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Niaspan increases axonal remodeling after stroke in type 1 diabetes rats[☆]

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Abstract

Background and objective—We investigated axonal plasticity in the bilateral motor cortices and the long term therapeutic effect of Niaspan on axonal remodeling after stroke in type-1 diabetic (T1DM) rats.

Experimental approaches—T1DM was induced in young adult male Wistar rats via injection of streptozotocin. T1DM rats were subjected to 2 h transient middle cerebral artery occlusion (MCAo) and were treated with 40 mg/kg Niaspan or saline starting 24 h after MCAo and daily for 28 days. Anterograde tracing using biotinylated dextran amine (BDA) injected into the contralateral motor cortex was performed to assess axonal sprouting in the ipsilateral motor cortex area. Functional outcome, SMI-31 (a pan-axonal microfilament marker), Bielschowsky silver and synaptophysin expression were measured. In vitro studies using primary cortical neuron (PCN) cultures and in vivo BDA injection into the brain to anterogradely label axons and terminals were employed.

Results—Niaspan treatment of stroke in T1DM–MCAo rats significantly improved functional outcome after stroke and increased SMI-31, Bielschowsky silver and synaptophysin expression in the ischemic brain compared to saline treated T1DM–MCAo rats ($p < 0.05$). Using BDA to anterograde label axons and terminals, Niaspan treatment significantly increased axonal density in ipsilateral motor cortex in T1DM–MCAo rats ($p < 0.05$, $n = 7/\text{group}$). Niacin treatment of PCN significantly increased Ang1 expression under high glucose condition. Niacin and Ang1 significantly increased neurite outgrowth, and anti-Ang1 antibody marginally attenuated Niacin induced neurite outgrowth ($p = 0.06$, $n = 6/\text{group}$) in cultured PCN under high glucose condition.

Conclusion—Niaspan treatment increased ischemic brain Ang1 expression and promoted axonal remodeling in the ischemic brain as well as improved functional outcome after stroke. Ang1 may partially contribute to Niaspan-induced axonal remodeling after stroke in T1DM-rats.

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Keywords

Type-one diabetes rats; Stroke; Angiopoietin; Axonal remodeling; Niaspan

Introduction

Axonal damage and degeneration are prominent components of acute neurological disorders such as stroke. Successful axonal outgrowth in the adult central nervous system (CNS) is central to the process of nerve regeneration and brain repair (Hou et al., 2008). Diabetes mellitus (DM) is a major health problem, and DM patients have a 3–4 fold higher risk of experiencing ischemic stroke. DM adversely influences the post-stroke level of disability, increasing the extent of the cerebral injured area and promoting worse outcome compared to the general population (Yong and Kaste, 2008). Diabetes also induces neuroaxonal dystrophy, synaptic dysplasia and defective axonal regeneration (Schmidt, 2002). Restriction of axonal regeneration and neuro-plasticity contributes to the worse functional recovery after stroke (Walmsley and Mir, 2007). Therefore, there is a compelling need to investigate axonal damage after stroke in DM rats and to develop therapeutic approaches specifically designed to remodel axons and thereby to reduce neurological deficits after stroke in the diabetic population.

Angiopoietin-1 (Ang1), a family of endothelial growth factors, promotes migration, sprouting, and survival of endothelial cells and mediates vascular remodeling (Suri et al., 1996). Ang1 plays a role in the recruitment of vascular smooth muscle cells (VSMCs) and pericytes during vascular maturation and the remodeling processes (Sato et al., 1995; Suri et al., 1996). However, overexpression of Ang1 in the brain not only increases vascularization but also alters neuronal dendrite configuration (Ward et al., 2004). Ang1 promotes neuronal differentiation in neural progenitor cells and neurite outgrowth in cultured dorsal root ganglion cells and in PC12 cells (Bai et al., 2009; Chen et al., 2009; Kosacka et al., 2005). In addition, neuritogenesis, expression of the presynaptic protein synaptophysin as well of the postsynaptic protein PSD-95 correlates with Ang-1 levels in culture (Kosacka et al., 2006).

Niacin (nicotinic acid) is the most effective medication in clinical use for increasing high density lipoprotein (HDL) cholesterol and is safely used in patients with diabetes (Elam et al., 2000). Niacin improves endothelial function, reduces inflammation (Rosenson, 2003) and has been used to improve endothelium-dependent vasodilatation in coronary heart disease patients (Chapman et al., 2004). Our previous studies have found that Niaspan treatment of stroke, a prolonged release formulation of Niacin, significantly increases Ang1 expression in the ischemic brain which promotes vascular remodeling and improves functional outcome after stroke in normal blood glucose wild-type (WT) rats (Chen et al., 2007). We also found that Niaspan treatment in T1DM stroke rats significantly increases Ang1 expression compared to non-treated T1DM–stroke rats (Ye et al., 2011). Whether Niaspan regulates axonal remodeling after stroke in DM rats and whether Ang1 plays a role in Niaspan-induced axonal remodeling have not been investigated. In this study, we investigated the effect of Niaspan treatment on axonal remodeling and functional outcome after stroke in type one diabetes rats (T1DM) and the role of Ang1 as underlying the axonal remodeling induced by Niaspan.

