Int J Physiol Pathophysiol Pharmacol 2023;15(4):115-124
www.ijppp.org /ISSN:1944-8171/IJPPP0149816

Original Article

The effect of laser radiation on glial fibrillary acidic protein and allograft inflammatory factor-1 expression in severed and surgically repaired sciatic nerve

Zinovii Yashchyshyn[1], Marko Kozyk[2], Kateryna Strubchevska[2], Sergiy Ziablitsev[3]

[1]Department of Physical and Occupational Therapy, Vasyl Stefanyk Precarpathian National University, No. 57 Shevchenka Street, Ivano-Frankivsk 76000, Ukraine; [2]Internal Medicine, Corewell Health William Beaumont University Hospital, 3601 W 13 Mile Road, Royal Oak, Michigan 48073, USA; [3]Department of Pathophysiology, Bogomolets National Medical University, Taras Shevchenko Boulevard 13, Kyiv 01601, Ukraine

Received February 22, 2023; Accepted July 12, 2023; Epub August 15, 2023; Published August 30, 2023

Abstract: Objective: Determine the effect of different spectrum laser radiations on the expression of Glial Fibrillary Acidic Protein (GFAP) and allograft inflammatory factor-1 (Iba-1) in the sciatic nerve during regeneration. Methods: The experiment was performed on 60 lab Wistar rats weighing 200-250 g. The left sciatic nerve was severed and subsequent end-to-end epineural suturing was performed 10, 20, 30, and 45 minutes after neurotomy. Western blot and immunohistochemistry analyses were performed by means of polyclonal anti-GFAP antibodies (Thermo Fisher Scientific, USA) and anti-Iba-1 antibodies (Invitrogen, USA) 90 days after nerve repair. Results: The use of green and blue spectrum laser radiation significantly increased GFAP protein expression regardless of the time when surgical nerve repair was performed after injury. The expression of Iba-1 and tubulin after blue spectrum laser radiation with a wavelength of 470 nm was significantly higher than the control values by 5.1-11.0 times. An increase in the expression of Iba-1 and tubulin was noted when a green spectrum laser with a wavelength of 560 nm was utilized and nerve suturing was performed 10 and 20 minutes after nerve injury. The green spectrum laser with a wavelength of 520 nm had no significant effect on the expression of Iba-1 and tubulin. Morphologically, the highest proliferative reaction of glia was recorded when using a blue spectrum laser. Conclusions: Laser radiation with blue (470 nm) and green (560 nm) spectra, promoted the activation of GFAP-positive Schwann cells and nerve regeneration. Activation of microglia is a necessary component of nerve regeneration and the content of Iba-1 represented the efficiency of regeneration.

Keywords: Nerve regeneration, GFAP, Iba-1, immunohistochemistry, immunoblotting, expression

Introduction

Peripheral nerve injuries of various etiologies occur in 2.8-5.0% of all traumas in North America and abnormal nerve regeneration often results in significant decrease in the quality of life [1, 2].

It is known that the activation of Schwann cells, which are responsible for maturation, proliferation, and growth of axons, is crucial for the process of nerve regeneration [3]. Thus, after sciatic nerve injury, Schwann cells begin to proliferate and gradually increase the production of specific proteins, including Glial Fibrillary Acidic Protein (GFAP), which is expressed by non-myelinating Schwann cells [4].

GFAP is a neuroglia-specific member of the intermediate filament family, which includes specific filament proteins with a scaffolding function to assemble and maintain the cytoskeleton [5]. In adults, only non-myelinating Schwann cells and Schwann cells that de-differentiate after nerve injury express GFAP. The injury of the peripheral nerve leads to Wallerian degeneration of the injured axons distal to the lesion and subsequent regeneration. Schwann cells provide nutrition and regulation of axonal regeneration in the injured area of the

nerve. In transgenic mice, which did not express GFAP and vimentin in glial cells, the regeneration of the sciatic nerve after injury was significantly delayed [6].

Approximately one-third of patients with peripheral nerve injury who had microsurgical interventions demonstrated incomplete functional recovery [7]. Most nerve recovery failures are associated with perineural scarring and fibrosis at the site of injury.

The effectiveness of reparative processes is controlled by the response of glia to peripheral nerve damage, which includes cell migration, proliferation, cytokine release, phagocytosis, antigen presentation, and T cell recruitment [8].

Ionized calcium-binding adapter molecule-1 (Iba-1) or allotransplant inflammatory factor-1 (AIF-1) is a cytoplasmic protein with a molecular weight of 17 kDa, which is expressed in macrophages and microglia at the time of activation [9]. Iba-1 protein expression increases in microglia after nerve injury, which activates the proliferation and migration of endothelial cells through the modular protein interaction domains that play a role in protein targeting and protein complex assembly (PDZ) [9, 10]. It has been previously established that the use of monochromatic radiation of the damaged nerve area with a low-frequency laser radiation significantly reduced the recovery time, improved the structure and function of the nerve, and increased the expression of neurofilament light chain in the sciatic nerve after injury and subsequent surgical repair [11, 12].

Materials and methods

General experimental conditions

For the experiment, white, sexually mature male Wistar rats weighing 200-250 g were selected. Keeping animals, feeding them, and manipulating them was carried out in compliance with the ethical and legal norms and requirements whilst performing scientific and morphological research, namely: Appendix 4 to the "Rules for conducting work with the use of experimental animals", approved by the order of the Ministry of Health of Ukraine No. 755 dated 12.08.1997, "On measures to further improve the organization of forms of work with the use of experimental animals" and the provisions of "General principles of experiments on animals", adopted by the First National Congress on Bioethics (Kyiv, 2001); in accordance with the Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruelty" dated 21.02.2006. The animals were kept in a vivarium, which was well ventilated and lit, at an air temperature of +20-+24°C, and a relative humidity of 45-65%, in single-tier plastic cages with an upper hinged wall, made in the form of a metal frame with a mesh. Daily housekeeping included changing the wood chip litter and washing the food dish.

Performing surgical intervention

The experiment was carried out on 60 male rats, which were transected under thiopental anesthesia (Thiopentalum-sodium, 50 mg/kg in 0.5 ml of physiological solution intraperitoneally) in compliance of the rules of asepsis and antiseptics and transection of the left sciatic nerve with suturing with an epineural suture end to end through 10, 20, 30 and 45 minutes after neurotomy.

