups (MCAo control, Niaspan treatment, Simvastatin treatment and combination treatment). The 4 groups were balanced at the baseline and there was no individual functional difference among 4 groups ($p=0.99$ based on the Global Test). On day 14, a marginal significant interaction was detected based on the Global Test ($p=0.08$), indicating a trend synergistic of the two treatments. A significant effect was observed in the Niaspan treated group ($p=0.0126$), in the Simvastatin treated group ($p=0.0015$) and in the combination treated group ($p=0.0029$), compared to the MCAo control group (Figure 1A-B). On day 7, no treatment interaction was detected based on the Global Test ($p=0.27$), indicating additivity of the two treatments. Based on the Global Test a significant effect was observed in the Niaspan treatment group ($p=0.038$), in the Simvastatin treatment group ($p=0.027$) and in the combination treatment group ($p=0.0087$), compared to the MCAo control group (Fig. 1A-B). Therefore, the data suggest that the combination treatment induces an additive up to 14 days after stroke.

Infarct Volume

No significant differences of ischemic lesion volumes in the combination treatment group ($27.16 \pm 3.99\%$) were detected compared with MCAo control rats ($29.74 \pm 3.12\%$, $p>0.05$).

Combination treatment of stroke with Niaspan and Simvastatin reduces axonal damage, increases axonal density, and decreases neuronal growth inhibitory molecule (NGR) and microglial cell marker (Iba-1) expression

APP is a marker of axon damage [35,36]. Bielschowsky silver is a marker for axons [37,38]. To test whether combination treatment with Niaspan and Simvastatin affects axonal damage and axonal density, APP and Bielschowsky silver immunostaining were performed. Fig. 2A-B show that combination treatment with Niaspan and Simvastatin significantly decreased APP expression in the ischemic brain (A) and significantly increased Bielschowsky silver expression in the striatal bundles (B) compared with control MCAo rats.

NGR is a neuronal growth inhibitory molecule [39]. Iba-1 is a specific marker for microglia [40]. To test whether combination treatment of stroke with Niaspan and Simvastatin inhibits the NGR-neuronal growth inhibitory molecule and microglia expression, NGR and Iba-1 expression in the ischemic brain were measured. Figure 2C-D show that NGR (C) and Iba-1 (D) expression were significantly decreased in the ischemic brain in the combination treatment rats compared with control MCAo animals. These data suggest that combination treatment with Niaspan and Simvastatin decreases axonal damage, increases axonal density and inhibits neuronal growth inhibitory molecule (NGR) and microglia (Iba-1) expression compared to non-treatment MCAo control.

Discussion

We demonstrated for the first time that combination treatment of stroke with Niaspan and Simvastatin starting 24h after MCAo significantly decreases axonal damage and activated microglia expression as well as increases axonal density in the ischemic brain and improves neurological functional outcome.

Injury to white matter, which results in a disruption of axoplasmic flow, can be detected immunohistochemically within a few hours by the accumulation of transported proteins in axonal swellings or bulbs [41]. Amyloid precursor protein (APP), one such protein, is carried along the nerve by fast axoplasmic flow and has been extensively validated as a sensitive marker for the detection of axonal injury [42-45]. Our data show that combination treatment with Niaspan and Simvastatin of stroke significantly decreased APP expression in the ischemic brain compared to non-treatment MCAo control, as well as significantly increased Bielschowsky silver expression in the striatal bundles in the ischemic brain compared to non-treatment MCAo control. Therefore, combination treatment with Niaspan and Simvastatin of stroke decreases axonal damage and promotes axonal density in the ischemic brain.

At least three proteins in the central nervous system (CNS) myelin, Myelin-associated glycoprotein (MAG), Nogo, and oligodendrocyte-myelin glycoprotein (OMgp), are capable of causing growth cone collapse and inhibiting neurite outgrowth in vitro [46]. MAG, Nogo and OMgp inhibit axonal growth by binding a common receptor, the Nogo66 receptor (NgR) [19,47-49]. Intraventricular infusion of soluble peptide fragment of the NgR (sNgR) that binds to and blocks all three NgR ligands promoted functional recovery after brachial dorsal root crush in rats [50]. Reduction of NgR function, using genetic and pharmacological methods, promotes anatomical plasticity and improves motor performance after stroke [51]. In this study, we found that combination treatment of stroke with Niaspan and Simvastatin significantly decreased NgR expression. Therefore, the combination Niaspan with Simvastatin treatment increase of axonal density may be regulated by decreasing NgR expression after stroke.

