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INTRODUCTION: Immunohistochemical analysis of the expression and localization of brain-derived neurotrophic factor (BDNF), choline acetyltransferase (ChAT), and activity of acetylcholine esterase (AChE) after adenovirus (AdV)-mediated BDNF gene transfer in and around the area of mechanical compression in the cervical spinal cord of the hyperostotic mouse (twy/twy).

OBJECTIVE: To investigate the neuroprotective effect of targeted AdV-BDNF gene transfection in the twy mouse with spontaneous chronic compression of the spinal cord anterior horn neurons.

BACKGROUND: Several studies reported the neuroprotective effects of neurotrophins on injured spinal cord motoneurons. However, no report has described the effect of targeted retrograde neurotrophic gene delivery on motoneuron survival in vivo in chronic compression lesions of the cervical spinal cord resembling lesions of myelopathy.

METHODS: The adenovirus Expression Vector Kit (Code#6150, Takara Biomedicals, Shiga, Japan) was used to allow recombinant adenovirus production. The final adenovirus vector titers contained $5.0 \times 10^{10}$ plaque forming units/ml. LacZ marker gene using adenoviral vector (AdV-LacZ) was used to evaluate retrograde delivery from the sternomastoid muscle to the cervical spinal cord anterior horn neurons in adult twy mice (16-week-old) and Institute of Cancer Research (ICR) mice (control). Four weeks after the AdV-LacZ or AdV-BDNF injection, the compressed cervical spinal cord was removed en bloc for immunohistological investigation of β-galactosidase activity and immunoreactivity and immunoblot analyses of BDNF. The number of anterior horn neurons was counted using Nissl, ChAT and AChE staining.

RESULTS: Spinal accessory motoneurons between C1 and C3 segments were successfully transfected by AdV-LacZ in both twy and ICR mice after targeted intramuscular injection. Immunoreactivity to BDNF was significantly stronger in AdV-BDNF-gene transfected twy mice than in AdV-LacZ-gene transfected...
mice. At the cord level showing the maximum compression in AdV-BDNF-transfected twy mice, the number of anterior horn neurons was significantly higher in the topographic neuronal cell counting of Nissl-, ChAT- and AChE-stained samples, than in AdV-LacZ-injected twy mice. Targeted AdV-BDNF-gene delivery via the sternomastoid muscle significantly increased Nissl-stained anterior horn neurons, which lacked nuclear chromatolysis and showed neurite arborization, and enhanced ChAT and AChE immunoreactivities in the twy mouse anterior horn neurons.

CONCLUSION: Viral vector-mediated gene delivery is feasible for therapeutic use in spinal cord injury in terms of efficacy and duration of transgene expression. However, direct in vivo gene administration raises serious problems with regard to the possible spread of traumatic damage to the spinal cord itself leading to further neural tissue necrosis and apoptosis. Our results suggest that targeted retrograde AdV-BDNF-gene in vivo delivery may enhance neuronal survival even under chronic mechanical compression without any damage.

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