BRAIN RESEARCH 1360 (2010) 147–158

available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Down-regulation of Nogo receptor promotes functional recovery by enhancing axonal connectivity after experimental stroke in rats

Tianzhu Wang[a], Jing Wang[a], Cheng Yin[a], Ruen Liu[b], John H. Zhang[c], Xinyue Qin[a,*]

[a]Department of Neurology, The First Affiliated Hospital of Chongqing Medical University, Chongqing 400016, China
[b]Department of Neurosurgery, China-Japan Friendship Hospital, Beijing100029, China
[c]Department of Neurosurgery, Loma Linda University School of Medicine, Loma Linda, CA, USA

ARTICLE INFO

Article history:
Accepted 30 August 2010
Available online 7 September 2010

Keywords:
Adenovirus-mediated RNAi
MCAO
NgR
Axonal connectivity

ABSTRACT

The inability of axons in central nervous system (CNS) to regenerate after injury is related partly to multiple endogenous axon growth inhibitors including Nogo receptor (NgR). This study tested the hypothesis that silencing NgR expression by adenovirus-mediated RNA interference (RNAi) (AD-NgR) may permit axonal connectivity after focal cerebral ischemia in rats. Male Sprague–Dawley rats (250–280 g, $n=97$) were assigned into seven groups: sham, MCAO (24 h and 2 weeks), MCAO plus AD-NgR (24 h and 2 weeks), and MCAO plus AD-HK (control oligonucleotides) (24 h and 2 weeks). After cerebral ischemia, NgR mRNA and protein in the cortex and hippocampus were significantly increased at 24 h and 2 weeks. However, in AD-NgR treated rats, NgR mRNA and protein were reduced by 40–60% in the cortex and hippocampus at both time points as compared to controls. Although there was no significant difference in the infarct volume between the two groups, the number of midline-crossing fibers projecting to the contralateral red nucleus and corticostriatal fibers in the dorsolateral striatum were increased in AD-NgR injected rats, accompanied by improved behavioral outcomes. Taken together, these results suggest that NgR knockdown may promote CNS axonal regeneration and functional recovery after ischemic cerebral injury.

1. Introduction

The inability of neuronal axons in CNS to regenerate after injury results severe limitations in the functional recovery after injury (Donoghue, 1997. Regenerative failure has been attributed in part to proteins associated with CNS myelin (Filbin, 2003) and with the glial scar that forms at the injury site (Silver and Miller, 2004). Several myelin inhibitors of axon growth, including Nogo (GrandPre et al., 2000), myelin-associated glycoprotein (MAG) (McKerracher et al., 1994) and

* *Corresponding author*. Fax: +86 23 89012478.
E-mail address: Qinxinyue@yahoo.com (X. Qin).

Abbreviations: AAV, adenovirus-associated virus; BDA, biotinylated dextran amine; BSA, bovine serum albumin; CNS, central nervous system; CST, corticospinal tract; TTC, 2,3,5-triphenyltetrazolium chloride; MAG, myelin-associated glycoprotein; MCAO, middle cerebral artery occlusion; NEP1–40, nogo extracellular peptide 1–40; NgR, nogo receptor; OMgp, oligodendrocyte myelin glycoprotein; RNAi, RNA interference; RT-PCR, reverse transcriptase-polymerase chain reaction

0006-8993/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2010.08.101

oligodendrocyte myelin glycoprotein (OMgp) (Wang et al., 2002a,b), exert their effects via the Nogo receptor (NgR) (Fournier et al., 2001) and other co-receptors, including $p75^{NTR}$, LINGO-1 and TROY (Wang et al., 2002a,b). Signaling through these receptors activate intracellular Rho GTPase signal pathways that results in the collapse of the growth cone (Niederost et al., 2002). Consequently, NgR is believed to play a pivotal role in the suppression of axonal growth pathways (McGee and Strittmatter, 2003). These pathways have been studied most intensively in traumatic spinal cord injury (Wang et al., 2006). The IgG fusion protein, NgR (310) Ecto-Fc, has been found to be more effective than Nogo extracellular peptide 1–40 (NEP1–40) in promoting axonal growth and recovery after spinal cord injury (Li et al., 2004). In behavioral assays in rats, NgR antagonism promotes functional recovery by enhancing axonal plasticity after stroke (Lee et al., 2004).

RNAi-induced gene knockdown is attractive for its faster speed, more usefulness, and lower cost, compared with the time-consuming conventional strategies such as gene targeting by homologous recombination (Kobayashi et al., 2004). In the present study, we examined whether adenovirus-mediated RNAi reduced the expression of NgR mRNA and protein in the cortex and hippocampus after cerebral ischemia/reperfusion. Furthermore, we determined whether knockdown of NgR promoted axon connectivity and functional recovery in rats after experimental stroke.

