Morphofunctional aspects of restorative processes in the optic nerve after traumatic injury under the influence of high doses of corticosteroids

N.M. Moiseenko, PhD in Medical Sciences

State Higher Education Institution "Ivano-Frankivsk National Medical University", Department of Otolaryngology and Ophthalmology

Ivano-Frankivsk (Ukraine)

E-mail: natalymoyseenko@ukr.net

Keywords: traumatic injury of the optic nerve, regenerative processes, remyelination, high doses of corticosteroids, the amplitude of the pupillary reaction.

Search of conditions conducive to the activation of regenerative processes and restoration of the functions of the optic nerve after traumatic injuries is relevant.

The purpose *of this research was to study morphological and functional aspects of the rehabilitation process in the optic nerve after traumatic injury under the influence of high doses of corticosteroids.*

Material and methods. *24 adult rabbits were used, 12 of them were from the group of rabbits injured without treatment and another 12 were treated with methylprednisolone on the second day after the injury. Morphological study was conducted on the 14th day, and the functional one (the amplitude of pupillary reaction) was carried out on the 14th day and in 1 month.*

Results *After the use of high doses of corticosteroids in the intracranial portion of the optic nerve, on the 24th day, improvement of microcirculation, decreasing swelling and remyelination of nerve fibers were detected. At the same time, the amplitude of the pupillary reaction has increased in one month.*

Conclusions. *It was established that in case of traumatic injury, usage of high-dose corticosteroids contributes not only to morphological but also to functional signs of activation of regenerative processes of the optic nerve.*

Introduction

The opportunities for the optic nerve regeneration have been searched for decades. It is believed that retinal ganglion cells naturally are not able to regenerate the optic nerve after the injury. In particular, in 2004 it was widely thought that an imbalance between inflammatory and anti-inflammatory factors in the damaged optic nerve prevents its recovery [14].

However, subsequent studies revealed a number of factors that could provide it. Thus, according to Ivanova N, regenerative processes in the eye occur in three stages: alteration, changes in circulation and proliferation [2]. By using apoptosis inhibitors and growth factors, it has been possible to reduce a loss of retinal ganglion cells after the optic nerve injury [10, 15].

The use of glucocorticoids, which in high doses (30 mg/kg) promote the development of personal protective factor of ganglion cells, ensuring their survival is considered the most effective way of direct neuroprotection in traumatic lesions of the optic nerve [11]. Therefore, the use of high doses of corticosteroids is considered as a standard of treatment for traumatic optic neuropathy.

If the searches for morphological base of regenerative processes of the optic nerve, despite still being debatable, have scientific rationale, the reconstituted fibers functioning is still doubtful and requires a more detailed study.

Purpose: to study morphological aspects of restorative processes in the optic nerve after traumatic injury under the influence of high doses of corticosteroids.

Material and methods

Twenty four mature male rabbits, weighing 3.5-4 kg, breed Soviet Chinchilla, the optic nerves of which were injured in the operating room conditions were used as an experimental model [4]. Twelve of them received no treatment (injured group) and the other 12, on the second day after the injury, were treated (treated group) using i. m. administration of methylprednisolone in a dose of 30 mg / kg during 3 days, then the dose was gradually reduced. The control group consisted of 12 rabbits.

In 2 weeks, a part of animals (6) from the both groups was withdrawn from the experiment via the guillotine. For morphological confirmation of damage, cranial portion of the optic nerve in both eyes was observed using light and electron microscopes. The corresponding structures of the control group were used as control ones.

Surgery and morphological studies were conducted [4] in the operating room of vivarium of the Department of Human Anatomy of Ivano-Frankivsk National University in compliance with the rules of asepsis. Animal management and withdrawal from the experiment were carried out in accordance with the "Requirements of bioethics under the Declaration of Helsinki on ethical principles of medical research".

We determined the heart rate and the amplitude of the pupillary reactions (the difference between the diameter of the pupil, measured with a ruler in the horizontal meridian between 3 and 9 o'clock at twilight illumination and under direct light-striking with flashlight with a light power of 5W) on the 14th day (all animals) and in 1 month (6 animals from experimental groups) after injury.