Modeling of traumatic neuropathy was carried out by cutting a peripheral nerve with a scalpel in the following way: After anesthesia in a sterile operating room, the animal was fixed and the operative field was treated with 10% Betadin (Povidonum-Iodum) solution three times. The skin was dissected and the muscles were separated by a blunt method. After removing the nerve into the surgical field, a ligature was placed on it and then cut. After the scheduled exposure time (10, 20, 30, or 45 minutes), the ends of the sciatic nerve were sutured end-to-end with an epineural suture. The surgical wound was sutured in layers.

Design of the study

The animals were divided into 4 groups - 1st (control, 5 animals), 2nd - application of a blue spectrum laser with a wavelength of 470 nm (20 animals), 3rd - application of a green spectrum laser with a wavelength of 560 nm (20 animals) and 4th - application of a green spectrum laser with a wavelength of 520 nm (15 animals). In experimental groups, 5 animals were taken for each period of suturing. The nerve in the control group was sutured 10 minutes after the neurotomy. The duration of

the experiment was 90 days. The research of the recovery of somato-visceral sensitivity was recorded by the reaction of the animals to pain and temperature stimuli, while motor function was detected by using a moving tape.

Use of laser radiation

The day after the operation, the area of surgical intervention was radiated with a laser of the appropriate spectrum for 10 consecutive days. Irradiation of the neurotomy site was carried out by a pulse laser irradiator "SPEKTR LC-02" (St. Petersburg, RF). The irradiator consists of a control unit and the actual matrix with replaceable nozzles capable of adjusting the wave frequency (470 nm, 520 nm, 560 nm) of the light beam. Frequency nozzles were selected according to the selected cohort of experimental animals. The daily exposure time was 5 minutes. The laser beam was directed at the affected area from a distance of 5 cm. The energy exposure dose was: for 560 nm - 0.15 J/cm^2, for 520 nm - 1.2 J/cm^2, for 470 nm - 1.5 J/cm^2.

Morphological and immunohistochemical studies

During the experiment, the research team was guided by Directive 2010/63/EU on the regulation of the use of animals for scientific research purposes. The process of euthanasia was selected in accordance with this directive and met the main requirements: quickly, painlessly, without unnecessary suffering. Thus Pentobarbital was administered intraperitoneally at a dose of 120 mg/kg. Taking into account the weight of the rats, an average dose of 36 mg was used.

After euthanasia, a fragment of the nerve in the repaired area was immediately isolated and fixed in a 10% solution of neutral buffered formalin (pH 7.4) between 24-36 hours. The material was processed and embedded in paraffin. Serial histological sections with a thickness of 2-3 μm were made from paraffin blocks on a rotary microtome NM 325 (Thermo Shandon, Knutsford, Cheshire, England). Sections were stained with hematoxylin and eosin for microscopy inspection. In all cases, an immunohistochemical study (IHCD) was conducted to determine the morpho-functional state of nerve cells and features of regenerative capabilities [13]. The slides were placed into adhesive glasses Super Frost Plus (Menzel, Berlin, Germany). For high-temperature processing of antigen epitopes, citrate buffer with pH 6 and EDTA buffer pH 8, the Vitro Master Polymer Plus Detection System (Peroxidase) detection system, and DAB Quanto chromogen (Master Diagnostica, Granada, Spain) were used. Mouse polyclonal antibodies to GFAP (Thermo Fisher Scientific, Waltham, MA, US) were also used. Sections were additionally stained with hematoxylin. Microscopic research and photo archiving were carried out using light-optical microscopes "ZEISS" (Köln, Germany) with the result processing system "Axio Imager.A2" "ZEISS" (Köln, Germany) with 5, 10, 20, 40× objective magnification, 1.5× binocular attachment and 10× eyepiece with ERc 5s, "Carl Zeiss" Primo Star and Axiocam cameras 105 color and optical microscope "Olimpus BX 40", additionally equipped with a digital camera "Olimpus C3030-ADU" digital camera and "Olimpus DP-Soft" software (Olimpus, Feasterville, PA, US).

The evaluation of expression severity was carried out according to the recommendations of D.J. Dabbs (2014) based on the visual-analog scale: 0 points - no coloring; 1 point (+) - weak color intensity; 2 points (++) - average color intensity; 3 points (+++) - high staining intensity [14].

Immunoblotting studies

For immunoblotting, sciatic nerve samples were ground with liquid nitrogen and homogenized in lysis buffer (Pierce Protease and Phosphatase inhibitor, ThermoScientific, Waltham, MA, US), disintegrated by ultrasound and centrifuged. The concentration of total protein was measured in the supernatant [15]. Electrophoresis was performed in an 8% polyacrylamide gel with sodium dodecyl sulfate according to the classical method in a chamber for vertical gel electrophoresis (BioRad, Hercules, CA, US) [16]. The protein concentration was 75 μg per gel lane. Concentration of samples was carried out at a voltage of 30-35 V (15-18 mA); separation - at 45-50 V (30-35 mA). Immunoblot analysis of the studied proteins of the sciatic nerve was performed according to the method of H. Towbin et al. [17]. After electrophoretic separation, proteins from the gel were transferred to a nitrocellulose

membrane by electroblotting. The voltage during the transfer was 35-45 V with a current of 200-230 mA. The duration of the transfer was 120 minutes.

Membranes were blocked in buffered saline with 5% nonfat dry milk powder at 37°C and then incubated with primary rabbit antibody to GFAP (Santa Cruz Biotechnology, Santa Cruz, CA, US, sc-9065, dilution 1:2000), mouse antibodies to Iba-1 (Invitrogen, Waltham, MA, US, MA5-27726, dilution 1:1500), and rat antibodies to tubulin (anti-tubulin YL1/2; Abcam, Waltham, MA, US, ab6160, dilution 1:5000). After incubation with primary antibodies, membranes were washed and treated with corresponding goat anti-species secondary antibodies conjugated with horseradish peroxidase (HRP) each diluted 1:8000 (anti-rabbit IgG HRP-conjugated, Abcam, Waltham, MA, US, ab6721; anti-mouse IgG HRP-conjugated, Abcam, Waltham, MA, US, ab197767; anti-rat IgG HRP-conjugated, Abcam, Waltham, MA, US, ab97057).

Immunoreactive zones were visualized using methods of enhanced chemiluminescence and autoradiography on X-ray films [18]. After development, the film was scanned, and semi-quantitative analysis of the obtained results was performed densitometrically using TotalLab software (TL120, Nonlinear Inc., San Marcos, CA, US). Page RulerTM Plus Prestained Protein Ladder markers (ThermoScientific, Vilnius, Lithuania) in the range of 10-230 kDa was used to determine the molecular weight of proteins.