Materials and methods

All experiments were strictly conducted in accordance with the Henry Ford Hospital Institutional Animal Care and Use Committee. Rats were housed individually in an enriched environment and in a temperature-controlled (70°–72 °F), humidity-controlled (30%–50%)

vivarium and maintained with free access to food and water. After surgery, rats were monitored continuously until they fully recovered from anesthesia and twice during the first 24 h after stroke.

Diabetes induction

Adult male Wistar rats (225–250 g) purchased from Charles River (Wilmington, MA) were used. T1DM was induced by a single intraperitoneal injection of streptozotocin (STZ, 60 mg/kg, Sigma Chemical Co., St. Louis, MO) to rats (Like and Rossini, 1976). The fasting blood glucose level was tested by using a glucose analyzer (Accu-Chek Compact System; Roche Diagnostics, Indianapolis, IN) and rats with fasting blood glucose >300 mg/dl were identified as diabetic. Animals were subjected to MCAo 2 weeks after diabetes induction.

MCAo model and experiment groups

Wild type (WT) non-diabetic Wistar rats and STZ-induced T1DM rats were anesthetized and transient (2 h) MCAo was induced by using a previously described method of intraluminal vascular occlusion (Chen et al., 2001a). Briefly, rats were initially anesthetized with 3.5% isoflurane and maintained with 1.0 to 2.0% isoflurane in 70% N₂O and 30% O₂ by a face mask. Rectal temperature was maintained at 37 °C throughout the surgical procedure by means of a feedback-regulated water heating system. A 4–0 nylon suture with its tip rounded by heating near a flame was inserted into the external carotid artery (ECA) through a small puncture. The length of nylon suture, determined according to the animal's weight, was gently advanced from the ECA into the lumen of the internal carotid artery (ICA) until the suture blocked the origin of the middle cerebral artery (MCA). After 2 h of MCAo, animals were reanesthetized with isoflurane, and restoration of blood flow was performed by withdrawal of the filament. Rats were randomly separated to three groups 24 h after MCAo and were gavaged with: 1) saline WT–MCAo control; 2) saline for T1DM–MCAo-control; 3) T1DM–MCAo–+Niaspan treatment: 40 mg/kg Niaspan (Kos Pharmaceuticals, Inc. Cranbury, NJ; dissolved in saline) started 24 h after MCAo and administered daily until sacrifice. A battery of functional test was performed. One set of rats was sacrificed at 14 days after MCAo for immunostaining (n=8/group). Another set of animals (n=7/group) was employed for biotinylated dextran amine (BDA) injection in the contralateral hemisphere at 14 days after MCAo and rats were sacrificed at 28 days to measure axonal density in the ipsilateral hemisphere.

Functional test

A battery of behavioral tests (modified neurological severity score, mNSS) and foot-fault tests were performed prior to and at 1, 7, 14, 21 and 28 days after MCAo by an investigator who was blinded to the experimental groups.

mNSS

Neurological function was graded on a scale of 0 to 18 (normal score 0; maximal deficit score 18. mNSS is a composite of motor, sensory, reflex and balance tests) (Chen et al., 2001b).

Foot-fault test

For locomotor assessment, rats were tested for placement dysfunction of forelimbs with the modified foot-fault test (Hernandez and Schallert, 1988). The percentage of foot-faults of the left paw to the total number steps was determined.

Blood glucose measurement

Blood glucose was measured at 24 h after MCAo and at 28 days prior to sacrifice by using test strips for glucose (Polymer Technology System, Inc. Indianapolis, IN 46268, USA) according to the manufacturer's instructions. The data are presented as milligrams per deciliter.

Biotinylated dextran amine (BDA) injection and measurements of axon density

To investigate whether T1DM-MCAo affects axonal length and axonal sprouting, BDA was injected into the contralateral cortex at 14 days after MCAo (n=7/group) for anterograde labeling of axons and terminals (Veenman et al., 1992) in the ipsilateral hemisphere. These rats were sacrificed 28 days after MCAo. Briefly, the animals were placed in a stereotaxic device, and a unilateral craniotomy was performed on the skull overlying the right cerebral cortex. BDA solution (100 nL; a 10% solution dissolved in 0.1 mol/l phosphate-buffered saline (PBS) (pH 7.4), molecular weight 10,000 mw; Molecular Probes, Eugene, OR, USA) was injected through a finely drawn glass capillary into the right stereotaxic coordinates (+0.5 mm or -0.5 mm rostral to the bregma, 2.5 mm lateral to the midline, 1.5 mm depth from the surface of the cortex) over a 3-min time period. The micropipette remained in place for 4-min after the injection was administered.