Computer data processing was performed using the statistical package Stat. Soft. Inc; Tulsa, OK, USA; Statistica 6.

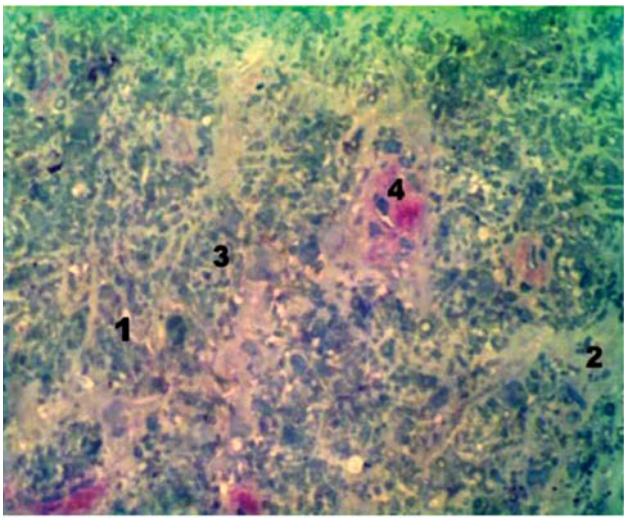

Figure 1. State of the intracranial portion of the right optic nerve in rabbit on day 14 after traumatic injury. Semifine sections were colored with polychrome dye. Coll .: x400. Note 1 — Destructive changes, 2 — Swelling changes, 3 — Reduction of the area of the axial cylinder, 4 — Newly formed connective tissue.

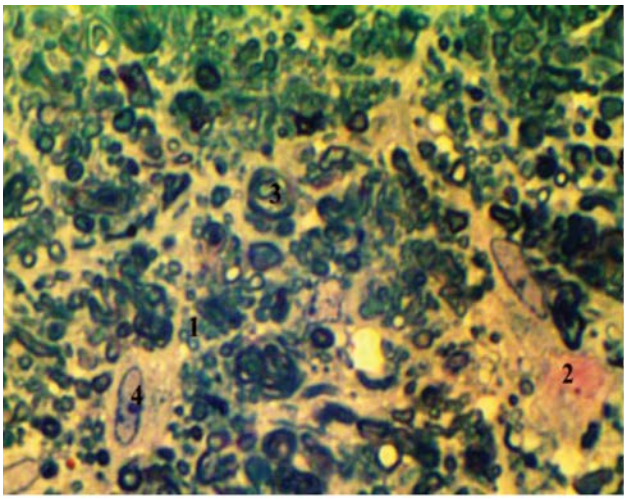

Figure 2. State of the intracranial portion of the right optic nerve in rabbit on day 14 after traumatic injury using high doses of corticosteroids. Semifine sections were colored with polychrome dye. Coll .: x1000. Note 1 — Newly formed MNFs, 2 — Newly formed collagen fibers, 3 — Destructively changed MNFs, 4 — Fibroblast nucleus, 5 — Reactive changes.

