The source and pathway of regenerating axons in end-to-side neurorraphy without epi/perineurial window model

**Introduction**

Since 1992, Vitebro and colleagues reintroduced the concept of end-to-side (ETS) neurorrhaphy and, over the following 10 years, several reports have been published. At present, this concept is being continually and used enthusiastically in clinical setting as repair of large peripheral nerve defect [1,2]. Although several experimental reports have showed histological and/or electrophysiological evidences of axonal regeneration in the distal nerve segment which coapted its end to the side of the intact nerve, the source of regenerating axons has been controversial. Several experimental reports indirectly showed that intact axons in the donor nerve give off collateral sprouts at the coaptation site [3]. However, a couple of recent basic studies on ETS neurorrhaphy showed findings suggesting regenerating axons derive far proximal to the coaptation site [4]. Normal peripheral nerve contains not only neurite promoting (such as laminin) but also inhibiting molecules (such as MAG, chondroitin sulfate proteoglycan, NG2) on/inside the Schwann cell basal lamina, which is thought to stabilize axons and prevent axons from sprouting collaterals. If collateral sprouting is the source of regenerating axons, those neurite inhibiting molecules must be downregulated around the coaptation site. To define the precise source of regenerating axons is indispensable for utilizing an ETS neurorrhaphy technique in clinical setting. To clarify the source of the regenerating axons in ETS neurorrhaphy, we carried out (1) Immunofluorescent study using antibodies against neurofilament (NF), MAG, CSPG and NG2, (2) electron microscopic study and (3) morphometric analysis.

**Materials and methods**

40 adult (12 to 16 week old) male Lewis rats were used. To exclude the chance of axonal injury of donor nerves on coaptation, we used T-shaped silicone chamber instead of suturing. The rat sciatic nerves were exposed at the mid-thigh level. The proximal stump was implanted into the major trochanter to avoid reinnervation. The intact tibial nerve and the distal peroneal nerve segment (10mm) were set in a T-shaped silicon chamber so that the two nerves attach in end-to-side fashion without sutures. (1) 1-4 weeks after surgery, the nerves were harvested and longitudinal and transverse sections were cut in a cryostat. Immunocytochemical investigation on the expression and localization of NF, MAG, CSPG and NG2 in experimental and normal sciatic nerves (control) were carried out. (2) 6 weeks after surgery, transverse sections were cut at following 3 levels for histological and morphometric analysis of the donor tibial nerve and the distal peroneal nerve segment (n=8). Level A: 10mm distal to the foramen intervertebrale, B: 5mm distal to the coaptation site, C: distal nerve segment of peroneal nerve. These sections were stained with Toluidine blue and/or anti-NF antibody examined by light microscopy for histological and morphometric analysis. Region of interest were outlined, cut ultra-thin (20 to 50 nm), and analyzed with electron microscopy.

**Results**

**Pattern of axonal regeneration in ETS neurorrhaphy** NF positive axons (regenerating axons) appeared in the distal peroneal nerve segment 3 weeks after surgery, and increased in number with time. On transverse sections at the coaptation site, many small diameter axons penetrating perineurium and invading into the epineurium were seen circumferentially. Under electron microscopy, myelinated axon and unmyelinated axons ensheathed with Schwann cell cytoplasm were confirmed in the epineurial connective tissue and between the perineurial cells (Fig. 1). In contrast, there were not any axons seen in epiperineurium at level A. Instead, a number of small diameter regenerating axons traveling in-group inside the fascicle were observed at level A (Fig. 2).

**Change in expression of the neurite inhibiting molecules** As mentioned in the introduction, downregulation of these molecules, especially those present around the intact axons such as MAG, CSPG, and NG2 appear to be a prerequisite for collateral sprouting from the intact axons in the tibial nerve. However, expression of MAG, CSPG and NG2 didn’t show any changes throughout the experiment period in the donor tibial nerve around the coaptation site.

**Axon Morphometry** At level A, the mean diameter of myelinated axon (3.945±1.839 µm) was significantly reduced after ETS coaptation compared with the control (4.304±2.405µm) (p< .0001). A frequency distribution showed that small diameter axons were increased at level A (Fig. 3).

**Discussion**

The expression of the neurite inhibiting molecules such as MAG, CSPG and NG2 at the coaptation site didn’t show any change, suggesting that the milieu around the coaptation site is inhibitory to formation of collateral sprouting. The present study demonstrated that in ETS neurorrhaphy without epi/perineurial window model regenerating axons originate far proximal to the coaptation site, possibly at the nerve cell body or very close to them, travel within the tibial nerve, penetrate the perineurium around the coaptation site, and invade into the distal peroneal nerve segment.

**References**