Effect of intrathecal FK506 administration on intraorbital optic nerve crush: an ultrastructural study

Levent Sarikcioglu,* PhD; Necdet Demir† PhD; Yusuf Akar‡ MD; Arife Demirtop§ BSc

ABSTRACT • RÉSUMÉ

Objective: Trauma to the optic nerve caused by fractures of the midface and (or) skull base has been simulated by an optic nerve crush injury model. Because the intraorbital segment of the optic nerve is surrounded by subarachnoidal cerebrospinal fluid and dura mater, we aimed to study the influence of intrathecal tacrolimus (FK506) administration after optic nerve crush injury and to determine its role in optic nerve protection or sparing after injury.

Study Design: Experimental study.

Participants: All optic nerves of the animals were included in the study.

Methods: A total of 48 female Wistar rats were randomly divided into 4 groups (control, sham operated, FK506 treated, and vehicle treated). In vehicle- and FK506-treated groups, intrathecal catheter implantation and crush injury to the intraorbital part of the optic nerve were performed and then the animals were treated intrathecally. The optic nerve samples were harvested on the 30th postoperative day. Optic nerve appearances were analyzed qualitatively.

Results: Light and electron microscopic evaluations revealed that numerous damaged myelin residues were present in the vehicle-treated group, whereas fibres of the optic nerve showed a well-shaped appearance in the FK506-treated group.

Conclusion: We propose that such an intrathecal administration route and small-dose regimen should be used to obtain lesser immunosuppression and neurotoxicity and higher protection or sparing after injury.

Nature: Étude expérimentale.

Participants: Tous les nerfs optiques des animaux compris dans l’étude.


Résultats: Les évaluations par microscope à la lumière ou électronique ont révélé que de nombreux résidus de myéline endommagée se trouvaient dans le groupe traité avec un véhicule, alors que les fibres du nerf optique semblaient bien formées chez le groupe traité au FK506.

Conclusions: Nous proposons qu’on devrait avoir recours à l’administration intrathécale et au régime à petites doses pour obtenir moins d’immunosuppression et de neurotoxicité et plus de protection ou d’ épargne après la blessure.

FK506 (tacrolimus) is a hydrophobic macrocyclic lactone isolated from a fermentation broth of *Streptomyces tsukubensis* from Mount Tsukuba in northern Japan.1,2 FK506 is a potent immunosuppressant and promotes graft survival in organ transplantation. It has a mechanism of action similar to that of cyclosporin A, but is more potent.3 The use of FK506 is of special interest in ophthalmology, because it may be effective in the treatment of immune-mediated diseases, such as corneal graft rejection, keratitis, scleritis, ocular pemphigoid, uveitis, and ocular Behçet disease.4 Although an increased rate of axonal regeneration has been noted in the central and peripheral nervous system, FK506 has some adverse effects, such as nephrotoxicity, hypertension, hyperesthesia, muscular weakness, insomnia, tremor, photosensitivity, gastrointestinal symptoms, and central nervous system alterations.4,5-8 The incidence of side effects may be increased when FK506 is administered systemically.4 Furthermore, systemically administered drugs penetrate poorly into the intraocular tissues because of the blood–ocular barrier. To avoid systemic side effects, topical application of an agent is preferred to the oral and intravenous routes.4 The optic nerve of the rat is a very vulnerable structure. Trauma to the optic nerve caused by fractures of the midface and (or) skull base has been simulated by an optic nerve crush injury model.9 The optic nerve, ontogenetically an outgrowth of the brain, is surrounded by subarachnoid-
that a small dose of FK506 should be beneficial to the optic nerve recovery because of its administration route. For this reason, we aimed to study the influence of intrathecal FK506 administration after optic nerve crush injury and to determine its effects on optic nerve crush from an ultrastructural point of view.

Methods

Animals
A total of 48 female Wistar rats (200–250 g) were randomly divided into 4 groups (control, sham operated, FK506 treated, and vehicle treated). The animals were housed in Makrolon cages (5 per cage) and maintained on a 12-hour light–dark cycle. Food and water were provided ad libitum. All procedures were reviewed and approved by the Animal Care and Use Committee of Akdeniz University.