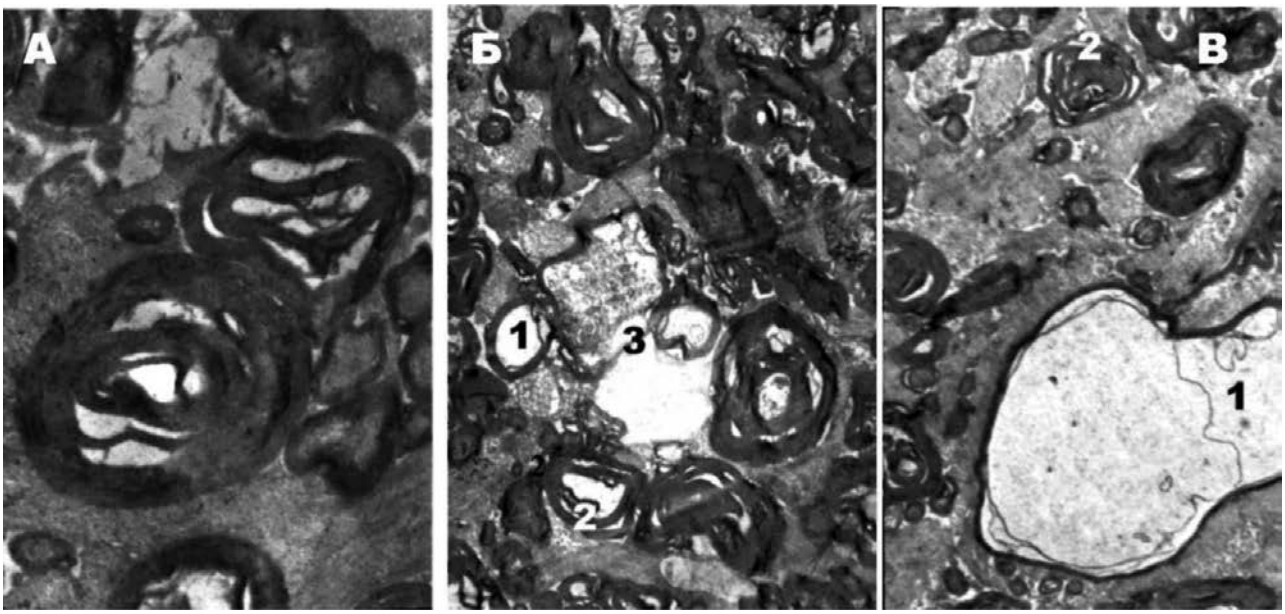

Figure 3. Ulrastructured changes of intracranial portion of optic nerve in rabbit on day 14 after traumatic injury without treatment. Coll .: А — x12000, Б, В — x4800. Note 1 — Swelling of the nerve fiber, 2 — Dissection of MS, 3 — Demyelination of nerve fibers, 4 — Vacuolization of MS.

Results

According to morphological studies on light-optical level of the intracranial portion of the optic nerve of rabbits from injured group on the 14th day (Fig. 1), significant swelling and destructive changes of the sheaths of myelinated nerve fibers (MNF) were observed, which led to a reduction of axial cylinders that were not detected in some nerve fibers, and the myelin sheath (MS) of these fibers occupied almost the entire area of the fiber. In endo- and perineurium the proliferation of connective tissue by newly formed collagen fibers was observed.

At the same time, a reduction of the swelling of endoneurial connective tissue has been observed after the treatment (Fig. 2). MNFs acquired a rounded or oval and irregular stellate and polygonal shape. In some MNFs, the external and internal contours of MS were concentric; in other ones, the inner layers of myelin formed protrusions of different shapes and heights, which is an evidence of the preservation of degenerative changes in these MNFs. [7]

According to morphometry, a decrease in MNF area ($p = 0.006972$) alongside with a statistically significant

