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Research report

Intrathecal gene delivery of glial cell line-derived neurotrophic factor ameliorated paraplegia in rats after spinal ischemia

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Abstract

Paraplegia is a catastrophic complication of thoracic aortic surgery. At present, there is no effective mean to prevent the ischemia-induced spinal cord trauma. Gene delivery of neurotrophic factors may hold promises for prevention of spinal injury. In the present study, we evaluated the effect of glial cell line-derived neurotrophic factor (GDNF) gene delivery on prevention of the pathological changes due to spinal ischemia. Recombinant adenovirus vectors encoding GDNF (Ad-GDNF) and green fluorescent protein (Ad-GFP) were used for gene transfer studies. Treatment with cobalt chloride induced dose-dependent bcl-2 and synaptophysin downregulation in spinal neuronal cells, which could be effectively reversed by GDNF gene transfer. Intrathecal injection of Ad-GDNF led to maximal GDNF expression in spinal cord within 2–7 days. Thus, after intrathecal administration of adenovirus vectors for 3 days, Sprague–Dawley rats received transient aortic occlusion to induce spinal ischemia and were monitored for behavior deficits. The Ad-GDNF-treated rats showed significantly lower paraplegia rate (40%) than that of Ad-GFP- or saline-treated groups (75–85%; $P < 0.01$). In addition, the Ad-GDNF-treated rats exhibited significantly improved locomotor function comparing with rats of control groups ($P < 0.001$). Histological analysis revealed that GDNF gene delivery profoundly attenuated the infiltration of leukocytes in spinal cord after ischemic insults. Furthermore, GDNF gene delivery prominently attenuated the ischemia-induced neuronal loss in dorsal horn lamina VI–VIII and reduction in synaptophysin expression in spinal cords. In conclusion, GDNF gene transfer confers protection to the neuronal cells and synapses networks, thereby alleviated the paraplegia due to spinal ischemia.

Theme: Development and regeneration
Topic: Regeneration

Keywords: GDNF; Adenovirus; Gene delivery; Spinal ischemia; Intrathecal

1. Introduction

In thoracoabdominal aortic surgery, aortic cross-clamping may induce transient spinal ischemia and lead to various degrees of spinal cord injury. Paraplegia is the most serious and catastrophic complication of spinal cord injury. It is one of the major causes of patient's perioperative morbidity and mortality. The incidence of paraplegia ranges was reported from 5.4% to 31% [12,32]. In the largest institutional experience reported, the overall incidence of spinal cord injury was 16% [32], in which approximately half of these

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cases were devastating paraplegia. Various approaches such as monitoring of somatosensory evoked potentials, evoked spinal cord potentials, and motor evoked potentials had been employed for early detection of spinal cord ischemia during operation. Moreover, temporary shunts, partial bypass, and hypothermia were applied attempt to prevent this complication [15]. Nevertheless, none of these methods effectively prevent the development of paraplegia. The mechanisms underlying spinal cord injury by transient ischemia, although not fully elucidated, may involve tissue ischemia, the loss of neuronal cells, and reperfusion injury.

Glial cell line-derived neurotrophic factor (GDNF) belongs to transforming growth factor-β superfamily [22] and promotes survival and neurite outgrowth of dopaminergic and motor neurons, as well as peripheral sensory and sympathetic neurons [14,21]. It has been reported that GDNF conferred protection to neurons during various types of injuries to nervous systems in vitro and in vivo [7,21,36,37]. However, continuous application of therapeutic proteins into central nerve system (CNS) is hampered by the short half-life of protein, concomitant injection trauma, and the difficulty to across blood–brain barrier to CNS including spinal cord.

Replication-defective adenoviral vector efficiently transduced expression of foreign genes in differentiated, non-dividing cells such as neuronal cells and became an effective approach for gene transfer in CNS [2,20,31]. In the present study, it was demonstrated that intrathecal injection of Ad-GDNF led to sustained GDNF expression in spinal cord. Subsequently, we investigated whether pretreatment with GDNF gene delivery attenuated the paraplegia or locomotor deficits in rats after spinal ischemia and we will discuss the mechanism of GDNF neuroprotection.

