FUNDAMENTAL SURGICAL TECHNIQUES OF CENTRAL NERVE SYSTEM REGENERATION EXPERIMENTS

Ağahan Ünlü*, Hasan Çağlar*, Efkan Çolpan*, Celal Bağdatoğlu**

* Ankara University Medical School, Department of Neurosurgery
** Mersin University Medical School, Department of Neurosurgery

SUMMARY
Neuronal recovery from stroke, trauma, or neurodegenerative disorders is very limited because the human CNS has a very restricted regeneration capacity. Lots of experiments have been done to improve the limited capacity of injured CNS neurons. Those experiments have mainly focused on improving the intrinsic capacity of the CNS neurons, and overcoming the inhibitory CNS environment which has been shown blocking the axonal regeneration. Optic nerve, sympathetic ganglia, dorsal root ganglia have been used for in vivo regeneration experiments. The main advantage of these experiments is that the cell body which is situated outside CNS have been easily manipulated and the results have been identified at nerve body level. In this article optic nerve and dorsal root ganglion model have been presented. All the techniques that have been used are previously defined techniques. The aim of this article is to collect these data and clarify with some simple modifications.

ÖZET
SANTRAL SINİR SİSTEMİ REJENERSAYON ÇALIŞMALARINDA TEMEL CERRAHİ TEKNİKLER

Corresponding Author:
Dr. Ağahan Ünlü
Ankara Üniversitesi Tıp Fakültesi
Beyin Cerrahisi Anabilim Dalı, Ankara
INTRODUCTION

Everyday a lot of people lost their lives or become morbid after trauma, stroke and neurodegenerative syndromes that injure CNS neurons. Although many attempts have been made to improve survival, unfortunately there is not a complete way for complete recovery. The ideal way should be the replacement of injured neurons to achieve a complete recovery.

Since Cajal, many studies have been done to have sufficient neuronal regeneration. Although peripheral nerve system has the capacity for neural regeneration, this capacity is limited in central type of neurons. In invitro studies cellular functions, cellular regeneration in different conditions, the effects of environment on cell regeneration have been well studied. Nowadays, genetics of cell is suggested to be important in regeneration. Application of the information gathered from in vitro studies or cellular function in cell’s natural environment are the principles of in vivo experiments. It is essential to define basic methods in experimental studies.

In this article we summarize the surgical basis of in vivo regeneration experiments. We believe this article will lighten the projects on Central Nerve Regeneration.

MATERIAL and METHODS

Twelve rats weighing 200-250 gr. were used according to the protocol of UW Animal Care Unit, for this experiment.

Rats were given Ketamine/ Xylasine combination IP for general anesthesia. Surgical microscope (Zeiss, Germany) was also used for surgery. Digital photographs were taken with Coolpix 950 (Nikon Co. Japan).

Optic Nerve Regeneration Model

Proximal injury

Prone position was used. After shaving and cleaning with alcohol - betadine solutions, a midline skin incision was made at the frontal region. Bended and taped needles were used for retraction of skin edges. Some sharp dissection was needed to approach periorbital structures. An incision should be done from lateral orbita to the medial side, just over the bone. Superior orbital nerve was cut and dissected over the intraorbital fat tissue. The fat tissue was retracted to medial side to periorbita in order to cover the orbital structures. The superior rectus muscle was exposed and cut 2 mm from globe. The proximal part of that muscle was used to retract the globe. After dissecting the tissues around the globe, the optic nerve was exposed at posterior medial part of the globe. Very small cotton balls retracted the fat tissue at two sides of optic nerve laterally. Vasculature of optic nerve and retina were at the inferior side of the optic nerve. It is essential to preserve those vessels in regeneration experiments. The perioptic tissues were dissected on superior side and all vessels were preserved that were situated on inferior side. The optic nerve was dissected from the perineural sheets. It is essential to identify the optic nerve wet and bright in color which provides sufficient dissection of membranes. A fine microforceps was inserted between the sheet and the nerve in order to protect vasculature of the retina that was situated inside the membranes. The optic nerve was cut 1-5 mm. to globe for proximal axotomy experiments.

In some of the experiments sciatic nerve grafting was used. For grafting two 10/0 sutures were used at each side of optic nerve. (Figure 1) The graft were put on and secured to frontal periosteum.