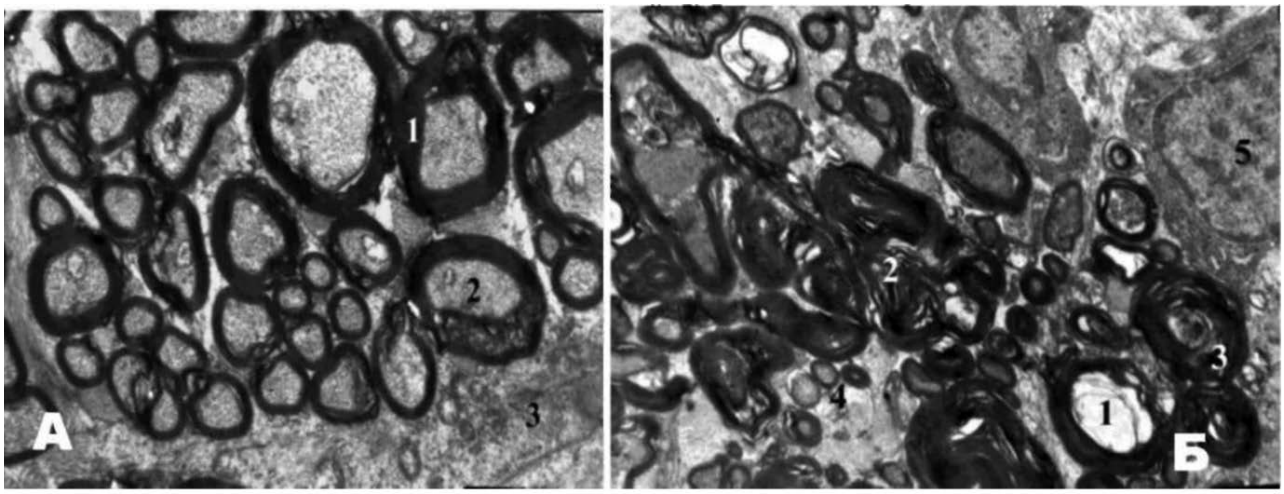

Figure 4. Restorative (A) and destructive (B) processes in MNFs of the intracranial portion of the right optic nerve in rabbit with traumatic injury on day 14 of treatment. Coll .: x6400. Note: A: 1 — MNFs with structured MS, 2 — Newly formed microtubules and neurofilament in axoplasm of MNFs, 3 — Cytoplasm of Schwann cell with young mitochondria and clearly ordered tanks of granular endoplasmic reticulum. B: 1 — Nerve fiber axolemma swelling, 2 — Dissection of lamella of myelin, 3 — Myelin sheath swelling, 4 — MNFs with preserved ultrastructure, 5 — Schwann cell's nidus.

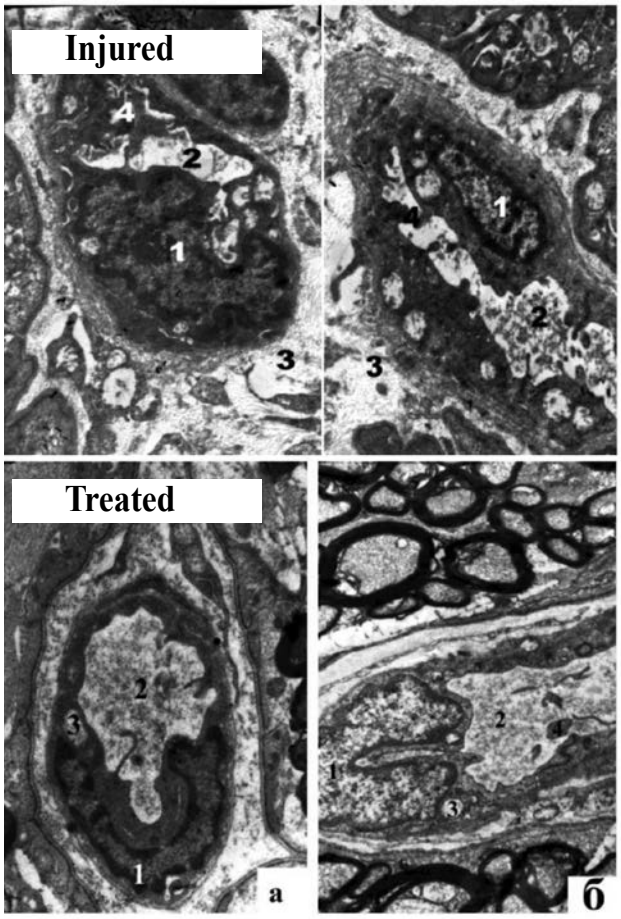

Figure 5. Hemodynamics of the right intracranial optic nerve with traumatic injury on day 14 in both groups. Coll .: Injured — x8000, Treated — x6400. Note: a — Capillary, б — Precapillary, 1 — Elongated endothelial nidus with marginally located chromatin, 2 — Capillary bore, 3 — Vacuoles in the cytoplasm of the endothelial cells, 4 — Microclasmatosis events.

increase in the g index ($p = 0.000423$) in intracranial portion of the right optic nerve, has been observed in the treated group compared to the injured one, indicating active processes of remyelination of these fibers.

The electron-diffraction patterns of optic nerves in the group without treatment (Fig. 3) show that fibers' axoplasm had a significant swelling of the MS of low electron density, and was vacuolated. The mitochondria were almost absent, and those that remained were swollen with enlightened matrix, deformed and partially destroyed by cristae. Strips of myelin were often chaotic, moving away from each other, having a wavy motion. All this, according to the studies, may indicate a disruption of axonal transport along the axial cylinders [6, 16, 17], caused by swelling of MS and can be interpreted as a periaxonal degeneracy. MNFs were often observed with symptoms of demyelination, specific manifestations of which was a deep disruption of the myelin sheath and a separation of myelin fragments that were freely located in the axoplasm or the cytoplasm of Schwann cell, indicating a disruption of the energy and protein metabolism [5, 8, 19].