Activated microglia may adversely contribute to cerebral injury after stroke [24]. Inhibition of microglial activation by the tripeptidemacrophage inhibitory factor (MIF, tuftsin fragment 1-3) was accompanied by neuroprotection against kainate-induced toxicity [52]. Edaravone, a novel free radical scavenger, significantly reduced the infarct volume and improved the neurological outcome after stroke through reduced microglial activation [53]. A tetracycline derivative, minocycline, provides significant protection against brain ischemia by inhibiting of microglial activation [54,55]. Our data show that combination treatment with Niaspan and Simvastatin reduced the expression of activated microglia (Iba-1) in the ischemic brain compared with nontreated MCAo control rats as well as improved functional outcome after stroke. Therefore, inhibition of microglial activity by combination treatment may play a role in improving of functional outcome after stroke.

In summary, we demonstrated that treatment of experimental stroke with combination of Niaspan and Simvastatin 24h after stroke significantly improves functional outcome, reduces axonal damage, increases axonal density and decreases expression of NgR and Iba-1 immunoreactive microglia. Decreased expression of the NGR and activated microglia may contribute to functional recovery after stroke.

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References

1. Elam MB, Hunninghake DB, Davis KB, Garg R, Johnson C, Egan D, Kostis JB, Sheps DS, Brinton EA. Effect of niacin on lipid and lipoprotein levels and glycemic control in patients with diabetes and peripheral arterial disease: the ADMIT study: A randomized trial. *Arterial Disease Multiple Intervention Trial JAMA* 2000;284(10):1263–1270.
2. Carlson LA. Niaspan, the prolonged release preparation of nicotinic acid (niacin), the broad-spectrum lipid drug. *Int J Clin Pract* 2004;58(7):706–713. [PubMed: 15311728]
3. Bays H. Safety of niacin and simvastatin combination therapy. *Am J Cardiol* 2008;101(8A):3B–8B. [PubMed: 18243856]
4. Vo AN, Kashyap ML. Fixed-dose combination of extended-release niacin plus simvastatin for lipid disorders. *Expert Rev Cardiovasc Ther* 2008;6(10):1303–1310. [PubMed: 19018682]
5. Ward S, Lloyd Jones M, Pandor A, Holmes M, Ara R, Ryan A, Yeo W, Payne N. A systematic review and economic evaluation of statins for the prevention of coronary events. *Health Technol Assess* 2007;11(14):1–160. iii–iv.
6. Aviram M, Rosenblat M, Bisgaier CL, Newton RS. Atorvastatin and gemfibrozil metabolites, but not the parent drugs, are potent antioxidants against lipoprotein oxidation. *Atherosclerosis* 1998;138(2):271–280. [PubMed: 9690910]
7. Diomedede L, Albani D, Sottocorno M, Donati MB, Bianchi M, Fruscella P, Salmona M. In vivo anti-inflammatory effect of statins is mediated by nonsterol mevalonate products. *Arterioscler Thromb Vasc Biol* 2001;21(8):1327–1332. [PubMed: 11498461]
8. Pandian A, Arora A, Sperling LS, Khan BV. Targeting multiple dyslipidemias with fixed combinations--focus on extended release niacin and simvastatin. *Vasc Health Risk Manag* 2008;4(5):1001–1009. [PubMed: 19183748]
9. Ballantyne CM, Davidson MH, McKenney J, Keller LH, Bajorunas DR, Karas RH. Comparison of the safety and efficacy of a combination tablet of niacin extended release and simvastatin vs simvastatin monotherapy in patients with increased non-HDL cholesterol (from the SEACOAST I study). *Am J Cardiol* 2008;101(10):1428–1436. [PubMed: 18471454]
10. Karas RH, Kashyap ML, Knopp RH, Keller LH, Bajorunas DR, Davidson MH. Long-term safety and efficacy of a combination of niacin extended release and simvastatin in patients with dyslipidemia: the OCEANS study. *Am J Cardiovasc Drugs* 2008;8(2):69–81. [PubMed: 18422390]
11. Taylor AJ, Sullenberger LE, Lee HJ, Lee JK, Grace KA. Arterial Biology for the Investigation of the Treatment Effects of Reducing Cholesterol (ARBITER) 2: a double-blind, placebo-controlled study of extended-release niacin on atherosclerosis progression in secondary prevention patients treated with statins. *Circulation* 2004;110(23):3512–3517. [PubMed: 15537681]
12. Chen J, Zhang ZG, Li Y, Wang Y, Wang L, Jiang H, Zhang C, Lu M, Katakowski M, Feldkamp CS, et al. Statins induce angiogenesis, neurogenesis, and synaptogenesis after stroke. *Ann Neurol* 2003;53(6):743–751. [PubMed: 12783420]
13. Chen J, Zhang C, Jiang H, Li Y, Zhang L, Robin A, Katakowski M, Lu M, Chopp M. Atorvastatin induction of VEGF and BDNF promotes brain plasticity after stroke in mice. *J Cereb Blood Flow Metab* 2005;25(2):281–290. [PubMed: 15678129]
14. Chen J, Cui X, Zacharek A, Ding GL, Shehadah A, Jiang Q, Lu M, Chopp M. Niaspan treatment increases tumor necrosis factor-alpha-converting enzyme and promotes arteriogenesis after stroke. *J Cereb Blood Flow Metab* 2009;29(5):911–920. [PubMed: 19223914]
15. Chen J, Cui X, Zacharek A, Jiang H, Roberts C, Zhang C, Lu M, Kapke A, Feldkamp CS, Chopp M. Niaspan increases angiogenesis and improves functional recovery after stroke. *Ann Neurol* 2007;62(1):49–58. [PubMed: 17557352]
16. Di Giovanni S. Molecular targets for axon regeneration: focus on the intrinsic pathways. *Expert Opin Ther Targets* 2009;13(12):1387–1398. [PubMed: 19922299]

