A standardized method to create optic nerve crush: 
Yasargil aneurysm clip

Levent Sarikcioglu a,*, Necdet Demir b, c, Arife Demirtop c

a Department of Anatomy, Akdeniz University, Faculty of Medicine, 07070 Antalya, Turkey
b Department of Histology and Embryology, Akdeniz University, Faculty of Medicine, 07070 Antalya, Turkey
c Electron Microscopy Unit (TEMGA), Akdeniz University, Faculty of Medicine, 07070 Antalya, Turkey

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Abstract

It is often difficult to compare results obtained by different investigators on nerve compression injuries, owing to differences in method of pressure application and noncomparable pressure levels. In the present study, we described a new method to crush the optic nerve by using a specially designed and commercially available device. We think that standardization of the compression methods is necessary to compare interlaboratory results.

Keywords: Yasargil aneurysm clip; crush injury; optic nerve

1. Introduction

The optic nerve of the rat is a very vulnerable structure (Gellrich et al., 2002). Isolated position of the optic nerve allows the scientist to create lesions conveniently, without damaging other parts of the brain. Another advantage of the optic nerve lesion model is that recovery of the function can be studied in a well-defined anatomical system with specific tests of visual behavior (Sabel and Aschoff, 1993).

Trauma to the optic nerve caused by either fractures of the midface and/or skull base has been simulated by optic nerve crush injury model (Gellrich et al., 2002). The main drawback of most of the methods described for optic nerve crush is that the injury cannot be quantified at the site of the trauma, but is rather semiquantitatively referred to as pressure in a balloon (Burke et al., 1985, 1986; Cottee et al., 1991), distance between the tips of a forceps (Buys et al., 1995; Duvdevani et al., 1990; Sautter and Sabel, 1993), or pulling force on a micro-sling (Matsuzaki et al., 1982).

In the literature, there are several methods to create optic nerve crush injury. However, the main disadvantages of these methods are standardization and their possible usage by investigators from all over the world. In our previous study we described a simple and reliable device, the Yasargil aneurysm clip, to create sciatic nerve injury. In this current study, our goal was to describe a simple method that simulates trauma to the optic nerve caused by fractures of the midface and/or skull base, in order to investigate the effects of optic nerve crush from a histological point of view.

2. Materials and methods

2.1. Animals

A total number of 20 female Wistar rats (200—250 g) were randomly divided into two groups (control, experimental). The animals were housed in Makrolon cages (5 per cage) and maintained on a 12-h light—dark cycle; lights on from 7.00—19.00 h. Food and water were provided ad libitum.
procedures were reviewed and approved by animal care and usage committee of Akdeniz University.

2.2. Yasargil aneurysm clip

Yasargil aneurysm clips (Aesculap AG & Co., Tutlingen, Germany) are designed for permanent occlusion of cerebral aneurysms (Yasargil, 2005). These permanent implantable clips are manufactured from a high grade Phynox cobalt–chrome alloy, or titanium which conforms to the ISO standards. Closing force of the clips is determined by a computerized electronic gauged scale. Closing force of the Yasargil aneurysm clip (catalog no. FE-752K) used in the present study was 185 gf (grams force) (approximately 1.82 N).

2.3. Optic nerve crush

The rats were anesthetized with a mixture of ketamine–xylazine (ketamine; 80 mg/kg IP, xylazine; 10 mg/kg IP) and their intraorbital optic nerves were exposed after a lateral canthotomy. All surgical procedures were performed under a trinocular operation microscope (Olympus SZ61) with fiber optic illumination. Parts of the lachrymal gland and the eye muscles were dissected 3 mm from the globe. Care was taken to ensure that the crush did not compromise the ophthalmic artery. The clip was placed 2 mm away from the optic nerve head. The dura was left intact and care was taken not to injure the optic nerve except with the subsequent crush. The optic nerve was crushed with a Yasargil aneurysm clip for 30 s. After completion of the crush, the canthotomy was sutured. Then, antibiotic ointment was applied, and the animals were allowed to recover from the surgery.

2.4. Light and electron microscopic evaluation

One month after the optic nerve crush, the animals were administered an overdose of chloral hydrate intraperitoneally. Each optic nerve was re-exposed and the dural sheath incised longitudinally 1 mm from the globe in both control and experimental groups. The optic nerves posterior to the crush site were then collected. Samples were fixed with 4% glutaraldehyde in 0.1 M Sorensen’s phosphate buffer solution (pH: 7.3), post-fixed with 2% osmium tetroxide in the same buffered solution, dehydrated through an ascending series of ethanol, and the samples were then embedded in epoxy resin (Araldite CY212, Agar Scientific Ltd., Stansted, UK). Semithin sections (1 μm thickness) stained with toluidine blue were examined with light microscope (Zeiss Axioplan). Then, ultrathin sections (40–60 nm thickness) were contrasted with uranyl acetate and lead citrate, and prepared sections were examined with Zeiss LEO 906E electron microscope.