Statistical procedures

For statistical analysis, the Statistica 10 program (StatSoft, Inc., Oklahoma, US) was used, the means (M) and their standard deviation (SD) were calculated and then compared using the analysis of variance (ANOVA), values of $P<0.05$ is considered significant.

Results

Condition of animals after surgery

Significant degenerative and dystrophic changes occurred in the nerve fibers after the sciatic nerve was severed and surgically repaired. The number of nerve fibers decreased. Demyelination, spasm of perineural blood vessels, and dissociation of the myelin sheath in individual nerve fibers developed [11]. Experimental rats did not have any reaction to pain and temperature stimuli. The animals did not step on injured extremity. Gradual remodeling of blood supply to the nerve and remyelination of nerve fibers started on the 15th day of experiment. Animals began to respond to temperature, pain stimuli, and step on the operated extremity by the 30th day. There was a complete return of blood supply, number of myelin nerve fibers, somatic, visceral, and motor sensitivity to the nerve by the 60th day. Satisfactory recovery of the nerve had occurred when surgical repair was performed within 30 minutes after injury. Delay in the surgery resulted in increased recovery period up to 75-90 days. Based on these results, the effect of laser radiation was assessed 90 days after surgery.

Immunoblotting study of GFAP

The GFAP content in the regenerating nerve was assessed using immunoblot analysis (**Figure 1**).

Compared to the control group, the GFAP protein content was 2.9-4.5 times higher in group 2 ($P<0.001$). The protein content was also increased by 2.9-4.1 times in group 3 compared to the control ($P<0.001$). The GFAP protein content in group 3 was the lowest if neurorrhaphy was performed 45 minutes after neurotomy compared to control ($P<0.05$). The GFAP protein content when neurorrhaphy was performed 10 minutes after neurotomy in group 4 was 1.4 times higher than the control value, but it was not statistically significant ($P=0.064$). Thus, the greatest protein content was achieved after radiation with a blue spectrum laser.

Immunohistochemical study of GFAP

The normal histologic structure of the sciatic nerve of the rat is shown (**Figure 2**). A small intensity of positive immunohistochemical staining was determined by perineurally located glial cells.

When regenerating cells were radiated with a blue spectrum laser (470 nm), a higher intensity of specific staining was achieved compared to the control. These results were revealed during Western blot analysis (Figures S1, S2, S3

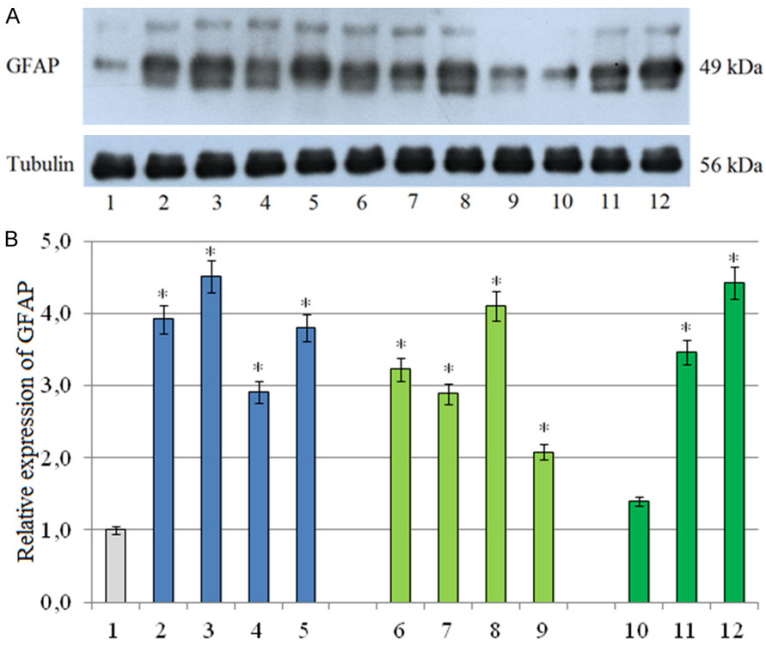

Figure 1. GFAP content in the sciatic nerve 90 days after injury. A. GFAP and tubulin contents in the nerves; B. The results of densitometric analysis of GFAP content are shown as a percentage of the control level; 1 - control; 2-5 - GFAP content after blue spectrum laser radiation (470 nm); 6-9 - GFAP content after green spectrum laser radiation (560 nm); 10-12 - GFAP content after green spectrum laser radiation (520 nm); 2, 6, 10 - GFAP content, neurorrhaphy 10 minutes after neurotomy; 3, 7 - GFAP content, neurorrhaphy 20 minutes after neurotomy; 4, 8, 11 - GFAP content, neurorrhaphy 30 minutes after neurotomy; 5, 9, 12 - neurorrhaphy 45 minutes after neurotomy. *P<0.05 compared to the control.

and S4). Cytoplasm and processes of the Schwann cells with intensity up to 2 points were detected around myelin fibers. However, staining of perineurial glial cells was up to 1 point.

When the cells were radiated with a green spectrum laser (560 nm), the total intensity of immunohistochemistry staining was similar to the 2nd group. Glial cells in the perineurium had staining intensity of 1 to 2 points. Intensity of staining in Schwann cells was up to 2 points.

Slightly lower staining intensity was observed in the nerves radiated with green spectrum laser (520 nm). Limited Schwann cells and their processes had staining intensity from 1 to 2 points. Rare glial cells in the perineurium were noted and had staining up to 1 point.

The content of GFAP in the regenerating nerves had increased. Thus, clear visualization of glial cells in regenerating nerves was the most prominent after radiation with blue (470 nm) and green (560 nm) laser. Further analysis revealed presence of GFAP-positive fibers and moderate amount of Schwann cells (**Figure 3**).

Immunoblotting and immunohistochemical study of Iba-1

The results of the immunoblotting studies revealed that the contents of Iba-1 and tubulin in nerves, which had radiation with blue spectrum laser, were higher compared to the control group by 5.1-11.0 times (**Figure 4A**, **4B**; columns 2-5). Iba-1 and tubulin levels were the highest when the nerve was radiated with blue laser and was repaired 10 minutes after injury.