Catheter implantation
Before all surgical operations, the animals were anesthetized with intramuscular injection of the mixture of xylazine HCl (10 mg/kg) and ketamine (80 mg/kg). The surgical area was shaved, and swabbed with an antiseptic solution. One longitudinal cutaneous incision was made over the dorsal midline. A small part of the muscles located between the spinous and transverse processes was retracted. The L4-5 or L5-6 vertebral level was adjusted according to the level of the tip of the iliac crest. The insertion needle of a 24-gauge catheter (Neoflon) was pushed forward and the spinal canal was entered through the L4-5 or L5-6 interlaminar space. During that time, the tail flick reflex was observed. The catheter was pushed in approximately 2 vertebral levels. The access port was placed subcutaneously. This implantation technique had been improved in a pilot study before the experimental studies. Additionally, the vertebral column of the animal was transected to ensure that the catheter was located in the spinal canal. The following day, an experienced author (Levent Sarikcioglu) examined the animals anatomically for impaired motor function to ensure that the catheter itself did not damage the spinal cord. Animals showing impaired motor function were excluded from the study.

Optic nerve crush
Crush injury to the intraorbital part of the optic nerve was performed 1 week after catheter implantation. During that time, the animals recovered from implantation surgery. Neurologically normal rats after catheter implantation were divided into 2 groups (FK506 treated and vehicle treated). These rats were anesthetized with a mixture of ketamine-xylazine and the intraorbital part of their optic nerves was exposed after a lateral canthotomy. Three millimetres from the globe, on the left side only, the optic nerve was crushed with a Yasargil aneurysm clip as described previously. A sham operation was performed in another group by exposing the optic nerve, but not crushing it. Then, antibiotic ointment was applied and the animals were allowed to recover.

FK506 administration
FK506 (0.05 mg/kg/day dissolved in sterile saline) was administered intrathecally from the day of the optic nerve crush to the day of animal sacrifice (postoperative day 30). FK506 administration was performed every day (including the weekends) at 10:00 AM. The same volume of saline was administered to the vehicle-treated animals. Before FK506 administration, the catheter's dead space was measured and then filled with saline. FK506 was administrated through the needle at the entry point.

Light and electron microscopic evaluations
Thirty days after optic nerve crush, the animals received an overdose of chloral hydrate intraperitoneally and were transcardially perfused with phosphate buffer solution. Each optic nerve was re-exposed in all groups. The optic nerve specimen was collected by cutting the optic nerve from the posterior of the crush site to the orbital orifice of the optic canal. Specimens were fixed with 4% glutaraldehyde in 0.1 mol/L Sorensen's phosphate buffer solution (pH 7.3) and postfixed with 2% osmium tetroxide in the same buffered solution, and, after dehydration through a graded ethanol series, embedded in epoxy resin (Araldite CY212, Agar Scientific, Stansted, U.K.). Semithin sections obtained from the middle part of the optic nerve specimens were stained with toluidine blue and examined under a light microscope (Zeiss Axioplan, Carl Zeiss, Oberkochen, Germany). Then, ultrathin sections (40–60 nm) were contrasted with uranyl acetate and lead citrate and examined with a Zeiss LEO 906E transmission electron microscope.

Results

Application of surgeries
Catheter implantation was the most difficult procedure of the study. We started to perform the catheter implantation on 24 animals, but in 7 animals, the catheter resulted in a neurological deficit. These animals were excluded from the study and new ones were included until the appropriate catheter implantation was achieved. As we described in a previous study, we reached the optic nerve via the lateral approach. By lateral chantoctomy, a small incision was performed and the optic nerve was exposed, then it was crushed with a Yasargil aneurysm clip. Immediately after the acute compression injury, the crushed area of the optic nerve became very thin, but nerve continuity was not interrupted grossly. After both surgeries, all rats survived and no wound infections were detected.