3. Results

3.1. Application of surgery

In our pilot study, the optic nerve was crushed by three approaches, supraorbital, lateral, and medial. In medial approach, it was difficult to use the Yasargil aneurysm clip to reach the optic nerve, since there was the lacrimal duct system in this region. In supraorbital approach, it was also difficult to reach the optic nerve. The frontal lobe of the brain and the skull bones make the surgery difficult. The easier approach was the lateral approach. By lateral chantotomy, a small incision was performed and the optic nerve was exposed, then it was crushed with a Yasargil aneurysm clip (Fig. 1).

3.2. Light and electron microscopic evaluation

The optic nerve was consisted of both myelinated and unmyelinated nerve fibers. Neither myelin debris nor damaged fibers was detected in control group in light microscopic observations. Myelinated and unmyelinated fibers showed well-shaped appearance (Fig. 2A, B).

Light microscopic evaluations revealed that numerous damaged myelin residues were present in the experimental group at the 30th postoperative day. Additionally, electron microscopic observations revealed that optic nerve crush resulted in severe degradation of the fibers of the optic nerve (Fig. 2C, D).

4. Discussion

Compression of nerve fibers can cause various clinical symptoms depending on the cause, magnitude and duration of the compression trauma (Rydevik and Lundborg, 1977). The pathophysiology of these various lesions is not fully understood and it has been debated whether the ischemia, secondary to compression, or the mechanical deformation of nerve fibers per se is the more significant etiological factor. Compression injury was, at first, attributed to ischemia due to following reasons: (1) nerve function was known to be dependent on blood supply and perfusion was impaired during compression; (2) nerve conduction failure did not occur even when in vitro nerve was compressed under high pressures (Powell and Myers, 1986); (3) nerve injury was greater with increased durations of compression; and (4) large myelinated fibers were especially vulnerable to ischemic injury.
the role of the ischemia has been stressed by some authors (Merrington and Nathan, 1949; Powell and Myers, 1986), the significance of the mechanical nerve fiber deformation was emphasized by others (Gelfan and Tarlov, 1956; Ochoa et al., 1972). The optic nerve axons may be severed by either crushing or cutting the optic nerve. In rabbits and rats this operation does not usually result in ischemic death of the retina, provided that the lesion is more than about 1 mm behind the eyeball. The central retinal artery in these animals enters the optic nerve just behind the optic disc. Posteriorly, the optic nerve contains no large internal vessels and appears to receive its blood supply through its meningeal covering (Ruskell, 1964). Postoperative changes in the retina and optic pathways can therefore be attributed with some confidence to the degeneration of axon (Kiernan, 1985). In the present study, the clip was placed 2–3 mm away from the optic nerve head and care was taken to ensure that the crush did not compromise the ophthalmic artery. Therefore, we think that the central retinal artery did not get affected from the compression.

Although the crush itself is an acute injury, the propagation of damage is a chronic event. This model can therefore simulate the spreading of neurodegenerative diseases, but not their initiation. Existing models for glaucoma are associated with increased intraocular pressure (Schwartz, 2004; Yoles et al., 1997). Each model has its specific advantages and disadvantages, and it is only by accumulating the relevant data from several models that it will be possible to make valid inferences for clinical situations. It is worth noting that whatever information was obtained from the optic nerve crush model turned out to be valid for other models as well (Schwartz, 2004; Schwartz and Cohen, 2000). It has been reported that chronic degeneration caused by an increase in intraocular pressure would better simulate the characteristics of glaucoma than crush injury (Schwartz, 2004; Schwartz and Cohen, 2000).

It is often difficult to compare the results obtained by different investigators on nerve compression injuries, owing to differences in method of pressure application and noncomparable pressure levels. In the present study, we tried to overcome some of these problems by using a specially designed and commercially available device. In our previous study (Sarikcioglu and Ozkan, 2003), this device was used to produce quantitative crush injury to the rat sciatic nerve. One of the most important advantages of this device is that, due to its relatively small size, it can reach the narrow approached areas, such as spinal nerve roots, facial nerve, and optic nerve as studied in the present study. The most important disadvantage of this device is that it cannot be used for graded compression injury. The investigator could only change the duration of the compression. We think that standardization of the compression is necessary to compare interlaboratory results.