The fat tissue that was retracted to lateral was reflected to its original position over anastomosis. Skin was sutured with 4/0 surgilene.
Fig 1: Suturing of the syatic nerve to cut optic nerve with 10/0 monofilament

Distal injury
The proximal part of the optic nerve was prepared as described above. Additionally, superior orbital rim was drilled out and a small frontal craniectomy was performed. The intracranial part of the optic nerve and chiasm were exposed after partial frontal lobectomy. The intracranial part of the optic nerve was cut just before the chiasm. Pulling the optic nerve from retrobulbar part was very easy after dissecting the nerve from perineural membranes in orbita. The cut optic nerve was used for distal injury and grafting experiments.

Dorsal Root Ganglion Model

High cervical Dorsal Column Injury
In prone position, cervical, occipital and upper thoracic regions were shaved and cleaned. A midline incision from occiput to lover cervical region was made. Skin was retracted laterally. Occiput and prominent of C2 were identified under fascia. Muscles were dissected at each side of C1 and C2. Dissection was also carried over occiput. Lamina of C2 was cleaned of muscle with a scissor and cotton balls. Spinous process of C2 was removed with rongeur and laminectomy was performed with electric drill. Dura was opened with sharp and bended needle tip. Dura edges were retracted laterally. Arachnoid over spinal cord should be peeled off with fine forceps. Dorsal column lesions were done at each side of midline which was defined by a central artery. Sharp and bended needles were used to inflict injury 1mm deep. Grafting was done using a sciatic nerve graft which was sutured to piamater with 10/0 sutures. (Figure 2) The distal end of sciatic graft was secured over occiput for further applications.

Proximal Lumbar root Injury
In prone position, lumbar area was shaved and cleaned. Midline skin incision was made and skin was retracted. On one side praspinal muscles were dissected sharply and L4, L5 laminas were identified. Lamina of L4 or L5 was removed with electric drill. Roots were exposed for crush injury or proximal axotomy. (Figure 3)

Sciatic Nerve Graft Harvesting
Prone position was used and a skin incision was made on the thigh parallel to extremity axes. Skin edges were retracted to each side with 4 needle retractors. Quadriceps femoris muscle was dissected from posterior side. Sciatic nerve was found beneath the muscle. Branches were also dissected near the popliteal region. (Figure 4) The main trunk and the branches were cut. Under microscope sciatic nerve was
prepared for grafting. The middle caliber branch was used for grafting side. The thinnest and thickest branches were cut and graft was cleaned. The graft should be protected from additional traumas.

Fig 3: A model of proximal root injury on rat made left lumbar 4 hemi-partial laminectomy

In order to make a sufficient injury and grafting, some points should be made. First, the dissection of the peri-orbita should begin on the superior orbital side, and venous structures should be carefully dissected before cutting to prevent excessive bleeding. Second, very small cotton balls are very useful for dissection and retraction. Third, vasculature of the retina should be preserved by the technique described above. Fourth, the grafting procedure is very important. The suture should first pass through the graft side and then optic nerve. The optic nerve should be held between the tips of microforceps without squeezing and the needle should pass very superficially. The material induced fibrotic changes can block regenerated axonal ends to pass through the anastomosis side.

Dorsal root ganglion regeneration models have been used to identify the regenerative capacity of sensorial neurons. The pseudobipolar cell body which is situated in ganglion is also easily accessible. Proximal injury has been made 2-3 mm. to ganglion and distal injury has been inflicted by sectioning of sciatic nerve. The axons of the cell body are also injured at high cervical level at dorsal column. The advantage of this system is to compare the peripheral and central responses in the same cell body. The system has been also used for peripheral axotomy induced central regeneration. Conditioning lesion, which is made by cutting the sciatic nerve, has been
suggested to promote cellular events that would improve the regeneration in central axons.

The cervical dorsal column lesion is used to cut the axons of DRG cells. The first point to mention is laminectomy. It is essential to make a sufficient laminectomy for lesion and grafting. The spinous process is removed by rongeurs, but the lamina has been removed with electric drill. The dura can be easily opened with a bended 22 gauge needle. The arachnoid should be separately opened with two microforceps for easy lesioning. The lesion can be made with free hand or stereotactic instruments. The graft should be secured very superficially to the spinal cord.

In the proximal DRG injury model, the partial laminectomy is done with electric drill. The ganglion is easily identified at the foraminal exit with its white-pinkish color. The proximal part of the root is cut or crushed. The site should be far enough to prevent the primary inflammatory effects of injury on the ganglion. It is essential to use the unique techniques for the experiments in order to test the effects of factors. The widely used techniques described above are the fundamental parts of the in vivo regeneration experiments. All researchers who are interested on nerve regeneration need to know about the parts of the experiments.

REFERENCES