The contents of Iba-1 and tubulin was also increased when green spectrum laser, with a wavelength of 560 nm, was used in nerves when surgically repaired 10 and 20 minutes after injury (**Figure 4B**, columns 6, 7). The green spectrum laser with a wavelength of 520 nm had no significant effect on the levels of Iba-1 and tubulin (**Figure 4B**, columns 10-12).

The histopathology of sciatic nerve regeneration zone showed activation of glial cells. The number of Schwann cells and microglia were the highest when blue spectrum laser was utilized (**Figure 5A-D**).

Discussion

Schwann cells respond to peripheral nerve injury by active involvement in the Wallerian degeneration of dissected peripheral axons [19]. Schwann cells are also responsible for regeneration and trophic support of the injured axons [20]. Normally, GFAP is present in Schwann cells [21]. Extracorporeal shock wave therapy has been shown to promote functional recovery, regenerate injured peripheral nerves, and increase expression of GFAP and other glial proteins, as well as improve motor function [22]. Radiation of the damaged facial nerve

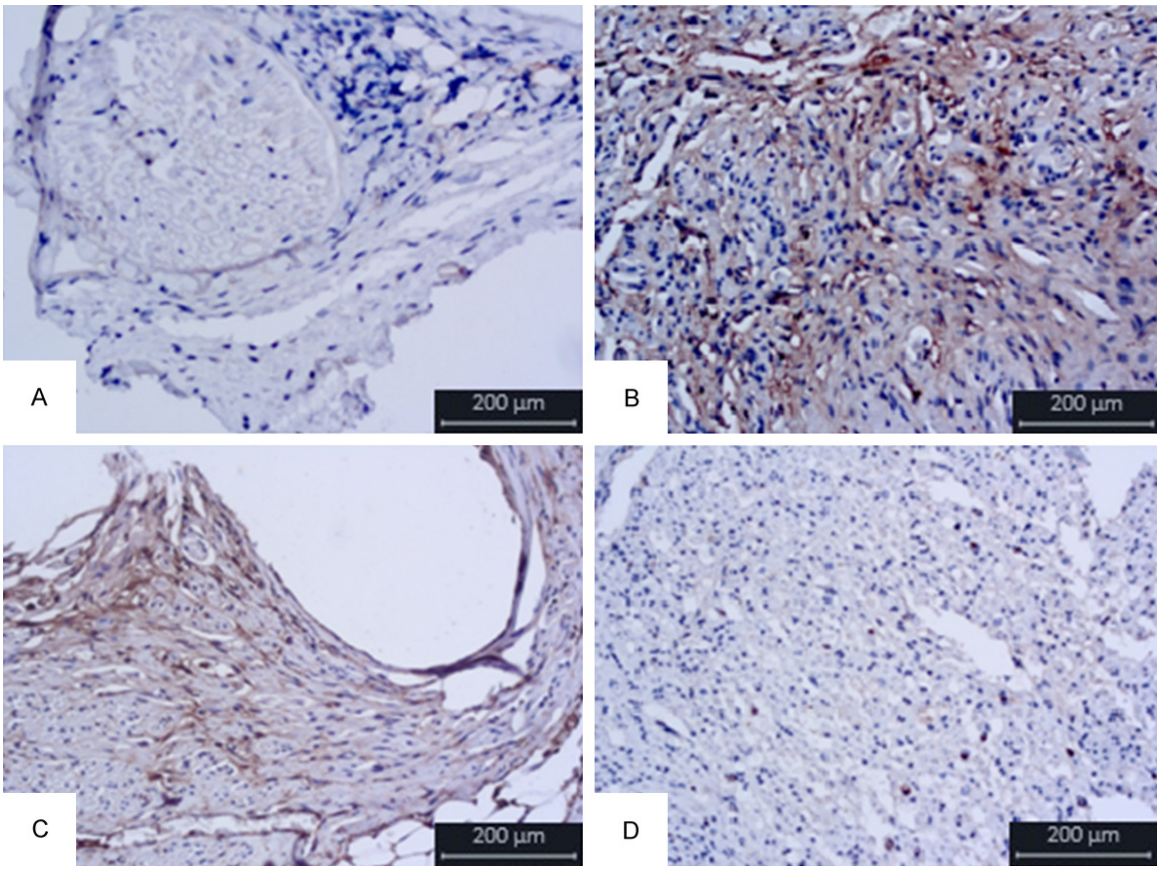

Figure 2. Representation of GFAP content in sciatic nerve before the injury (A) and 90 days after injury (B-D) by immunohistochemical assay. Neurorrhaphy was done 30 minutes after neurotomy. Additional staining was done with hematoxylin. (A) Low intensity (up to 1 point) of immunospecific staining, mainly in the perineurium; (B) Laser irradiation with the blue spectrum (470 nm); a moderate number of Schwann cells with a staining intensity of 1 to 2 points, in glial cells - up to 1 point; (C) Green spectrum laser irradiation (560 nm); positive cells in the perineurium with a staining intensity of 1 to 2 points, in Schwann cells - up to 2 points; (D) Green spectrum laser irradiation (520 nm); single immunopositive Schwann cells with a staining intensity of 1 to 2 points, single positive cells in the perineurium (up to 1 point).

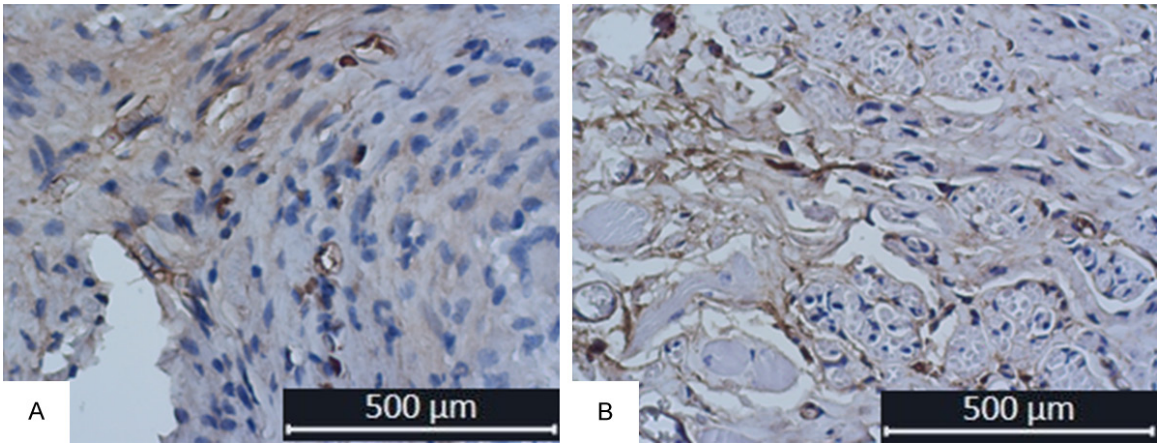

Figure 3. The immunohistochemical assay of GFAP content in sciatic nerve 90 days after neurotomy and subsequent neurorrhaphy 30 minutes after injury. Additional staining was done with hematoxylin. Blue spectrum laser with a wavelength of 470 nm (A) and green spectrum laser with a wavelength of 560 nm (B) were applied.