Tissue preparation

Two weeks after BDA injection, animals were anesthetized with ketamine and transcardially perfused with saline, followed by 4% paraformaldehyde. The brain tissues were processed to acquire adjacent 100-µm-thick coronal sections using a vibratome. Ten sections from the center of the lesion (bregma -2 mm to +2 mm) were used to detect BDA labeling. Briefly, free-floating sections were incubated with 0.5% H₂O₂ in 50 mmol/l Tris-buffered saline (pH 7.4) for 20 min, and washed with Tris-buffered saline containing 0.25% Triton X-100 three times for 30 min each at room temperature. Then the sections were incubated with avidin-biotin-peroxidase complex (Vector Laboratories, Burlingame, CA, USA) in Tris-buffered saline/Triton X-100 at 41 °C for 3 days, and BDA labeling was visualized with 3, 3'-diaminobenzidine-nickel (Vector Laboratories) for light microscopy examination. Image was used for subsequent quantitative measurements of BDA-positive axonal densities in the ipsilateral cortex (six fields per section) from 10 sections from each individual animal divided by the total mean density in all animals and shown as percentage of proportional areas.

Histological and immunohistochemical assessment

The brains were fixed by transcardial perfusion with saline, followed by perfusion and immersion in 4% paraformaldehyde before being embedded in paraffin. For immunostaining, 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 of 5 sections was used for immunohistochemical staining. Antibody against synaptophysin (monoclonal antibody; dilution 1:1000, Chemicon, Temecula, Calif) and SMI-31 (a pan-axonal neurofilament marker, Neurofilaments, phosphorylated monoclonal antibody, 1:1000, Covance, CA) was employed. Bielschowsky silver immunostaining was used to demonstrate axons (Irving et al., 2001). Briefly, for Bielschowsky staining, slides were placed in 20% silver nitrate in the dark, then ammonium hydroxide was added, and were then treated with NaOH and sodium thiosulfate. Control experiments consisted of staining brain coronal tissue sections as outlined above, but non-immune serum was substituted for the primary antibody. The immunostaining analysis was performed by an investigator blinded to the experimental groups.

Bielschowsky silver, SMI-31 and synaptophysin expression quantification

For quantitative measurements of Bielschowsky silver, SMI-31 and synaptophysin, five slides from each brain, with each slide containing 4 fields from striatum for Bielschowsky silver, and 8 fields from the ischemic border (IBZ, cortex and striatum) for SMI-31, synaptophysin 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) (Calza et al., 2001; Chen et al., 2003). Positive areas of immunoreactive cells were measured in the IBZ. Data were analyzed in a blinded manner.

Double immunohistochemical staining

Our previous studies have found that Niaspan treatment of stroke in T1DM stroke rats significantly increased Ang1 expression in the ischemic brain (Ye et al., 2011). To identify cell type of Ang1-reactive cells, double immunofluorescence staining (Ang1/SMI-31) was performed. SMI-31 and Ang1 (rabbit polyclonal IgG, 1:2000, Abcam, Cambridge, MA, USA) were used. FITC (Calbiochem, Darmstadt, Germany) and cyanine-5.18 (CY5, Jackson ImmunoResearch, West Grove, PA) were used for double-label immunostaining. Each coronal section was first incubated with the primary and anti-SMI-31 antibodies with Cy5, and was then followed by Ang1 antibody with FITC. Control experiments consisted of staining brain coronal tissue sections as outlined above, but omitted the primary antibodies (Li et al., 1998).

Primary cultured neuron (PCN) culture and quantification of dendrite outgrowth in PCN

PCNs were obtained from pregnant 17 day Wistar rat embryos and cultured with Neuralbasal-A medium (GIBCO) containing 2% B27 medium-supplement (GIBCO). One hour of oxygen–glucose deprivation (OGD) was induced.

To test whether Niacin regulates Ang1 expression, the OGD-PCN cultures were treated without or with Niacin (1 mM) under high glucose (HG) condition (HG, 37.5 mmol/l glucose) for 3 days. Ang1 immunostaining was performed (n=4/group). The percentage of Ang1 positive cell number in total cells was presented.