17. Benowitz LI, Yin Y. Combinatorial treatments for promoting axon regeneration in the CNS: strategies for overcoming inhibitory signals and activating neurons' intrinsic growth state. *Dev Neurobiol* 2007;67(9):1148–1165. [PubMed: 17514713]
18. Park E, Velumian AA, Fehlings MG. The role of excitotoxicity in secondary mechanisms of spinal cord injury: a review with an emphasis on the implications for white matter degeneration. *J Neurotrauma* 2004;21(6):754–774. [PubMed: 15253803]
19. Wang F, Liang Z, Hou Q, Xing S, Ling L, He M, Pei Z, Zeng J. Nogo-A is involved in secondary axonal degeneration of thalamus in hypertensive rats with focal cortical infarction. *Neurosci Lett* 2007;417(3):255–260. [PubMed: 17382469]
20. Bramlett HM, Dietrich WD. Pathophysiology of cerebral ischemia and brain trauma: similarities and differences. *J Cereb Blood Flow Metab* 2004;24(2):133–150. [PubMed: 14747740]
21. Schilling M, Besselmann M, Leonhard C, Mueller M, Ringelstein EB, Kiefer R. Microglial activation precedes and predominates over macrophage infiltration in transient focal cerebral ischemia: a study in green fluorescent protein transgenic bone marrow chimeric mice. *Exp Neurol* 2003;183(1):25–33. [PubMed: 12957485]
22. Kaushal V, Schlichter LC. Mechanisms of microglia-mediated neurotoxicity in a new model of the stroke penumbra. *J Neurosci* 2008;28(9):2221–2230. [PubMed: 18305255]
23. Block F, Peters M, Nolden-Koch M. Expression of IL-6 in the ischemic penumbra. *Neuroreport* 2000;11(5):963–967. [PubMed: 10790864]
24. Wang Q, Tang XN, Yenari MA. The inflammatory response in stroke. *J Neuroimmunol* 2007;184(1-2):53–68. [PubMed: 17188755]
25. Lehnardt S, Massillon L, Follett P, Jensen FE, Ratan R, Rosenberg PA, Volpe JJ, Vartanian T. Activation of innate immunity in the CNS triggers neurodegeneration through a Toll-like receptor 4-dependent pathway. *Proc Natl Acad Sci U S A* 2003;100(14):8514–8519. [PubMed: 12824464]
26. Zhang Z, Chopp M, Powers C. Temporal profile of microglial response following transient (2 h) middle cerebral artery occlusion. *Brain Res* 1997;744(2):189–198. [PubMed: 9027378]
27. Chen J, Zacharek A, Li A, Zhang C, Ding J, Roberts C, Lu M, Kapke A, Chopp M. Vascular endothelial growth factor mediates atorvastatin-induced mammalian achaete-scute homologue-1 gene expression and neuronal differentiation after stroke in retired breeder rats. *Neuroscience* 2006;141(2):737–744. [PubMed: 16730914]
28. Chen J, Li Y, Wang L, Zhang Z, Lu D, Lu M, Chopp M. Therapeutic benefit of intravenous administration of bone marrow stromal cells after cerebral ischemia in rats. *Stroke* 2001;32(4):1005–1011. [PubMed: 11283404]
29. Chen J, Sanberg PR, Li Y, Wang L, Lu M, Willing AE, Sanchez-Ramos J, Chopp M. Intravenous administration of human umbilical cord blood reduces behavioral deficits after stroke in rats. *Stroke* 2001;32(11):2682–2688. [PubMed: 11692034]
30. Swanson RA, Morton MT, Tsao-Wu G, Savalos RA, Davidson C, Sharp FR. A semiautomated method for measuring brain infarct volume. *J Cereb Blood Flow Metab* 1990;10(2):290–293. [PubMed: 1689322]
31. Li Y, Jiang N, Powers C, Chopp M. Neuronal damage and plasticity identified by microtubule-associated protein 2, growth-associated protein 43, and cyclin D1 immunoreactivity after focal cerebral ischemia in rats. *Stroke* 1998;29(9):1972–1980. 1980–1971. discussion. [PubMed: 9731626]
32. Chen J, Zhang ZG, Li Y, Wang L, Xu YX, Gautam SC, Lu M, Zhu Z, Chopp M. Intravenous administration of human bone marrow stromal cells induces angiogenesis in the ischemic boundary zone after stroke in rats. *Circ Res* 2003;92(6):692–699. [PubMed: 12609969]
33. Calza L, Giardino L, Giuliani A, Aloe L, Levi-Montalcini R. Nerve growth factor control of neuronal expression of angiogenic and vasoactive factors. *Proc Natl Acad Sci U S A* 2001;98(7):4160–4165. [PubMed: 11259645]
34. Lu M, Chen J, Lu D, Yi L, Mahmood A, Chopp M. Global test statistics for treatment effect of stroke and traumatic brain injury in rats with administration of bone marrow stromal cells. *J Neurosci Methods* 2003;128(1-2):183–190. [PubMed: 12948561]