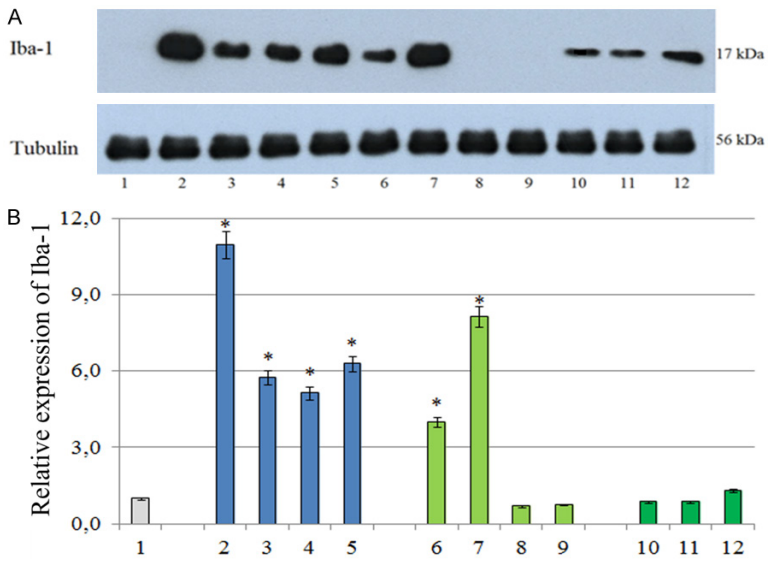

Figure 4. Iba-1 and tubulin contents in the sciatic nerve 90 days after injury. A. Iba-1 and tubulin contents in the nerves. B. The results of densitometric analysis of Iba-1 and tubulin contents are shown as a percentage relative to the control level. 1 - control; 2-5 - Iba-1 and tubulin contents after blue spectrum laser radiation (470 nm); 6-9 - Iba-1 and tubulin contents after green laser spectrum radiation (560 nm); 10-12 - Iba-1 and tubulin contents after green spectrum laser radiation (520 nm). 2, 6, 10 - Iba-1 and tubulin contents, neurorrhaphy 10 minutes after neurotomy; 3, 7 - Iba-1 and tubulin contents, neurorrhaphy 20 minutes after neurotomy; 4, 8, 11 - Iba-1 and tubulin contents, neurorrhaphy 30 minutes after neurotomy; 5, 9, 12 - Iba-1 and tubulin contents, neurorrhaphy 45 minutes after neurotomy. *P<0.05 compared to the control.

with a wavelength of 980 nm promoted the regeneration of axons due to PI3K/Akt signaling pathway [23].

Improper nerve regeneration is caused by disturbed regulation of Schwann cells, axons, and extracellular matrix [3, 4]. Peripheral nerves develop and function properly in transgenic mice lacking GFAP. However, axon regeneration after injury was observed to be delayed in mice lacking GFAP [24]. Mutant Schwann cells kept their ability to differentiate but showed impaired proliferation, which is a key event in nerve regeneration. The GFAP signaling pathway involves the integrin alphavbeta8 that initiates mitotic signals after nerve injury by interacting with fibrin, which is absent in GFAP-null cells. Consequently, GFAP initiates differential signaling required for nerve regeneration.

GFAP has an important role in the initiation of pro-inflammatory reactions in peripheral nerve injury. The signaling pathway of interleukin 6 (IL-6) and signal transducer and activator of transcription 3 (STAT3) induces GFAP gene expression [25]. This pathway is important for neuroprotection and reactive gliosis after injury. GFAP production in sciatic nerves was significantly suppressed in IL-6-deficient mice. Therefore, activation of the IL-6/STAT3 pathway stimulates an increased GFAP expression after neurotomy.

It could be argued that the experimental results and analysis of the literature has shown that a prerequisite for successful nerve regeneration after nerve injury is activation of GFAP-positive Schwann cells. Blue spectrum laser radiation with a wavelength of 470 nm and green spectrum laser radiation with a wavelength of 560 nm promotes GFAP activation and nerve regeneration. Iba-1 and tubulin contents were the highest when blue spectrum laser was utilized. The contents of Iba-1 and tubulin were also increased when a green spectrum laser with a wavelength of 560 nm was used and suturing was done 10 and 20 minutes after injury. The use of green spectrum laser with a wavelength of 520 nm had no statistically significant effect on the content of Iba-1 and tubulin.

The use of low-intensity laser therapy in physical medicine and rehabilitation is a highly effective method in restoring nerve's functions [26]. Monochromatic laser radiation activates mitochondrial cytochrome oxidase, increases adenosine triphosphate (ATP) formation, promotes cell proliferation, and stimulates protein synthesis [27, 28]. Low-intensity laser radiation promoted the regeneration and restoration of the injured peripheral nerve, stimulated the proliferation of Schwann cells [29, 30], activated the secretion of neurotrophic factors in macrophages [31], increased the expression of nerve growth factor (NGF) and vascular endothelial growth factor (VEGF) [32].

