Sciatic nerve regeneration using calcium phosphate coated conduit and Brain-derived neurotrophic factor gene-transfected Schwann cell in rat

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Purpose of Study

Peripheral nerve damage due to cancer surgery or surgical trauma results in detrimental effects. Peripheral nerve regeneration depends on neurotrophism of distal nerve stump, recovery potential of neuron, supporting cell like Schwann cell and neurotrophic factors such as BDNF. Peripheral nerve regeneration can be enhanced by the conduit which connects the both sides of transected nerve. The conduit maintains the effects of neurotrophism and BDNF produced by Schwann cells which can be made by gene therapy. In this study, we tried to enhance the peripheral nerve regeneration by using calcium phosphate coated Millipore conduit and BDNF-Ad infected Schwann cells in sciatic nerve of rats.

Materials and Methods

Microporous filter which permits the tissue fluid essential for nerve regeneration and does not permit infiltration of fibroblasts, was made in 2mm diameter and 17mm length. To improve the Schwann cell adhesion and survival, the Millipore filter was coated with calcium phosphate. The coated filter was evaluated by SEM examination and MTT assay.

For effective allogenic Schwann cell culture, dorsal root ganglia of 1-day old rat were extracted and treated with enzyme and antimitotic Ara-C. Human BDNF cDNA was obtained from cDNA library and amplified using PCR. BDNF gene was inserted into adenovirus shuttle vector pAACCMVpARS in which E1 was deleted. We infected the BDNF-Ad into 293 human mammary kidney cell-line and obtained the virus plaque 2 days later. RT-PCR was performed to evaluate the secretion of BDNF in infected Schwann cells. To determine the most optimal m.o.i of BDNF-Ad, we infected the Schwann cells with LacZ adenovirus in 1, 20, 50, 75, 100, 250 m.o.i for 2 hours and stained with ß-galactosidase.

Rats(n=24) weighing 300g were used for peripheral nerve regeneration experiment. Total 14mm nerve defect was made and connected with calcium phosphate coated conduits. Schwann cells(1x106) and BDNF-Ad infected Schwann cells(1x106) were inserted in conduit and media(MEM) was injected in control group. 12 weeks after surgery, we evaluated the state of nerve regeneration by gait analysis, electrophysiologic measurements and histomorphometric analysis.

Results.

1. Microporous Millipore filter was effective conduit which permitted the adhesion of Schwann cells and inhibited the adhesion of fibroblast. We could enhance the Schwann cell adhesion and survival by coating Millipore filter with calcium phosphate.
2. Schwann cell culture technique using repeated treatment of Ara-C and GDNF was established. The mean number of Schwann cells obtained 1 and 2 weeks after the culture were $1.54\pm 4.0; 10^6$ and $9.66\pm 9.6; 10^6$.
3. The mRNA of BDNF in BDNF-Ad infected Schwann cells was detected using RT-PCR. In Schwann cell 0.69 ìg/ìl of DNA was detected and in BDNF-Adenovirus transfected Schwann cell 0.795 ìg/ìl of DNA was detected. The most effective infection concentration was determined by LacZ Adenovirus and 75 m.o.i was found the most optimal.

Conclusion
BDNF-Ad transfected Schwann cells successfully regenerated the 14mm nerve gap which was connected with calcium phosphate coated Millipore filter. The BDNF-Ad group showed better results compared with Schwann cells only group and control group in aspect to sciatic function index, electrophysiologic measurements and histomorphometric analysis.

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- Keywords: peripheral nerve regeneration, Calcium phosphate coating, nerve conduit, Schwann cell, BDNF (Brain-derived Neurotrophic factor), Adenovirus.
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