Nerve Regeneration in Conditions of HSV-Infection and an Antiviral Drug Influence

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ABSTRACT

Herpes simplex virus type I (HSV-I) is a latent neuroinfection which can cause focal brain lesion. The role of HSV-infection in nerve regeneration has not been studied so far. The aim of the work was to study sciatic nerve regeneration in the presence of HSV-infection and the influence of an antiviral drug. BALB/c line mice were divided into five groups. Group 1 animals were infected with HSV-I. After resolution of neuroinfection manifestations the sciatic nerve of these animals was crushed. Group 2 mice were administered acyclovir following the same procedures. Groups 3–5 mice served as controls. Thirty days after the operation distal nerve stumps and *m.gastrocnemius* were studied morphologically and biochemically. Ultrastructural organization of the sciatic nerve in control animals remained intact. Morphometric parameters of the nerves from the experimental groups have not reach control values. However, in the group 1 diameter of nerve fibers was significantly smaller than in the group 2. Both nerve regeneration and *m.gastrocnemius* reinnervation were confirmed. The muscle hypotrophy was found in groups 1, 2, and 3 (the muscle fibers diameter decreased). Metabolic changes in the muscles of the infected animals (groups 1 and 2) were more pronounced than in control groups 3 and 4. The levels of TBA-active products, conjugated dienes, carbonyl and SH-groups were reduced in *m.gastrocnemius* of the experimental groups, however no significant difference associated with acyclovir administration was found. HSV-infection is not limited to the local neurodegenerative changes in the CNS but affects regeneration of the injured sciatic nerve. Anat Rec, 00:000–000, 2018.

Key words: nerve crush; nerve regeneration; HSV-infection; antiviral drugs; acyclovir

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INTRODUCTION

Although the regeneration of an injured nerve is relevant, it remains an inadequately solved problem. While a significant number of pathogenic components of traumatic peripheral nerve damage, specific features of nerve regeneration in the setting of partial or complete nerve intersection have been found so far (Dubovy, 2011), the role of latent viral neuroinfection in the injured nerve regeneration and neuro-muscular recovery was not studied earlier.

Herpes simplex virus type I (HSV-I), one of the most common viruses in human beings (Xu et al., 2006), is one of these neuroinfections. It is a neurotropic virus having the unique ability to remain latent in sensory and autonomic ganglia (Roizman et al., 2007, Yao et al., 2014). HSV-I was shown to cause the development of neurodegenerative changes in the brain cortex and hippocampus of the lab animals and to reactivate after the simulated hemorrhagic stroke (Gumenyuk et al., 2016), yet the current study has focused on the evaluation of the central nervous system, leaving the peripheral units aside. Reactivation of HSV-I was found in the superior cervical ganglion after distal neurectomy (Price and Schmitz, 1978). In another pilot study, it was found that the introduction of hepatocyte growth factor in HSV vector had significantly accelerated the recovery of the facial nerve function after crushing. These data also suggest replication of viral DNA in the facial nerve ganglion following the nerve injury (Esaki et al., 2011).

Although no precise evidence regarding reactivation of latent HSV-I were found on the molecular level, many factors (such as inflammation, diabetes, cytokines, prostaglandins, growth factors NGF, CNTF) are considered stress-inducing factors of infection reactivation (Kriesel, 2002; Toma et al., 2008; Al-Dujaili et al., 2011; Esaki et al., 2015). The effect of neuroinfection on the peripheral nerve regeneration has not been studied earlier. Such research seems interesting for identifying factors that negatively impact the potential for recovery. However, regeneration of the nerve does not necessarily result in a successful reinnervation of skeletal muscles. Study of histological and biochemical changes in the muscles can provide interesting and important information for determining the recovery of the sciatic nerve.

The current work aimed at studying sciatic nerve regeneration in the presence of HSV-infection and effect of an antiviral drug.

MATERIALS AND METHODS

Twenty-five BALB/c line mice weighing 18–20 g were randomly divided into five groups (five mice in each):

Group 1—animals with HSV-I infection and sciatic nerve injury;

Group 2—animals with HSV-I infection and sciatic nerve injury with subsequent administration of acyclovir;

Group 3—animals with sciatic nerve injury (control 1);

Group 4—animals which were injected normal saline and sham-operated (control 2, sham-operated group);

Group 5—animals injected normal saline and sham-operated with subsequent administration of normal saline (control 3).

Herpes virus meningoencephalitis model was used in the experiment. Animals were infected with lyophilized HSV-I. For 30 days the animals have been suffering from the acute infection, with the further procedures conducted after the gradual resolution of the viral infection manifestations (fatigue, stiffness, reduced need of food and water).