Our data confirms the high efficiency of restoration of the structural components of the sciatic

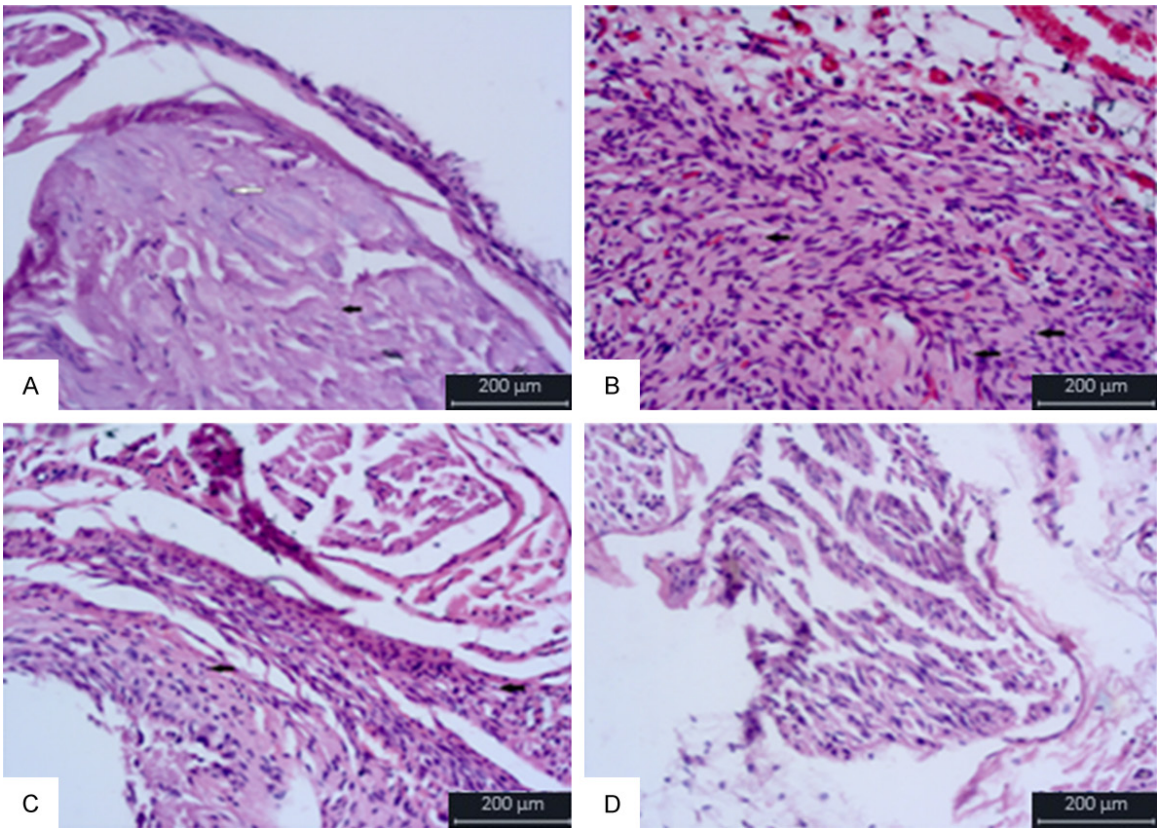

Figure 5. The sciatic nerve before injury (A) and 90 days after injury (B-D); hematoxylin-eosin staining. (A) A small number of Schwann cells and microglia. (B) Blue spectrum laser with a wavelength of 470 nm, large number of Schwann cells and microglia. (C) Green spectrum laser with a wavelength of 560 nm, intermediate number of microglia and Schwann cells. (D) Green spectrum laser with a wavelength (520 nm), the number of glial cells is the smallest compared to other groups.

nerve after its injury with end-to-end suturing and the monochromatic low-frequency laser radiation.

The peripheral nervous system injury causes immediate release of proinflammatory cytokines by glial cells and neurons [33]. These cytokines trigger microgliosis and astrogliosis, recruit immune cells, and activate all stages of nerve regeneration. The inflammasome is activated in the motoneurons of the ventral horn, which causes a complex proinflammatory reaction and activation of microglia [34]. Radiation of damaged alveolar nerve with wavelength of 810 nm and 980 nm promoted the neurosensory recovery [35]. The application of a laser with a wavelength of 808 nm enhanced the regeneration of the damaged inferior alveolar nerve and increased the number of axons per square micrometer [36].

The results obtained indicate that the activation of microglia is a necessary component of nerve recovery, and thus the content of Iba-1 and tubulin reflects the efficiency of regeneration. At the same time, the level of microglia per high power field was the highest when blue spectrum laser with a wavelength of 560 nm was utilized.

Conclusions

The use of laser radiation during nerve regeneration significantly (2.9-4.5 times; $P<0.001$) increased GFAP protein production, which led to restoration of the structure and functions of the severed nerve. The most pronounced effect was observed with the use of blue spectrum laser radiation with a wavelength of 470 nm.

The level of Iba-1 and tubulin in regenerating nerve was significantly higher than the control by 5.1-11.0 times ($P<0.05$) when the blue spectrum laser (470 nm) was used. An increase in the content of Iba-1 and tubulin was noted when using a green spectrum laser (560 nm)

coinciding under the conditions of nerve suturing, 10 and 20 minutes after injury. The use of green spectrum laser (520 nm) had no significant effect on the content of Iba-1.

Limitations of the study

Our studies have shown the effect of laser radiation of different spectrum on the key processes of sciatic nerve regeneration. A positive effect of the use of laser radiation with the blue spectrum with a wavelength of 470 nm was revealed. At the same time, the cellular and molecular mechanisms of these phenomena remained unclear. From our perspective, it is promising to study the mechanisms of implementation of pro-inflammatory processes and in particular, the signaling pathway of the pro-inflammatory cytokine intelekin 6 and signal transducer and activator of transcription 3 (IL-6/STAT3). This would make it possible to substantiate a possible anti-inflammatory correction, which could positively complement the effect of laser radiation.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Marko Kozyk, Internal Medicine, Corewell Health William Beaumont University Hospital, 3601 W 13 Mile Road, Royal Oak, Michigan 48073, USA. E-mail: marko.kozyk@beaumont.org

References

[1] Novak CB, Anastakis DJ, Beaton DE and Katz J. Patient-reported outcome after peripheral nerve injury. J Hand Surg Am 2009; 34: 281-287.

[2] Kouyoumdjian JA. Peripheral nerve injuries: a retrospective survey of 456 cases. Muscle Nerve 2006; 34: 785-788.

[3] De la Hoz CL, Oliveira AL, Queiroz Lde S and Langone F. Wallerian degeneration in C57BL/6J and A/J mice: differences in time course of neurofilament and myelin breakdown, macrophage recruitment and iNOS expression. J Anat 2003; 203: 567-78.

[4] Bhatheja K and Field J. Schwann cells: origins and role in axonal maintenance and regeneration. Int J Biochem Cell Biol 2006; 38: 1995-1999.

[5] Triolo D, Dina G, Lorenzetti I, Malaguti M, Morana P, Del Carro U, Comi G, Messing A, Quattrini A and Previtali SC. Loss of glial fibrillary acidic protein (GFAP) impairs Schwann cell proliferation and delays nerve regeneration after damage. J Cell Sci 2006; 119: 3981-3993.