Our previous studies found that high glucose (HG) significantly decreases PCN neurite outgrowth (Chen et al., 2011). To test whether Niacin regulates neurite outgrowth under HG condition, the OGD-PCN cultures were then divided into (n=6/group): 1) HG control (HG, 37.5 mmol/l glucose); 2) HG+Niacin (1 mM); 3) HG+Ang1 (100 ng/ml, Chemicon, Temecula, CA); 4) HG+Niacin+anti-Ang1 antibody (1 µg/ml, Abcam, Cambridge, MA); 5) HG+Niacin+Tie2-FC (recombinant mouse Tie2/FC, 2 µg/ml, Chimera, R&D System, Cambridge, MA) for 3 days. The PCN cultures were performed for TUJ1 immunofluorescent staining using a monoclonal anti-TUJ1 antibody (1:1000, Covance, Princeton, NJ) with Cy3 for dendrite outgrowth measurement. To trace the dendrite outgrowth of fluorescently labeled neurons, the fluorescent photomicrographs were captured at 20× magnification with a digital camera. The total dendrites of 20 TUJ1 positive neurons were measured using MCID analysis system (Markus et al., 2002). The average length of neuronal dendrite outgrowth was presented.

Statistical analysis

All measurements and analyses were performed by normality of distribution, and the homogeneity of variances was tested including the functional outcome, biochemistry, immunostaining and cell culture. One-way ANOVA was used for the evaluation of functional tests, immunostaining and neurite outgrowth analysis, respectively. Pearson's partial correlation after bivariate correlation analysis was used to analyze the correlation of

neurologic functional outcome with axonal density in the ischemic brain. All data are presented as mean±standard error of the mean (SE).

Results

Niaspan treatment in T1DM-MCAo rats does not alter blood glucose levels but increases HDL levels after stroke in T1DM rats

There were no significant differences in blood glucose levels between the two groups prior to treatment (T1DM-MCAo control: 367.2±43.6 mg/dl; T1DM-MCAo+Niaspan: 379.2±53.1 mg/dl) and after treatment at 28 days after MCAo (T1DM-MCAo control: 423.7±65.9 mg/dl; T1DM-MCAo+Niaspan: 458.6±59.1 mg/dl). Niaspan treatment significantly increased HDL level (39.3±2.2 mg/dl) compared to T1DM-MCAo control (31.6±1.3 mg/dl, $p<0.05$).

Niaspan treatment of stroke in T1DM rats significantly improves functional outcome after stroke (Fig. 1)

To test whether Niaspan treatment regulates long term functional outcome after stroke, a battery of behavioral tests was performed. Figs. 1A–B show that T1DM-MCAo rats exhibit significantly worse functional outcome until one month after MCAo compared to WT-MCAo rats ($p<0.05$). Niaspan treatment of stroke in T1DM-MCAo rats significantly attenuated neurological deficit on mNSS (Fig. 1A) and foot-fault (Fig. 1B) tests compared to non-treatment T1DM-MCAo rats ($p<0.05$, $n=7$ /group).

Cerebral axonal density is decreased in T1DM-MCAo rats

Niaspan treatment of T1DM-MCAo rats increases cerebral axonal density (Fig. 2). To investigate whether T1DM-MCAo affects axonal density, BDA was injected into the contralateral cortex at 14 days after MCAo and rats were sacrificed at 28 days after MCAo to anterogradely label axons and terminals. Fig. 2 shows that the density of BDA positive axons was significantly decreased in T1DM-MCAo rats compared to WT-MCAo rats ($p<0.05$, $n=7$ /group). However, Niaspan treatment significantly increased the axonal density in the ischemic brain compared to T1DM-MCAo control rats ($p<0.05$, $n=7$ /group). Correlation coefficient analysis shows significant negative correlations between mNSS score (28 days after MCAo) and axonal density (D , $r=-0.72$, $P<0.05$), and foot-fault (28 days after MCAo) and axonal density (D , $r=-0.74$, $P<0.05$) in stroke animals. These data suggest that T1DM-MCAo rats exhibit decreased ability of axonal outgrowth after stroke compared to WT-MCAo rats and that Niaspan treatment significantly increases axonal density in T1DM-MCAo rats. In addition, the significant correlation between axonal density and functional response suggests that axonal density contributes to neurological recovery.

Niaspan treatment promotes axonal remodeling and synaptic plasticity in T1DM rats after MCAo (Figs. 3A–C)

Synaptophysin is a marker for presynaptic plasticity and synaptogenesis (Ujike et al., 2002). SMI-31 is a pan-axonal neurofilament marker; Bielschowsky silver is a marker for axons. Figs. 3A–C show that T1DM rats exhibit significantly decreased SMI-31 expression in the striatum of the ischemic border zone (IBZ) compared to WT-MCAo control rats ($p<0.05$). T1DM-MCAo did not significantly decrease synaptophysin and Bielschowsky silver density compared to WT-MCAo rats ($p>0.05$). Niaspan treatment significantly increased SMI-31, Bielschowsky silver and synaptophysin expression in the ischemic hemisphere in T1DM-MCAo+Niaspan rats compared to non-treatment T1DM-MCAo control rats ($p<0.05$). These data suggest that Niaspan promotes axonal density and synaptic density in T1DM-MCAo rats.