To simulate nerve injury the right sciatic nerve was accessed and crushed at the upper third with a surgical needle holder (compression lasted 40 sec, lesion site area was 4 mm^2). The muscle and skin damage was closed with 3-0 prolene suture (Ethicon, Inc.). The skin incision site was treated with 10% povidone-iodine (Betadine). In sham operated animals only skin incision and access to the nerve were made without any nerve damage. The wound was closed in the same way.

Acyclovir (in a dose of 50 mg/kg) was administered intraperitoneally to the infected animals. 0.03 mL of normal saline were administered intraperitoneally with insulin syringe in control groups.

All laboratory (virology and experimental) procedures on animals were performed in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments, and the National Institutes of Health Guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978). The research was approved by the Bioethical committee for human subjects or animal research at Bogomolets National Medical University, Minutes №12, December 30, 2015.

To confirm the presence of the inflammatory process in the brain of infected animals, histological examination was performed. The murine brain was fixed in 10% formalin solution in 0.1 M phosphate buffer (pH 7.4), dehydrated in ascending concentrations of ethanol and embedded in paraffin as per the standard procedure. The paraffin slices 6–8 μm thick were stained with toluidine blue.

Fifteen μ thick cryosections were obtained from the sciatic nerve. Longitudinal cryosections were stained with toluidine blue, H&E and silver impregnated. Nerve fibers (which contained axon, axial cylinder), the contour of the myelin sheath, the Schwann cells nuclei were recorded in microslides.

To assess structural changes in the sciatic nerve, nerve regeneration, and condition of the *m.gastrocnemius* histological examination was conducted. 30 days after the nerve injury nerves and muscles were harvested and fixed in 10% neutral formalin (in cold phosphate buffer, PBS solution). Twenty-four hours later, the samples were dehydrated and embedded in paraplast (Leica Surgipath Paraplast Regular).

Six to eight micrometer thick sections were obtained on a rotary microtome (Thermo Microm HM 360 Rotary Microtome) and stained with H&E and toluidine blue.

The nerve structure elements were further studied with scanning electron microscopy (SEM). For this purpose, the fixed samples were dehydrated in ethanol and dried under the conditions of a critical point of CO_2. The dried samples were covered with 15 nm thick gold using the 682 Gatan PECS device. SEM micrograph studies were performed on TescanMira 3 LMU.

Quantitative changes were evaluated using a morphometric method. For this purpose histological microsections were photographed at the magnification of ×400

and ×1000. The following criterion was used in comparing the groups: similarity of nerve and muscle samples in size and level of excision from the limb. The number of nerve fibers was measured using an eyepiece micrometer. The method of morphometric quantification of nerve fibers was based on the random selection of several test-zones and their assessment in each studied nerve. First, the 15 μ thick longitudinal sections through the entire nerve diameter were selected. Subsequent assessment of 4–6 test zones from each nerve allowed the best evaluation of the nature of nerve fibers growth in the distal nerve stump. Only the distal nerve stump was measured. The number of nerve fibers impregnated by nitric silver was counted under the eyepiece micrometer and translated into the density per nerve thickness according to the formula. By the formula, the number of fibers in the test zone was converted to a density of mm^2 (units/test-zone).

$$\text{The density of nerve fibers} = \frac{N \times 10^6}{T \times 170}$$

N—quantity of nerve fibers; *T*—the thickness of microsections (15 μ);

170—the length of test-zone (170 μ)

This approach is evidence-based and has been used in numerous studies since 1965 (Strelin and Evsyukov, 1965). The advantage of this method is its capability of measuring the distance from the site of the damage in the section area.

Morphometric analysis was conducted using the Olympus BX 51 microscope and CarlZeiss (AxioVision SE64 Rel.4.9.1) software with the magnification of ×400. The nerve fibers diameter (μ) was measured. Several measurements were made on a separate fiber on the longitudinal and transverse sections of the nerve fiber. Morphometry of the nerve fibers diameter was also based on the random principle. The diameters of individual nerve fibers were measured using Carl Zeiss software. Since the diameter of the nerve fiber is not uniform along its entire length, each fiber was measured several times. This allowed obtaining the mean diameter of each individual nerve fiber.

Quantitative evaluation of structural changes of *m.gastrocnemius* included several indicators: muscle fiber diameter (μ) and nuclei density/test-zone. Density was estimated as the number of stained nuclei per the test-zone. Microphotographs with ×400 magnification were randomly chosen as the test-zones. Histological slides for morphometry have been preselected to evaluate the changes in the most similar samples of the muscle. Metabolic changes in the *m.gastrocnemius* were studied by biochemical methods. The level of TBA-active products, conjugated dienes, carbonyl groups, free SH-groups, and NAD(P)H-quinone-oxidoreductase activity have been determined.