Although crush of the intraorbital part of the optic nerve has been extensively performed (Becker et al., 2000; Buys et al., 1995; Campbell et al., 1999; Chen and Weber, 2001; Freeman and Grosskreutz, 2000; Meyer and Miotke, 1990; Okada et al., 2005), crush of the intracranial part of the optic nerve has also been reported. Chierzi et al. (1999) performed intracranial optic nerve crush injuries in wild-type mice and in
bcl-2 transgenic mice, which overexpress bcl-2 in neurons, including retinal ganglion cells. These investigators found that 100% of retinal ganglion cells were still viable 1 month after optic nerve crush in bcl-2 transgenic mice, compared with 45% retinal ganglion cell survival after crush in wild-type mice.

In the literature, there are several types of indirect or direct injuring models, neither of which allows quantitative or standard application of compression. The balloon method was used on the optic nerve of the cat (Burke et al., 1985, 1986; Cottee et al., 1991) and micro-sling was used on the optic nerve of Japanese monkeys (Matsuzaki et al., 1982). Forces was also used to cause a defined trauma to the optic nerve of rats (Buys et al., 1995; Duvdevani et al., 1990; Kiernan, 1985; Sautter and Sabel, 1993), mice (Li et al., 1999; Tezel et al., 2004), and opossum (Araujo Couto et al., 2004).

Chen and Weber (2001) used a smooth-faced bulldog clamp that exerts approximately 1024 gf (grams force) to crush the optic nerve for 15 s. They used this clamp in the cat. Additionally, ligation is also a popular method to crush the optic nerve for 15 s. They used this clamp in the cat. Addition-

al forces modified by attaching a screw to calibrate the force

of the post-traumatic changes in the retinal ganglion cell layer (Gellrich et al., 2002). To solve the standardization problem few attempts have been reported. Duvdevani et al. (1990) developed a Castroviejo’s cross-action (self-closing) capsule forceps modified by attaching a screw to calibrate the force applied to the optic nerve. They changed the screw nut position to achieve various crush forces. They studied the consequences of the lesion with different severities both electrophysiologically and behaviorally. On the electrophysiological examination, they observed a lesion-dependent loss of conduction of the compound action potential across the crush site; and more severe the crush, the more severe the initial deficit and the smaller the recovery of the compound action potential. On behavioral examination they observed that the loss of visual function, as defined by rat’s ability to orient toward a visual stimulus, and the subsequent recovery also depended on the severity of the lesion. To improve their method, Sautter et al. (1991) attached a micrometer screw to the handle cross-action forceps. These cross-action forceps have also been used by numerous studies (Hanke, 2002; Kipnis et al., 2000; Mawrin et al., 2003; Sautter and Sabel, 1993; Schmitt and Sabel, 1996).

Another attempt to calibrate the optic nerve crush was performed by Klocker et al. (2001). They described a model of crush lesion of the rat optic nerve inducing retrograde ganglion cell degeneration that can be carefully controlled in its extent by a newtonmeter device. They studied the histological consequences of the injury, and also monitored the functional integrity of the retinal ganglion cell projection. They observed that the extent of secondary retinal ganglion cell death increased linearly with the applied crush force. Moreover, visually evoked potentials were used to characterize the consequences of controlled optic nerve lesion on the functional integrity of the visual projection. Their method is based on a calibrated device in combination with the supra-orbital approach. Their method has several advantages in comparison with previously reported crush methods and lateral-orbital approaches. Gellrich et al. (2002) used the same microinjuring device for making optic nerve injury, and quantified the histological changes after this calibrated crush.

To perform the same crush injury model described above, investigators need to obtain the same calibrated device. However, Yasargil aneurysm clip is a specially designed device and is also commercially available all over the world. The results of the present study revealed that the crush injury of the optic nerve resulted in degeneration, approved by appearance of the numerous damaged myelin residues in the optic nerve. The present device would be sterilized and successively applied. No inflammatory response was observed during the postoperative days. Another advantage of this device is that, due to its relatively small size, it can be reached to the narrow approached areas. The most important disadvantage of this device is that it cannot be used for graded compression injury. In our previous report (Sarikcioglu and Ozbek, 2003), we used Yasargil aneurysm clip to create 5, 10, 20 min compressions to the rat sciatic nerve and found a strong relationship between nerve damage and subsequent recovery. Increment of the time resulted in severe axonal degeneration in sciatic nerve. We think that such increment of time might also be studied for the optic nerve.

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