Niaspan treatment increases Ang1 expression in T1DM-MCAo rats (Figs. 3D–E)

Axons and blood vessels share molecular signals for purposes of navigation, regeneration and terminal arborization (Ebenezer et al., 2011). Ang1 regulates vascular maturation (Thurston et al., 2000). In our previous study, we found that T2DM-MCAo mice exhibit significantly decreased Ang1 protein expression in the ischemic brain and Niaspan treatment increased Ang1 expression in T1DM stroke rats (Ye et al., 2011). In the present study, we found that Ang1 colocalized with SMI-31 (3D) in the ischemic brain, indicating that Ang1 is expressed in neurons.

Niacin and Ang1 increase neurite outgrowth in cultured PCN (Fig. 4)

To test whether Ang1 participates in Niaspan-induced axonal outgrowth, in vitro primary cortical neuron (PCN) culture was employed. Under conditions of high glucose (HG), Niacin significantly increased PCN Ang1 expression compared to non-treatment HG-control (Figs. 4A–C, $p < 0.05$). Both Niacin and Ang1 treatment increased neurite outgrowth in cultured PCN under condition of HG (Figs. 4E–F) compared to non-treatment control (Fig. 4D, $p < 0.05$). Anti-Ang1 marginally decreased Niacin induced neurite outgrowth (Fig. 4G, $p = 0.06$). However, Tie2-FC did not decrease Niacin induced neurite outgrowth (Fig. 4H, $p > 0.05$). These data suggest that Ang1 partially contributes to Niacin-induced neurite outgrowth, while it is not mediated by the Tie2 pathway.

Discussion

In this study, we are the first to demonstrate that T1DM-MCAo rats exhibit significantly decreased axonal density after stroke compared to WT-MCAo rats. Niaspan treatment of stroke in T1DM rats increased Ang1 expression, promoted axonal remodeling in the IBZ and improved functional outcome after stroke. High glucose (HG) decreased neurite outgrowth in PCN cultures (Chen et al., 2011). Niaspan treatment increased Ang1 expression in the ischemic brain and in the cultured PCN. Niacin and Ang1 significantly increased neurite outgrowth in PCN culture under HG condition. Inhibition of Ang1, but not Tie2-FC partially attenuated Niacin-induced PCN dendrite outgrowth. Therefore, Niacin induced increase of Ang1 may partially contribute to axonal remodeling in T1DM rats after stroke.

Axonal density is decreased in T1DM rats after stroke compared to WT-MCAo rats

Niaspan treatment of stroke increases axonal density in T1DM-MCAo rats. Functional recovery following acute CNS injury in humans, such as stroke, is exceptionally limited, leaving the affected individual with life-long neurological deficits. Axonal remodeling is related with functional outcome after stroke. Enhancement of plasticity by induction of axonal density has been shown to compensate for formerly lost function in spinal cord injury (Galtrey and Fawcett, 2007), as well as in stroke models (Liu et al., 2008; Papadopoulos et al., 2006). In this study, anterograde tracing with BDA injected into the right motor cortex was used to assess axonal sprouting in the contralateral motor cortex and ipsilateral rostral forelimb area. We found that T1DM-MCAo rats show decreased axonal density in the ipsilateral in T1DM-MCAo rats compared to WT-MCAo rats. Niaspan treatment significantly attenuated the decreased axonal density in T1DM-MCAo rats. Cortical neurons surviving in the peri-infarct motor cortex undergo axonal sprouting to restore connections between different cerebral areas after stroke (Carmichael, 2003; Carmichael et al., 2001; Dancause et al., 2005). Spontaneous functional recovery after stroke maybe attributed to axonal remodeling in the corticospinal system (Liu et al., 2009). Therefore, the increased axonal remodeling may contribute to the Niaspan-induced functional outcome after stroke in T1DM-MCAo rats. In addition, we found that functional benefit is present from 7 days after Niaspan treatment and persists to 28 days after treatment. The long term beneficial effect

after stroke is related to axonal remodeling. The early beneficial effect may be related to vascular protection/remodeling, such as decrease of BBB leakage and brain hemorrhage. Previous studies have found that axonal remodeling starts from 2 to 3 weeks after stroke and could be detected at 28 days after MCAo (Liu et al., 2009, 2010). Our data also indicate that functional outcome after stroke is significantly correlated with axonal density in the ischemic brain at 28 days after MCAo. Axonal remodeling may be needed for the maintenance of improved neurological benefit.