Light and electron microscopic observations
The optic nerve consists of both unmyelinated and myelinated nerve fibres. Neither myelin debris nor damaged fibres were detected in the control and sham-operated
Intrathecal FK506 administration—Sarikcioglu et al.

Sheaths around the optic nerve showed normal structure. We could not observe endoneural edema or damage in these groups. Myelinated and unmyelinated nerve fibres showed a well-shaped appearance and normal axoplasm (Figs. 1 and 2).

Severe damage was observed in the vehicle-treated group on the 30th postoperative day. Electron microscopic evaluations showed that numerous damaged myelin residues were present in the vehicle-treated group. Additionally, nerve crush resulted in severe degradation in the myelinated and unmyelinated fibres of the optic nerve. Normal myelinated fibres were destroyed and the fibres’ borders were not clear. There was an enormous amount of myelin debris and myelin residue. Normal axon structure, axoplasm, neurofilaments, and neurotubules could not be seen (Fig. 3).

However, light and electron microscopic observations revealed that a well-shaped appearance on the same postoperative day was present in the FK506-treated group (Figs. 1 and 3). Additionally, evaluation of the cross-section of the optic nerve specimens revealed that there were very small degenerative sites in the optic nerve. These sites located diffusely in the optic nerve (Fig. 1). Therefore, we observed less myelin debris in the cytoplasm in the FK506-treated group compared with the vehicle-treated group. Axonal structures, neurofilaments, and neurofibrils were clear and could be defined (Fig. 2).

**Conclusions**

In this study, we showed that intrathecal administration of FK506 affected the optic nerve after injury of its intraorbital part. Different dosages of FK506 for the regeneration of various nerves have been reported in the literature. Although Gold reported that the optimal dose of FK506 was 5 mg/kg, there is no consensus on the optimal dose of FK506 to achieve the best regeneration rate. The wide range of these dosages may be due to the administration route or to individual variability in absorption, distribution, and elimination of FK506.

Intrathecal drug delivery places medication directly into the cerebrospinal fluid that surrounds the spinal cord. For instance, morphine delivered directly to the intrathecal space is particularly effective because it does not have to circulate systemically to reach the cerebrospinal fluid and the dorsal horn of the spinal cord. As a result, much smaller doses are needed (e.g., approximately 1/300 of an oral morphine dose), and the frequency of side effects is reduced. For this reason, we selected intrathecal administration of FK506 with a small dose. Compared with the dose (5 mg/kg)
Intrathecal FK506 administration—Sarikcioglu et al.

proposed by Gold,\textsuperscript{21} we used a 100-times smaller dose for intrathecal administration.

Gillon et al.\textsuperscript{24} reported that FK506 did not promote more axonal regeneration into the peripheral nerve graft but did reduce inflammation while not reducing the number of regenerated axons of the retinal ganglion cell, in comparison with the findings in sham-injected animals. In the present study, we showed a protective effect of the FK506 by qualitative electron microscopic evaluations. We think that further studies should be performed using specific tracers or intrinsic markers specific to regenerated retinal ganglion cell axons to evaluate the effect of FK506 on axonal regeneration in the optic nerve.

Topical,\textsuperscript{25,26} intravitreal,\textsuperscript{26,27} intramuscular,\textsuperscript{26} subcutaneous,\textsuperscript{12} and oral\textsuperscript{18} application of FK506 have been reported in the literature. Intrathecal administration has not, to our knowledge, been investigated in ocular diseases. In our previous report, intrathecal administration of the FK506 showed beneficial effects to the sciatic nerve recovery.\textsuperscript{28} It might be possible that FK506 treatment avoids the loss of myelin and therefore plays a protective or sparing role after injury. Because the daily dose of FK506 reported in the literature is very high, we used a small dose (0.05 mg/kg) of FK506. We propose that the intrathecal administration route and dose regimen should be used to obtain lesser immunosuppression and neurotoxicity and higher protection or sparing after injury.

This study was supported by the Akdeniz University Research Fund. The authors have no proprietary or commercial interest in any materials discussed in this article.

References


Keywords: intrathecal administration, FK506, crush injury, optic nerve, protection; optic nerve crush in vivo.