2. Materials and methods

2.1. Cell cultures

Primary spinal neuronal cells were isolated from rat spinal lumbar regions at postnatal days 5–10 and cultured in DMEM (Gibco BRL, Rockville, MD) containing 10% fetal calf serum, 2 mM glutamine, and 20 ng/ml bFGF (R&D, Minneapolis, MN) in poly-lysine-coated dishes as previously described [33]. Rat pituitary GH_3 cells were purchased from American Type Culture Collection (ATCC; Rockville, MD) and cultured in DMEM/F-12 medium (Gibco BRL) containing 10% fetal calf serum, 2 mM glutamine, 100 mg/ml streptomycin, and 100 U/ml penicillin at 37 °C in 5% CO_2. For production and propagation of Ad5 adenovirus, E1a-transformed human embryonic kidney 293 cells were purchased from Microbix Biosystems (Toronto, Canada) and maintain them at low passage. The 293 cells were cultured in DMEM (Life Technologies, Rockville, MD) medium containing 10% fetal calf serum, 2 mM glutamine, 100 mg/ml streptomycin, and 100 U/ml penicillin at 37 °C in 5% CO_2 incubator.

2.2. MTT assay

Cells were cultured in a 96-well plate at a density of 4×10^4 cells per ml. After treatment, cells were supplemented with fresh medium containing 3-[4,5-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT; 0.456 mg/ml) and incubated for 1–2 h at 37 °C. The formazan in viable cells were dissolved with 100 μl of dimethyl sulfoxide and determined by reading optical densities in microplate reader (Dynex ELISA reader) at an absorption wavelength of 570 nm.

2.3. Generation and purification adenovirus vectors

The recombinant adenovirus vectors encoding GDNF (Ad-GDNF) or enhanced green fluorescent protein (Ad-GFP) were prepared as described previously [3,33]. The virus was amplified in 293 cells, purified by two rounds of cesium chloride gradient ultracentrifugation, and dialyzed against buffer containing 10 mM Tris, pH 7.5, 1 mM $MgCl_2$, 10% glycerol at 4 °C. The titer of virus solution was determined by measuring optical density at wavelength of 260 nm and plaque forming assay in 293 cells before storage at −80 °C.

2.4. Western blot analysis

Cells were homogenized with lysis buffer containing 40 mmol/l HEPES, 1% Triton X-100, 10% glycerol, and 1 mmol/l phenylmethanesulfonyl fluoride. The cell lysate was centrifuged at 6000 rpm for 5 min at 4 °C with a microcentrifuge and collected to determine protein concentration with a BCA protein assay kit (Pierce, Rockford, IL). An aliquot of 20 μg of protein from each sample was separated on 10% SDS-polyacrylamide gel. Proteins were subsequently transferred onto polyvinylidene difluoride membranes (Immobilon-P membrane, Millipore, Bedford, MA). Membranes were incubated for 1 h with a mouse IgG monoclonal antibody to bc-2 (1:1000; Transduction Laboratories) or synaptophysin (1:1000; Santa Cruz, USA). Membranes were then washed and incubated with a horseradish peroxidase-conjugated goat anti-mouse IgG antibody (1:5000; Vector Laboratories) for 40 min. Immunoreactivity was detected by ECL plus chemiluminescence kit (Amersham, UK).

2.5. Animals and surgical procedures

All animals were handled in compliance with the "Principles of Laboratory Animal Care" and the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health (NIH publication 85-23, revised 1985). Furthermore, the protocol was reviewed and approved by the Animal Care Committee of the Chang Gung Memorial Hospital, Kaohsiung. Rats were caged in groups of three to four in separate plastic cages with soft bedding and allowed free access to laboratory chow and tap water under a 12:12-h day/night cycle. The rats were kept at