35. Sherriff FE, Bridges LR, Sivaloganathan S. Early detection of axonal injury after human head trauma using immunocytochemistry for beta-amyloid precursor protein. *Acta Neuropathol* 1994;87(1):55–62. [PubMed: 8140894]
36. Leclercq PD, McKenzie JE, Graham DI, Gentleman SM. Axonal injury is accentuated in the caudal corpus callosum of head-injured patients. *J Neurotrauma* 2001;18(1):1–9. [PubMed: 11200244]
37. Karnezis T, Mandemakers W, McQualter JL, Zheng B, Ho PP, Jordan KA, Murray BM, Barres B, Tessier-Lavigne M, Bernard CC. The neurite outgrowth inhibitor Nogo A is involved in autoimmune-mediated demyelination. *Nat Neurosci* 2004;7(7):736–744. [PubMed: 15184901]
38. Pluchino S, Quattrini A, Brambilla E, Gritti A, Salani G, Dina G, Galli R, Del Carro U, Amadio S, Bergami A, et al. Injection of adult neurospheres induces recovery in a chronic model of multiple sclerosis. *Nature* 2003;422(6933):688–694. [PubMed: 12700753]
39. Li S, Kim JE, Budel S, Hampton TG, Strittmatter SM. Transgenic inhibition of Nogo-66 receptor function allows axonal sprouting and improved locomotion after spinal injury. *Mol Cell Neurosci* 2005;29(1):26–39. [PubMed: 15866044]
40. Ito D, Imai Y, Ohsawa K, Nakajima K, Fukuuchi Y, Kohsaka S. Microglia-specific localisation of a novel calcium binding protein, Iba1. *Brain Res Mol Brain Res* 1998;57(1):1–9. [PubMed: 9630473]
41. Imai H, McCulloch J, Graham DI, Masayasu H, Macrae IM. New method for the quantitative assessment of axonal damage in focal cerebral ischemia. *J Cereb Blood Flow Metab* 2002;22(9):1080–1089. [PubMed: 12218413]
42. Kalara RN, Bhatti SU, Palatinsky EA, Pennington DH, Shelton ER, Chan HW, Perry G, Lust WD. Accumulation of the beta amyloid precursor protein at sites of ischemic injury in rat brain. *Neuroreport* 1993;4(2):211–214. [PubMed: 8453062]
43. Kawarabayashi T, Shoji M, Harigaya Y, Yamaguchi H, Hirai S. Expression of APP in the early stage of brain damage. *Brain Res* 1991;563(1-2):334–338. [PubMed: 1786548]
44. Blumbergs PC, Scott G, Manavis J, Wainwright H, Simpson DA, McLean AJ. Staining of amyloid precursor protein to study axonal damage in mild head injury. *Lancet* 1994;344(8929):1055–1056. [PubMed: 7523810]
45. McKenzie KJ, McLellan DR, Gentleman SM, Maxwell WL, Gennarelli TA, Graham DI. Is beta-APP a marker of axonal damage in short-surviving head injury? *Acta Neuropathol* 1996;92(6):608–613. [PubMed: 8960319]
46. Hunt D, Coffin RS, Anderson PN. The Nogo receptor, its ligands and axonal regeneration in the spinal cord; a review. *J Neurocytol* 2002;31(2):93–120. [PubMed: 12815233]
47. Domeniconi M, Cao Z, Spencer T, Sivasankaran R, Wang K, Nikulina E, Kimura N, Cai H, Deng K, Gao Y, et al. Myelin-associated glycoprotein interacts with the Nogo66 receptor to inhibit neurite outgrowth. *Neuron* 2002;35(2):283–290. [PubMed: 12160746]
48. Wang KC, Koprivica V, Kim JA, Sivasankaran R, Guo Y, Neve RL, He Z. Oligodendrocyte-myelin glycoprotein is a Nogo receptor ligand that inhibits neurite outgrowth. *Nature* 2002;417(6892):941–944. [PubMed: 12068310]
49. McKerracher L, Winton MJ. Nogo on the go. *Neuron* 2002;36(3):345–348. [PubMed: 12408839]
50. Harvey PA, Lee DH, Qian F, Weinreb PH, Frank E. Blockade of Nogo receptor ligands promotes functional regeneration of sensory axons after dorsal root crush. *J Neurosci* 2009;29(19):6285–6295. [PubMed: 19439606]
51. Lee JK, Kim JE, Sivula M, Strittmatter SM. Nogo receptor antagonism promotes stroke recovery by enhancing axonal plasticity. *J Neurosci* 2004;24(27):6209–6217. [PubMed: 15240813]
52. Rogove AD, Siao C, Keyt B, Strickland S, Tsirka SE. Activation of microglia reveals a non-proteolytic cytokine function for tissue plasminogen activator in the central nervous system. *J Cell Sci* 1999;112(Pt 22):4007–4016. [PubMed: 10547361]
53. Zhang N, Komine-Kobayashi M, Tanaka R, Liu M, Mizuno Y, Urabe T. Edaravone reduces early accumulation of oxidative products and sequential inflammatory responses after transient focal ischemia in mice brain. *Stroke* 2005;36(10):2220–2225. [PubMed: 16166574]
54. Yrjanheikki J, Keinänen R, Pellikka M, Hokfelt T, Koistinaho J. Tetracyclines inhibit microglial activation and are neuroprotective in global brain ischemia. *Proc Natl Acad Sci U S A* 1998;95(26):15769–15774. [PubMed: 9861045]