Ang1 partially contributes to Niaspan-induced axonal outgrowth in T1DM-MCAo rats

Many factors may regulate axonal remodeling after stroke, such as brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) and Ang1, among others. In our previous study, we found that Niaspan significantly increases both BDNF/TrkB and Ang1 expression in WT-stroke rats (Cui et al., 2010; Chen et al., 2007). However, we found that Niaspan significantly increases Ang1 expression (Ye et al., 2011), but does not increase BDNF expression in the ischemic brain in diabetes stroke rats (data not shown). The response to stroke and treatment differ between diabetic and wild-type stroke animals. In addition, Ang1 is particularly interesting because of its complementary and important role in angiogenesis and vascular maturation (Iurlaro et al., 2003). Ang1 not only promotes angiogenesis and vascular maturation, but is also a neurotrophic/neuritotrophic factor (Ward et al., 2005). In this study, we found that T1DM-MCAo rats exhibit decreased Ang1 expression as well as decreased axonal density compared to WT-MCAo rats. Inhibition of Ang1 using an anti-Ang1 antibody in cultured PCN partially decreases Niacin-induced neurite outgrowth. In addition, HG treatment in cultured PCN also decreases neurite outgrowth (Chen et al., 2011). In this study, we found that Ang1 treatment in cultured PCN in vitro attenuates the HG induced decrease of neurite outgrowth. Therefore decreased Ang1 expression in T1DM-MCAo rats may contribute to the increased axon damage. Although the mechanism of the observed Ang1-induced rescue of axonal outgrowth on HG remains to be elucidated, our initial experiments suggest it is not mediated via Tie2 pathway because function-blocking experiments using Tie2-FC did not affect neurite outgrowth on Niacin treated group. Our Ang1 findings do not exclude possible contributions to axonal remodeling from other factors.

Ang1 may regulate both the vascular and nervous systems. For instance, the networking properties of the nervous and vascular systems are governed by common cues that in the brain regulate axon connections and vasculature remodeling (Arese et al., 2011; Serini and Bussolino, 2004). Key axon guidance molecules direct axons to their targets and also help blood vessels to navigate to their targets (Carmeliet and Tessier-Lavigne, 2005). In the current study, we found that Niaspan treatment in DM stroke rats increases Ang1 expression. Ang1 is an angiogenic factor and also promotes vascular stabilization and maturation. We found that Ang1, not only regulates vascular change but also directly promotes neurite outgrowth in cultured primary cortical neurons. Therefore, Ang1 may couple the vascular and axon remodeling. In addition, vascular remodeling includes angiogenesis, vascular stabilization and maturation. Increase of vascular stabilization and maturation will decrease BBB leakage and brain hemorrhagic transformation. In our previous study, we found that diabetes significantly decreased Ang1, but increased Ang2 and MMP9 expression, which may contribute to the increased vascular damage, BBB leakage and brain hemorrhagic transformation (Cui et al., 2011). Increase of BBB leakage and brain hemorrhage may induce neurotoxicity into the ischemic brain and thereby promotes axonal and white matter damage. Therefore, it is also possible that the increase of Ang1 may decrease BBB leakage and brain hemorrhage and thereby indirectly decrease axonal damage in the ischemic brain. Ang1 may have both direct and indirect effects on the regulation of axonal remodeling.

There are a number of limitations and caveats to the present study. In this study, we only measured treatment outcome under hyperglycemic conditions for one month after stroke. Additional studies are warranted under conditions of hyperglycemic controls and longterm outcomes.

Conclusion

In summary, we demonstrate that treatment of experimental stroke in T1DM rats with Niaspan at 24 h after stroke significantly increased synaptic plasticity and axonal remodeling as well as long term functional outcome. The AngI appears to partially contribute to Niaspan-induced synaptic plasticity and axonal remodeling after stroke in T1DM rats.