2. Results

2.1. *Adenoiviral gene delivery effect on gene expression in the peri-infarct cortex and hippocampus*

GFP-adenovirus was injected as control indicator to measure how far adenovirus could reach from injection sites. There were no GFP-positive cells detected in contralateral cortex or hippocampus 24 h after viral transfection (Fig. 1, A1 and A2). GFP-positive neurons were observed among injected peri-infarct cortex (Fig 1, C1 and C2), hippocampal regions CA1–3 and dentate gyrus (Fig. 1, B1 and B2).

2.2. *Infarct volume analysis*

Infarct volume (white colored areas) was evaluated using TTC staining at 24 h and 2 weeks after MCAO. The infarct volumes at 24 h (Fig. 2, A1, B1 and C1) were not significantly different among MCAO, MCAO plus AD-NgR and MCAO plus AD-HK groups ($P>0.05$). Even though TTC values were lower at 2 weeks when compared with those at 24 h in each group respectively ($P<0.01$), no significance was observed among the three groups at 2 weeks ($P>0.05$) (Fig. 2, A2, B2 and C2).

2.3. *Expression of NgR mRNA in infarcted cortex and hippocampus*

At 24 h (Fig. 3, A1 and B1) and at 2 weeks (Fig. 3, A2 and B2) after MCAO, a marked increase ($P<0.01$) in the expression of NgR mRNA in the infarcted cortex and ipsilateral hippocampus was observed when compared to the sham group. AD-NgR but not AD-HK treatment significantly ($P<0.01$) suppressed the level of NgR mRNA (Fig. 3, A3 and B3).

2.4. *Protein expression of NgR*

A significant elevation ($P<0.01$) of NgR protein was observed at 24 h and 2 weeks after MCAO (Fig. 4). Only AD-NgR treatment abolished the elevation of NgR in the ischemic cortex (Fig. 4, A1 and A2) and ipsilateral hippocampus (Fig. 4, B1 and B2).

2.5. *Sensorimotor function assessment*

Rats were trained daily for 2 weeks to establish limb preference and baseline measurements prior to MCAO (Fig. 5). No significant difference among the groups was recorded before MCAO ($P>0.05$). Animals that received MCAO surgery showed marked deficits in successfully obtaining pellets with the stroke-affected limb after 1 week. In AD-NgR treatment groups, a 50% recovery (3.2±0.44) of the baseline behavioral performance (6.2±0.45) was demonstrated after 3 weeks, which was significantly different from the values obtained from MCAO and MCAO plus AD-HK treatment groups. From 5 to 9 weeks, the behavioral performance of the rats which received AD-NgR treatment (4.8±0.89) was able to recover to 75% of the baseline behavioral performance, significantly better than MCAO and MCAO plus AD-HK treatment groups (Fig. 5).

2.6. *Tracing corticorubral and corticospinal tracts*

At the level of the midbrain, the lateral branches between the red nucleus and the primary motor cortex, was examined. A distinct characteristic of this unilateral projection to the red nucleus was that only a small number of fibers crossed the midline to project to the contralateral nucleus. In MCAO and AD-HK treatment groups, the corticorubral tract was ipsilateral dominant, with little evidence of fibers crossing to the contralateral red nucleus (Fig. 6, A2 and C2). In contrast, animals that underwent treatment with AD-NgR showed much more BDA-positive fibers crossing the midline and terminating in the contralateral red nucleus at appropriate target areas (Fig. 6, B2). Quantitative analysis (Fig. 6, D) demonstrated a significant ($P<0.01$) increase in the fibers crossing the midline treated with AD-NgR (520±56) as compared to animals in MCAO (220±33) and AD-HK treatment groups (158±67).

2.7. *Tracing corticostriatal tract*

At the level of striatum, there were large numbers of labeled fibers entering the ipsilateral striatum, most of which were located in the dorsolateral quadrant. There was no apparent difference in the intensity of labeling fibers in the ipsilateral striatum between these three groups (Fig. 7, A4, B4 and C4). In contrast with MCAO and AD-HK treatment groups, more BDA-positive fibers were seen coursing through the corpus callosum into the contralateral dorsolateral striatum in AD-NgR group (Fig. 7, A2, B2 and C2). Following quantitative analysis, the ratio of contralateral striatum BDA-positive fibers divided by ipsilateral striatum was significantly higher