The content of lipid peroxidation products in the *m.gastrocnemius* was determined by reaction with thiobarbituric acid (TBA-positive products) (Peyroux and Sternberg, 2006). Two hundred milliliter of 10% cooled TCAA solution were added to 100 mL homogenate and cold incubated for 15 min. Samples were centrifuged at 2,200 g for 15 min. 4°C. Two hundred milliliter 0.67% solution of TBA were added to 200 μL of supernatant and kept for 20 min in the boiling water bath.

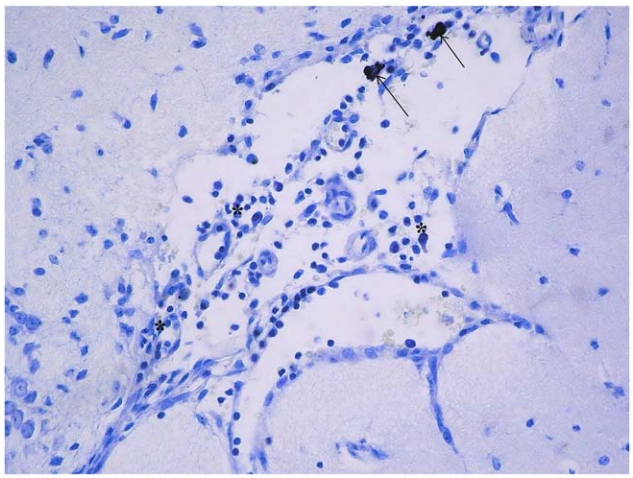

Fig. 1. Focus of inflammation in the hippocampus of animals after HSV-I infection. * - infiltration by monocytes/macrophages; ← basophils. Nissl staining, ×400.

The samples were cooled and their absorbancy measured at 532 nm using an automatic spectrophotometer μQuant («Biotek», USA). The content of TBA-positive products in the sample was determined by a standard curve, which was built using tetraethoxypropane standard.

To determine the content of conjugated dienes 4 mL of heptane-isopropanol (1: 1) mixture were added to 0.2 mL aliquots and shaken up for 10–15 min on a laboratory shaker. Then, 1 mL of HCl (pH = 2) and 2 mL of heptane were added to the test tube and vigorously shaken, with the top heptane layer collected after settling and separation of the mixture (after 20–25 min). This layer was used for spectrophotometric determination of the conjugated dienes at a wavelength of 232 nm.

The content of carbonyl groups in proteins was determined in lysates assay according to O.V. Zaĭtseva (Zaĭtseva and Shandrenko, 2012) using an automatic spectrophotometer μQuant («Biotek», USA).

The content of low-molecular weight SH-groups was determined in lysates with o-phthalaldehyde (Fluka, Austria) assay by M. L. Hu (Hu, 1994). Excitation wavelength for o-phthalaldehyde was 360 nm and emission was detected at 420 nm by FL800 (Biotek, USA). Reduced glutathione was used as standard (Sigma, USA).

The activity of NAD(P) H-quinone oxidoreductase in rats was determined in the reaction mixture containing a NADPH-generating glucose-6-phosphate dehydrogenase system, menadione (2-methyl-1,4-naphthoquinone) and MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide)]. NAD(P)H-quinone oxidoreductase catalyzes the NADPH-dependent reduction of menadione to menadiol. The subsequent formazan formation resulting from non-enzymatic MTT reduction under the effect of menadiol was observed at the wavelength range of 550–640 nm (Lind et al., 1990).

To detect signs of apoptosis and necrosis DNA was isolated by the enzymatic method from *m.gastrocnemius* samples and electrophoresis was performed in agarose gel. Samples of muscle weighing 200 mg were preincubated at 37°C in a buffer containing 10 mmol/L of