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References

- Arese M, et al. Nervous vascular parallels: axon guidance and beyond. *Int J Dev Biol*. 2011; 55:439–445. [PubMed: 21858769]
- Bai Y, et al. Ectopic expression of angiopoietin-1 promotes neuronal differentiation in neural progenitor cells through the Akt pathway. *Biochem Biophys Res Commun*. 2009; 378:296–301. [PubMed: 19028450]
- Calza L, et al. Nerve growth factor control of neuronal expression of angiogenic and vasoactive factors. *Proc Natl Acad Sci U S A*. 2001; 98:4160–4165. [PubMed: 11259645]
- Carmeliet P, Tessier-Lavigne M. Common mechanisms of nerve and blood vessel wiring. *Nature*. 2005; 436:193–200. [PubMed: 16015319]
- Carmichael ST. Plasticity of cortical projections after stroke. *Neuroscientist*. 2003; 9:64–75. [PubMed: 12580341]
- Carmichael ST, et al. New patterns of intracortical projections after focal cortical stroke. *Neurobiol Dis*. 2001; 8:910–922. [PubMed: 11592858]
- Chapman MJ, et al. Raising high-density lipoprotein cholesterol with reduction of cardiovascular risk: the role of nicotinic acid—a position paper developed by the European Consensus Panel on HDL-C. *Curr Med Res Opin*. 2004; 20:1253–1268. [PubMed: 15324528]
- Chen J, et al. Therapeutic benefit of intravenous administration of bone marrow stromal cells after cerebral ischemia in rats. *Stroke*. 2001a; 32:1005–1011. [PubMed: 11283404]
- Chen J, et al. Intravenous administration of human umbilical cord blood reduces behavioral deficits after stroke in rats. *Stroke*. 2001b; 32:2682–2688. [PubMed: 11692034]
- Chen J, et al. Statins induce angiogenesis, neurogenesis, and synaptogenesis after stroke. *Ann Neurol*. 2003; 53:743–751. [PubMed: 12783420]
- Chen J, et al. Niaspan increases angiogenesis and improves functional recovery after stroke. *Ann Neurol*. 2007; 62:49–58. [PubMed: 17557352]
- Chen X, et al. Angiopoietin-1 induces neurite outgrowth of PC12 cells in a Tie2-independent, beta1-integrin-dependent manner. *Neurosci Res*. 2009; 64:348–354. [PubMed: 19379779]
- Chen J, et al. White matter damage and the effect of matrix metalloproteinases in type 2 diabetic mice after stroke. *Stroke*. 2011; 42:445–452. [PubMed: 21193743]
- Cui X, et al. Niacin treatment of stroke increases synaptic plasticity and axon growth in rats. *Stroke*. 2010; 41:2044–2049. [PubMed: 20671245]
- Cui X, et al. Angiopoietin/Tie2 pathway mediates type 2 diabetes induced vascular damage after cerebral stroke. *Neurobiol Dis*. 2011; 43:285–292. [PubMed: 21515377]
- Dancause N, et al. Extensive cortical rewiring after brain injury. *J Neurosci*. 2005; 25:10167–10179. [PubMed: 16267224]

- Ebenezer GJ, et al. Impaired neurovascular repair in subjects with diabetes following experimental intracutaneous axotomy. *Brain*. 2011; 134:1853–1863. [PubMed: 21616974]
- Elam MB, et al. 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; 284:1263–1270.
- Galtrey CM, Fawcett JW. The role of chondroitin sulfate proteoglycans in regeneration and plasticity in the central nervous system. *Brain Res Rev*. 2007; 54:1–18. [PubMed: 17222456]
- Hernandez TD, Schallert T. Seizures and recovery from experimental brain damage. *Exp Neurol*. 1988; 102:318–324. [PubMed: 3197789]
- Hou ST, et al. Permissive and repulsive cues and signalling pathways of axonal outgrowth and regeneration. *Int Rev Cell Mol Biol*. 2008; 267:125–181. [PubMed: 18544498]
- Irving EA, et al. Assessment of white matter injury following prolonged focal cerebral ischaemia in the rat. *Acta Neuropathol*. 2001; 102:627–635. [PubMed: 11761724]
- Iurlaro M, et al. Rat aorta-derived mural precursor cells express the Tie2 receptor and respond directly to stimulation by angiopoietins. *J Cell Sci*. 2003; 116:3635–3643. [PubMed: 12876214]
- Kosacka J, et al. Angiopoietin-1 promotes neurite outgrowth from dorsal root ganglion cells positive for Tie-2 receptor. *Cell Tissue Res*. 2005; 320:11–19. [PubMed: 15714275]
- Kosacka J, et al. Adipocyte-derived angiopoietin-1 supports neurite outgrowth and synaptogenesis of sensory neurons. *J Neurosci Res*. 2006; 83:1160–1169. [PubMed: 16493688]
- Li Y, et al. 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; 29:1972–1980. (discussion 1980–1). [PubMed: 9731626]
- Like AA, Rossini AA. Streptozotocin-induced pancreatic insulinitis: new model of diabetes mellitus. *Science*. 1976; 193:415–417. [PubMed: 180605]
- Liu Z, et al. Contralateral axonal remodeling of the corticospinal system in adult rats after stroke and bone marrow stromal cell treatment. *Stroke*. 2008; 39:2571–2577. [PubMed: 18617661]
- Liu Z, et al. Remodeling of the corticospinal innervation and spontaneous behavioral recovery after ischemic stroke in adult mice. *Stroke*. 2009; 40:2546–2551. [PubMed: 19478220]
- Liu Z, et al. Bone marrow stromal cells enhance inter- and intracortical axonal connections after ischemic stroke in adult rats. *J Cereb Blood Flow Metab*. 2010; 30:1288–1295. [PubMed: 20125183]
- Markus A, et al. Raf and akt mediate distinct aspects of sensory axon growth. *Neuron*. 2002; 35:65–76. [PubMed: 12123609]
- Papadopoulos CM, et al. Dendritic plasticity in the adult rat following middle cerebral artery occlusion and Nogo—a neutralization. *Cereb Cortex*. 2006; 16:529–536. [PubMed: 16033928]
- Rosenson RS. Antiatherothrombotic effects of nicotinic acid. *Atherosclerosis*. 2003; 171:87–96. [PubMed: 14642410]
- Sato TN, et al. Distinct roles of the receptor tyrosine kinases Tie-1 and Tie-2 in blood vessel formation. *Nature*. 1995; 376:70–74. [PubMed: 7596437]
- Schmidt RE. Neuropathology and pathogenesis of diabetic autonomic neuropathy. *Int Rev Neurobiol*. 2002; 50:257–292. [PubMed: 12198813]
- Serini G, Bussolino F. Common cues in vascular and axon guidance. *Physiology (Bethesda)*. 2004; 19:348–354. [PubMed: 15546852]
- Suri C, et al. Requisite role of angiopoietin-1, a ligand for the TIE2 receptor, during embryonic angiogenesis. *Cell*. 1996; 87:1171–1180. [PubMed: 8980224]
- Thurston G, et al. Angiopoietin-1 protects the adult vasculature against plasma leakage. *Nat Med*. 2000; 6:460–463. [PubMed: 10742156]
- Ujike H, et al. Gene expression related to synaptogenesis, neuritogenesis, and MAP kinase in behavioral sensitization to psychostimulants. *Ann N Y Acad Sci*. 2002; 965:55–67. [PubMed: 12105085]
- Veenman CL, et al. Biotinylated dextran amine as an anterograde tracer for single- and double-labeling studies. *J Neurosci Meth*. 1992; 41:239–254.

