Purpose of study

Despite considerable advances in the techniques used to repair damaged facial nerve, functional recovery is often not complete and the time required for nerve regeneration remains quite long. Therefore, a facial nerve crush injury model was constructed and the nerve regeneration after crush injury was evaluated using exogenous agents in an effort to overcome this problem. The aim of this study was to propagate a brain-derived neurotrophic factor (BDNF) adenovirus and to evaluate the efficacy of BDNF gene delivery and the immunosuppressant FK506 to regenerate crushed facial nerve in rats.

Materials & methods

Human BDNF cDNA was obtained from the cDNA library and amplified using PCR. The BDNF gene was delivered into an adenovirus shuttle vector in which E1 was deleted. A BDNF-adenovirus was infected into 293 cells and a virus plaque was obtained 2 days later. RT-PCR was performed to evaluate the secretion of BDNF in the infected cells. The ¥â-gal staining method was used after the transduction of adenovirus-LacZ with various MOIs in order to measure the transduction efficiency of the adenoviral vector. An ELISA specific for this protein was used in order to quanitate the BDNF levels in the viral infected cell lines and mock cell lines.

In the in vivo study, a two millimeter crushed injury was made on the right facial nerve main trunk just before the furcation of the branch ( n=72, Sprague Dawley rat ). In the experimental groups, the BDNF group (n=24) was injected with adenoviral BDNF(3§¡, 1011 plaque forming unit (pfu) /§¡) alone and the BDNF+FK506 group (n=24) was injected with adenoviral BDNF once on the day of crush injury; after the wound closure, it is followed by subcutaneous injections of FK506(5mg/kg/day) on the upper part of the injured site 8 days on a daily basis. In the control group(n=24), an equal amount of saline was injected into the crushed facial nerve. The nerve regeneration was evaluated with functional test (vibrissae and ocular movement), electrophysiological studies (threshold, peak voltage and conduction velocity) and a histomorphometric study at four intervals after treatment ( postoperative days (PODs) 10, 20, 30 and 40).

Results

The BDNF mRNA in the BDNF-adenovirus infected Schwann cells was detected using RT-PCR. The DNA concentration in the Schwann cell was 0.690§¶/§¡. However, it was 0.795§¶/§¡ in the BDNF-adenovirus infected Schwann cells. The most effective infection concentration was determined by a ¥â-gal staining method and 50MOI was found to be optimal.

In the in vivo study, there was a significant difference between the control group and the BDNF+FK506 group in the vibrissae movement score at POD20 (p<0.05). At POD 30, there was a significant difference between the control group and BDNF group (p<0.05). The mean scores of vibrissae movement at 30 and 40 days were significantly different between the control and experimental groups (p<0.05). However, there was no difference between the BDNF group and BDNF+FK506 group in the mean score of vibrissae movement. There was a significant difference in the mean score of vibrissae movement and ocular movement with time in each group (p<0.05).

The mean threshold value in the electrophysiological test showed a significant difference between the control group and BDNF+FK506 group at POD 20 (p<0.01). There was a significant difference in the mean conduction velocity value between the control group, BDNF group and BDNF+FK506 group at POD 30 and POD 40 (p<0.01). There was a significant difference in the mean threshold value and the conduction velocity value with time in all groups (p<0.01). The mean axon density values obtained on POD 20, significantly increased in the BDNF+FK506 group in comparison to the other groups (p<0.01). However, there was no significant difference between the BDNF group and BDNF+FK506 group at POD 30 and
40. A statistically significant improvement of the total axon count in the BDNF+FK506 group was initially noted on POD 20 (p<0.01). The myelin thickness increased in all of the groups between 20 to 40 days after the injury and it was always higher in experimental groups than in the control group. The histomorphometric analysis values of all groups, except for the mean myelin thickness value in the BDNF group showed significant increases with time.

Conclusion

The generation of a BDNF-adenovirus and the induction of BDNF overexpression were successful in host cells. In the in vivo study, the degree of nerve regeneration was significantly higher in the experimental groups than in the control groups during the 40 days of analysis, and the functional recovery rate after facial nerve crush considerably increased in the BDNF-adenovirus with FK506 groups at 30 days after the operation. The results of this study also suggest that the FK506 effect may have initiated at approximately 20 days.

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