MNFs, the MS of which consisted only of several myelin lamellas, being in clear order have been detected in the animals from the treated group (Fig. 4 A). Axoplasm of such MNFs contained young elongated mitochondria with dense matrix and ordered cristae, moderate amount of clearly structured microtubules and neurofilaments, indicating a recovery of their backbone and conductivity [12]. In the Schwann cells' cytoplasm, it has been discovered a large nidus with multiple dispersed chromatin and clear nucleolemma with shallow invaginations, young mitochondria and clearly ordered tanks of granular endoplasmic reticulum, having a large number of ribosomes located on their surface. These morphological features are evidence of active energy supplying and biosynthetic processes that occur in Schwann cells [7].

Such changes in the morphological structure of the treated group occurred against the background of microcirculation restoration. Endoneurium capillaries had rounded shape with oval or rounded clearance. The endotheliocyte niduses became elongated with marginally located chromatin (Fig. 5). The cytoplasm in endotheliocytes had an increased electron optical density and contained young mitochondria and single micropinocytotic vesicles, i.e. young endothelial cells [10].

Regarding the functional activity, according to the pupillary reactions data, a complete lack of direct reaction to light on the affected side (amplitude was 0 mm) (Fig. 6) has been observed in the injured group. In contrast, within 1 month after the injury, a nonsignificant increase in the amplitude of the pupillary reaction to (1 ± 0,5) mm was observed in the treated group, indicating an activation of the afferent link of the optic tract and, possibly, a partial restoration of the functional ability of nerve fibers (Fig . 7).

Thus, on the 14th day after the use of high doses of corticosteroids, an improvement of microcirculation, a reduction of swelling and a remyelination of nerve fibers were discovered in the intracranial portion of the optic nerve. Along with this, amplitude of the pupillary response has increased in one month, indicating a recovery of functional activity of the optic nerve.

However, despite a direct neuroprotective effect of high doses of corticosteroids, this method of treatment has a strong negative effect in connection with its high toxicity and mortality of experimental animals in the both groups in terms of 1 month.

Conclusion

Consequently, it has been established that the use of high doses of corticosteroids after the traumatic injury of the optic nerve contributes to the appearance not only of morphological but also of functional features of recovery processes activation in the optic nerve.

Figure 6. State of direct pupillary response in rabbits with traumatic lesions of the optic nerve without and after 1month of treatment

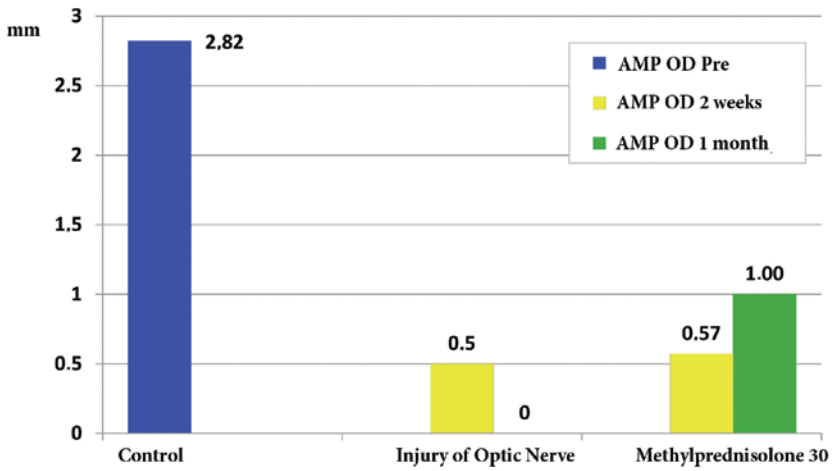

Figure 7. Dynamics of pupillary reactions during the injuries of the optic nerve within 1 month and when using high doses of corticosteroids in rabbits