55. Yrjanheikki J, Tikka T, Keinanen R, Goldsteins G, Chan PH, Koistinaho J. A tetracycline derivative, minocycline, reduces inflammation and protects against focal cerebral ischemia with a wide therapeutic window. *Proc Natl Acad Sci U S A* 1999;96(23):13496–13500. [PubMed: 10557349]

Fig. 1. Neurological outcome after stroke

Panel **A** shows mNSS test after stroke in the 4 experimental groups (MCAo control, Niaspan treatment, Simvastatin treatment and combination treatment). Panel **B** shows footfault test after stroke in the 4 experimental groups (MCAo control, Niaspan treatment, Simvastatin treatment and combination treatment). SE = standard error. *p< 0.05 vs MCAo.

Fig. 2. Combination treatment of stroke with Niaspan and Simvastatin reduces axonal damage, increases axonal density, and decreases NGR and Iba-1 expression

Panel A-B Show that combination treatment with Niaspan and Simvastatin significantly decreased APP expression in the ischemic brain (A) and significantly increased Bielschowsky silver expression in the striatal bundles (B) compared with control MCAo rats. Panel C-D show that NGR (C) and Iba-1 (D) expression were significantly decreased in the ischemic brain in the combination treatment rats compared with control MCAo animals. SE = standard error.

* $p < 0.05$ vs MCAo. N=8/group. The scale bar in A,B,C and D is 0.1 mm.