- Walmsley AR, Mir AK. Targeting the Nogo-A signalling pathway to promote recovery following acute CNS injury. *Curr Pharm Des.* 2007; 13:2470–2484. [PubMed: 17692015]
- Ward NL, et al. Angiopoietin 1 expression levels in the myocardium direct coronary vessel development. *Dev Dyn.* 2004; 229:500–509. [PubMed: 14991706]
- Ward NL, et al. Vascular-specific growth factor angiopoietin 1 is involved in the organization of neuronal processes. *J Comp Neurol.* 2005; 482:244–256. [PubMed: 15690488]
- Ye X, Chopp M, Cui X, Zacharek A, Cui Y, Yan T, Shehadeh A, Roberts C, Liu X, Lu M, Chen J. Niaspan enhances vascular remodeling after stroke in type 1 diabetic rats. *Exp Neurol.* 2011; 232:299–308. [PubMed: 21963653]
- Yong M, Kaste M. Dynamic of hyperglycemia as a predictor of stroke outcome in the ECASS-II trial. *Stroke.* 2008; 39:2749–2755. [PubMed: 18703813]

Fig. 1. T1DM-MCAo rats have worse neurological outcome after stroke compared to WT-MCAo rats ($p<0.05$). Niaspan treatment of stroke in T1DM rats significantly improves functional outcome after stroke. A: mNSS test; B: Foot-fault tests. * $p<0.05$ compared to T1DM-MCAo. $n=7/\text{group}$.

Fig. 2.

T1DM-MCAo rats exhibit decreased axonal density in the ischemic brain compared to WT-MCAo rats. Niaspan treatment of stroke in T1DM-MCAo rats significantly increased axonal density in the ischemic brain. A: Low magnification of BDA injection in the contralateral cortex (brown color, right side) and labeled axon in the ipsilateral cortex (brown color, left side). B: Quantitative data of axonal density (n=7/group). C: Axons are labeled with BDA in the ipsilateral hemisphere in WT-MCAo, T1DM-MCAo and T1DM-MCAo+Niaspan treatment animals. D: Correlation analysis of neurological outcome and axonal density.

Fig. 3.

Niaspan treatment of stroke in T1DM rats promotes axonal remodeling and synaptic plasticity. Ang1 expression is colocalized with SMI31 in the ischemic brain. A: Bielschowsky silver (a marker for axons) immunostaining and quantitative data; B: SMI-31 (a pan-axonal neurofilament marker) immunostaining and quantitative data; C: Synaptophysin (a marker for presynaptic plasticity) immunostaining and quantitative data; D: Ang1/SMI31 double immunostaining.

Fig. 4.

Niacin increases Ang1 expression in cultured hypoxic PCN. Niacin and Ang1 increase neurite outgrowth under HG conditioned media. Inhibition of the Ang1, but not Tie2-FC decreased neurite outgrowth in cultured hypoxic PCN under HG conditions. A–B: Ang1 immunostaining in cultured hypoxic PCN treated without (A) or with Niacin (B). C: Ang1 expression quantitative data (n=4/group). D–H: Neurite outgrowth measured by TUJ1 immunostaining in cultured hypoxic PCN under HG condition control (D); HG+Ang1 (E); HG+Niacin (F); HG+Niacin+anti-Ang1 (G); HG+Niacin+Tie-2-FC (H) and neurite outgrowth quantitative data (I). N=6/group.