[6] Berg A, Zelano J, Pekna M, Wilhelmsson U, Pekny M and Cullheim S. Axonal regeneration after sciatic nerve lesion is delayed but complete in GFAP- and vimentin-deficient mice. PLoS One 2013; 8: e79395.

[7] Wang ML, Rivlin M, Graham JG and Beredjiklian PK. Peripheral nerve injury, scarring, and recovery. Connect Tissue Res 2019; 60: 3-9.

[8] Calvo M and Bennett DL. The mechanisms of microgliosis and pain following peripheral nerve injury. Exp Neurol 2012; 234: 271-282.

[9] Tian Y, Jain S, Kelemen SE and Autieri MV. AIF-1 expression regulates endothelial cell activation, signal transduction, and vasculogenesis. Am J Physiol Cell Physiol 2009; 296: C256-C266.

[10] Hung AY and Sheng M. PDZ domains: structural modules for protein complex assembly. J Biol Chem 2002; 277: 5699-5702.

[11] Yashchyshyn ZM, Zaiats LM, Nebesna ZM, Poliuk MI, Rohuzhynska VH and Kerniakevych SIA. Morfofunktsionalni zminy v strukturi sidnychnoho nerva pislia neirotomii z ushyvanniam epinevralnym shvom cherez rizni promizhky chasu ta podalshoiu lazernoiu korektsiiu. Aktualni problemy transportnoi medytsyny. Morphofunctional changes in the structure of the sciatic nerve after neurotomy with epineural suture at different time intervals and subsequent laser correction. Actual Problems of Transport Medicine 2020; 60: 85-91.

[12] Yashchyshyn ZM, Ziablitsev SV and Zaiats LM. Immunohistochemical detection of neurofilaments in the sciatic nerve, which regenerates after neurotomy and surgical suturing. World of Medicine and Biology 2022; 81: 252-256.

[13] Griffin J and Treanor D. Digital pathology in clinical use: where are we now and what is holding us back? Histopathology 2017; 70: 134-145.

[14] Dabbs D. Diagnostic Immunohistochemistry Theranostic and Genomic Applications. 4th edition. 2014. pp. 960.

[15] Stoscheck CM. Quantitation of protein. Methods Enzymol 1990; 182: 50-68.

[16] Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970; 227: 680-685.

[17] Towbin H, Staehelin T and Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci U S A 1979; 76: 4350-4354.

[18] Alegria-Schaffer A. Western blotting using chemiluminescent substrates. Methods Enzymol 2014; 541: 251-259.

[19] Fawcett JW and Keynes RJ. Peripheral nerve regeneration. Annu Rev Neurosci 1990; 13: 43-60.
[20] Madduri S and Gander B. Schwann cell delivery of neurotrophic factors for peripheral nerve regeneration. J Peripher Nerv Syst 2010; 15: 93-103.
[21] Jessen KR and Mirsky R. The origin and development of glial cells in peripheral nerves. Nat Rev Neurosci 2005; 6: 671-682.
[22] Seo M, Lim D, Kim S, Kim T, Kwon BS and Nam K. Effect of botulinum toxin injection and extracorporeal shock wave therapy on nerve regeneration in rats with experimentally induced sciatic nerve injury. Toxins (Basel) 2021; 13: 879.
[23] Li B and Wang X. Photobiomodulation enhances facial nerve regeneration via activation of PI3K/Akt signaling pathway-mediated antioxidant response. Lasers Med Sci 2022; 2: 993-1006.
[24] Triolo D, Dina G, Lorenzetti I, Malaguti M, Morana P, Del Carro U, Comi G, Messing A, Quattrini A and Previtali SC. Loss of glial fibrillary acidic protein (GFAP) impairs Schwann cell proliferation and delays nerve regeneration after damage. J Cell Sci 2006; 119: 3981-3993.
[25] Lee HK, Seo IA, Suh DJ, Hong JI, Yoo YH and Park HT. Interleukin-6 is required for the early induction of glial fibrillary acidic protein in Schwann cells during Wallerian degeneration. J Neurochem 2009; 108: 776-786.
[26] de Oliveira RF, de Andrade Salgado DM, Trevelin LT, Lopes RM, da Cunha SR, Aranha AC, de Paula Eduardo C and de Freitas PM. Benefits of laser phototherapy on nerve repair. Lasers Med Sci 2015; 30: 1395-1406.
[27] Hashmi JT, Huang YY, Osmani BZ, Sharma SK, Naeser MA and Hamblin MR. Role of low-level laser therapy in neurorehabilitation. PM R 2010; 2 Suppl 2: S292-S305.
[28] Passarella S and Karu T. Absorption of monochromatic and narrow band radiation in the visible and near IR by both mitochondrial and non-mitochondrial photoacceptors results in photobiomodulation. J Photochem Photobiol B 2014; 140: 344-358.
[29] Anders JJ, Geuna S and Rochkind S. Phototherapy promotes regeneration and functional recovery of injured peripheral nerve. Neurol Res 2004; 26: 233-239.
[30] Gigo-Benato D, Geuna S, de Castro Rodrigues A, Tos P, Fornaro M, Boux E, Battiston B and Giacobini-Robecchi MG. Low-power laser biostimulation enhances nerve repair after end-to-side neurorrhaphy: a double-blind randomized study in the rat median nerve model. Lasers Med Sci 2004; 19: 57-65.
[31] Zhang J, Sun J, Zheng Q, Hu X, Wang Z, Liang Z, Li K, Song J, Ding T, Shen X, Zhang J and Qiao L. Low-level laser therapy 810-nm up-regulates macrophage secretion of neurotrophic factors via PKA-CREB and promotes neuronal axon regeneration in vitro. J Cell Mol Med 2020; 24: 476-487.
[32] Dias FJ, Fazan VPS, Cury DP, de Almeida SRY, Borie E, Fuentes R, Coutinho-Netto J and Watanabe IS. Growth factors expression and ultrastructural morphology after application of low-level laser and natural latex protein on a sciatic nerve crush-type injury. PLoS One 2019; 14: e0210211.
[33] Bastien D and Lacroix S. Cytokine pathways regulating glial and leukocyte function after spinal cord and peripheral nerve injury. Exp Neurol 2014; 258: 62-77.
[34] Molnár K, Nógrádi B, Kristóf R, Mészáros Á, Pajer K, Siklós L, Nógrádi A, Wilhelm I and Krizbai IA. Motoneuronal inflammasome activation triggers excessive neuroinflammation and impedes regeneration after sciatic nerve injury. J Neuroinflammation 2022; 19: 68.
[35] Hakimiha N, Dehghan MM, Manaheji H, Zaringhalam J, Farzad-Mohajeri S, Fekrazad R and Moslemi N. Recovery of inferior alveolar nerve by photobiomodulation therapy using two laser wavelengths: a behavioral and immunological study in rat. J Photochem Photobiol B 2020; 204: 111785.
[36] Diker N, Aytac D, Helvacioglu F and Oguz Y. Comparative effects of photobiomodulation therapy at wavelengths of 660 and 808 nm on regeneration of inferior alveolar nerve in rats following crush injury. Lasers Med Sci 2020; 35: 413-420.