References

1. Abdulaev MI. [Nerves in the history of anatomy and their myeloarchitectonics. Rising degeneracy of nerves. The vegetative nature of the neuron]. Nurlan (Baku). 2002:140. Russian.
2. Ivanova NV. Pathogenetic role of sex steroid hormones and fibronectin at posttraumatic reparative processes in the eye, and the effectiveness of the correction of their damages. [dissertation]. [Simferopol]. 2004:409. Doctor of Medical Science. Russian.
3. Levytskyj VA, Yurah OM, Yurah GY. [Degeneracy and reparative regeneration of of nerve fibers in the sciatic nerve condition of laser irradiation]. Galytskyi likarskyi vistnyk. 2009;(1):49-52. Ukrainian.
4. Moiseenko NM, Zhurakivska OY, Leskiv GM. [Structural changes of the cranial portion of optic nerve under traumatic damage of its orbital part in the experiment]. Ophtalmol. Zhurn. 2014;(6):101-105. Ukrainian.
5. Gerashchenko SB, Dyeltsova OI, Kolomiytsev AK, Tchaikovsky YB. [Peripheral nerve (neuro-vascular desmal intercourse to health and disease)]. Ukrmedknyha (Ternopol). 2005:342. Ukrainian.
6. Pivneva TA, Kolotushkina EV, Melnik NA. [Mechanism of the process of demyelination and its modeling]. Neurofisiologia.1999. 31(6):497-509. Russian.
7. Kupriyanov VV, Bobryk II, Karaganov YL. [Vascular endothelium]. Zdorovia. 1986:286. Ukrainian.
8. Tverdokhlib IV. [Characteristics of the structural changes of myelinated nerve fibers in conditions of demyelination and remyelination in the central nervous system (CNS)]. Karpovski chytannya: Materials of II Ukrainian scientific morphological conference. Dnipropetrovsk, 12-15 April 2005. Porogy (Dnepropetrovsk. 2005:93. Ukrainian.
9. Bove L, Picardo M, Maresca V, et al. A pilot study on the relation between cisplatin neuropathy and vitamin E. J. Exp. Clin. Cancer. Res. 2001;20(2):277-280.
10. Weibel D, Georg W, Kreutzberg, Schwab ME. Brain-derived neurotrophic factor (BDNF) prevents lesion-induced axonal die-back in young rat optic nerve. Brain Res. 1995;679:249-254.
11. Guy J, Simon B. Traumatic Brain Injury Induced Neuroprotection of Retinal Ganglion Cells to Optic Nerve Crush. Journal of Neurotrauma. 2006;23(7):1072-1082.
12. Helwig BG, Parimi S, Ganta CK, et al. Aging alters regulation of visceral sympathetic nerve responses to acute hypothermia. Am J Physiol Regul Integr Comp Physiol. 2006;291(3):573-579.
13. Li Y, Irwin N, Yin Y, Lanser M, Benowitz LI. Axon regeneration in goldfish and rat retinal ganglion cells: differential responsiveness to carbohydrates and cAMP. J. Neurosci. 2003;23:7830-7838.
14. Marcus Ohlsson. Complement Activation following Optic Nerve Crush in the Adult Rat. Journal of Neurotrauma. 2004;20(9):895-904.
15. Meyerfranke A, Kaplan MR, Pfrieger FW. Characterization of the signalling interactions that promote the survival and growth of developing retinal ganglion cells in culture. Neuron. 1995;15:805-819.
16. Sarikcioglu L, Demirel BM, Utuk A. Walking track analysis: an assessment method for functional recovery after sciatic nerve injury in the rat. Folia Morphol (Warsz). 2009;68(1):1-7.
17. Schmidt AP, Paniz L, Schallenberger C, et al. Guanosine prevents thermal hyperalgesia in a rat model of peripheral mononeuropathy. J Pain. 2009;(4):1007-1011.
18. Shin RH, Friedrich PF, Crum BA, et al. Treatment of a segmental nerve defect in the rat with use of bioabsorbable synthetic nerve conduits: a comparison of commercially available conduits. J Bone Joint Surg Am. 2009; 91(9):2194-2204.
19. Yang Q, Zhang YS. Effects of qichu fujin recipe on regeneration and repair of injured sciatic nerve in rats. Zhong Xi Yi Jie He Xue Bao. 2009;7(9):848-854.