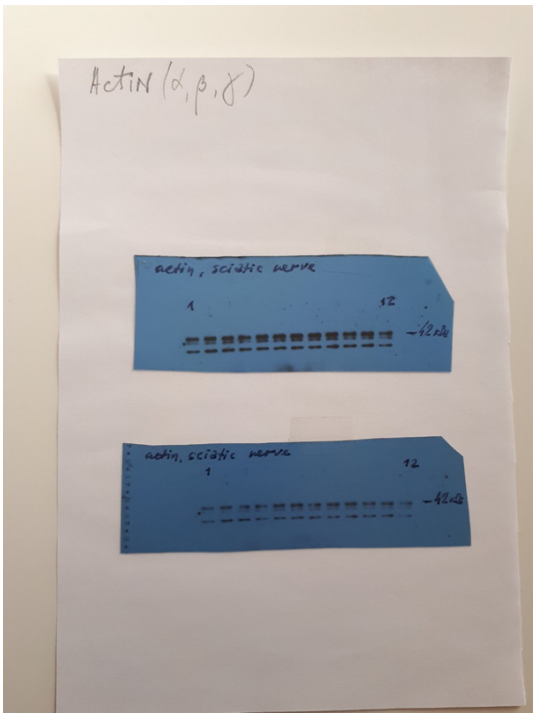

Figure S1. Full-length gel and Western blot image of actin, sciatic nerve.

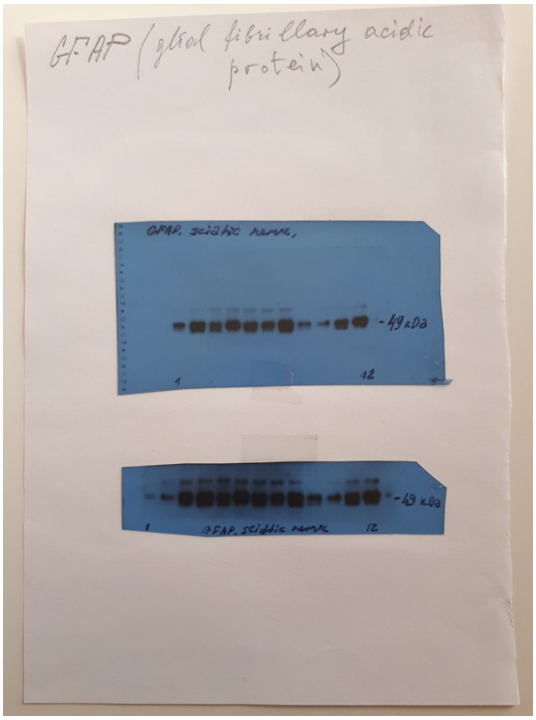

Figure S2. Full-length gel and Western blot image of GFAP, sciatic nerve.

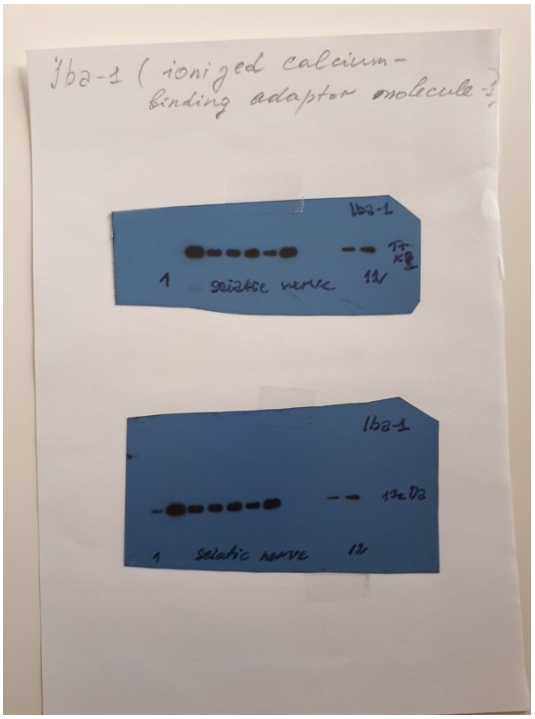

Figure S3. Full-length gel and Western blot image of Iba-1, sciatic nerve.

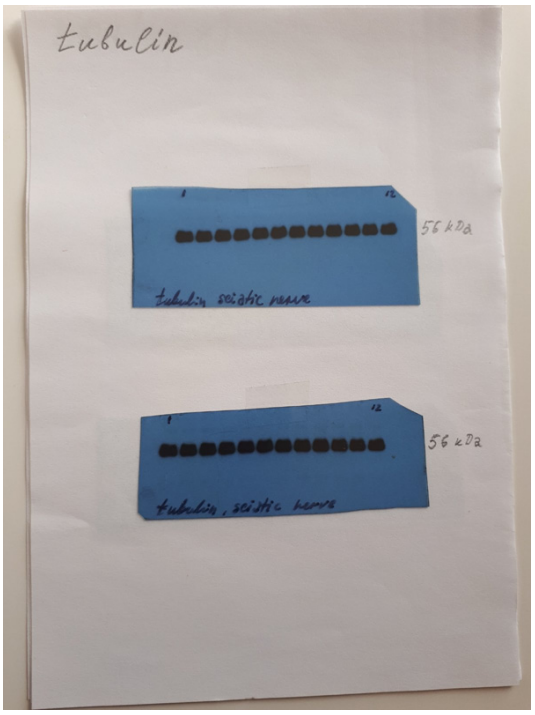

Figure S4. Full-length gel and Western blot image of tubulin, sciatic nerve.