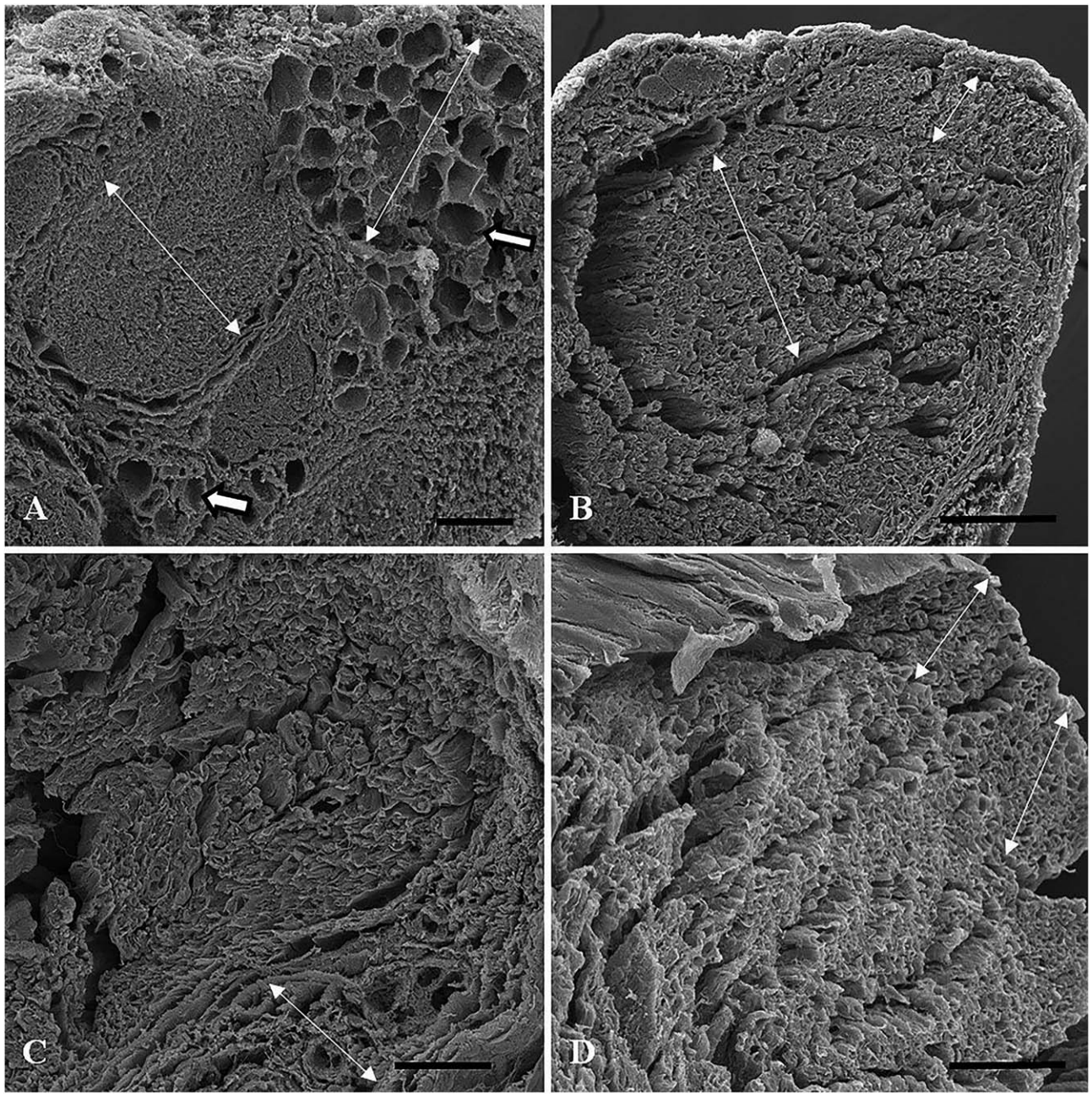

Fig. 2. Ultrastructural organization of the sciatic nerve of control and experimental animals. (A) HSV-I infected mice with nerve crush (group 1); (B) HSV-I infected mice, nerve crush, and acyclovir (group 2); (C) sciatic nerve crush (group 3); (D) intact sciatic nerve (group 4); disrupted endoneurial tube; ↔ fascicle. SEM. Scale bar 25 μm.

Na_2HPO_4, 1 mmol/L of NaH_2PO_4, 130 mmol/L of NaCl, 1,27 mmol/L $MgSO_4$, 2 mmol/L of $CaCl_2$, 20 mmol/L of HEPES (pH 7,4), 2 mg/mL of BSA. The incubation completed, the incubation medium was removed, the samples cooled, the tissue cold homogenized in a glass homogenizer and procedures for DNA isolation performed. DNA fragments were separated in 1.7% agarose gel (Agarose Serva Premium, «Serva», Germany) prepared in TE buffer containing 10 mmol/L of Tris and 10 mmol/L of EDTA (pH 8.0). Electrophoresis lasted for 1.5–2 hr at a voltage of 100 mV. After electrophoresis, the gels were photographed by the digital video camera in transilluminator and scanned using «Photo Capt Mw» software. The amount of protein homogenates was determined by Bradford MM method (Bradford, 1976).

Statistical Analysis

The results were expressed as the mean ± standard error of the mean (SEM). The data were analyzed using

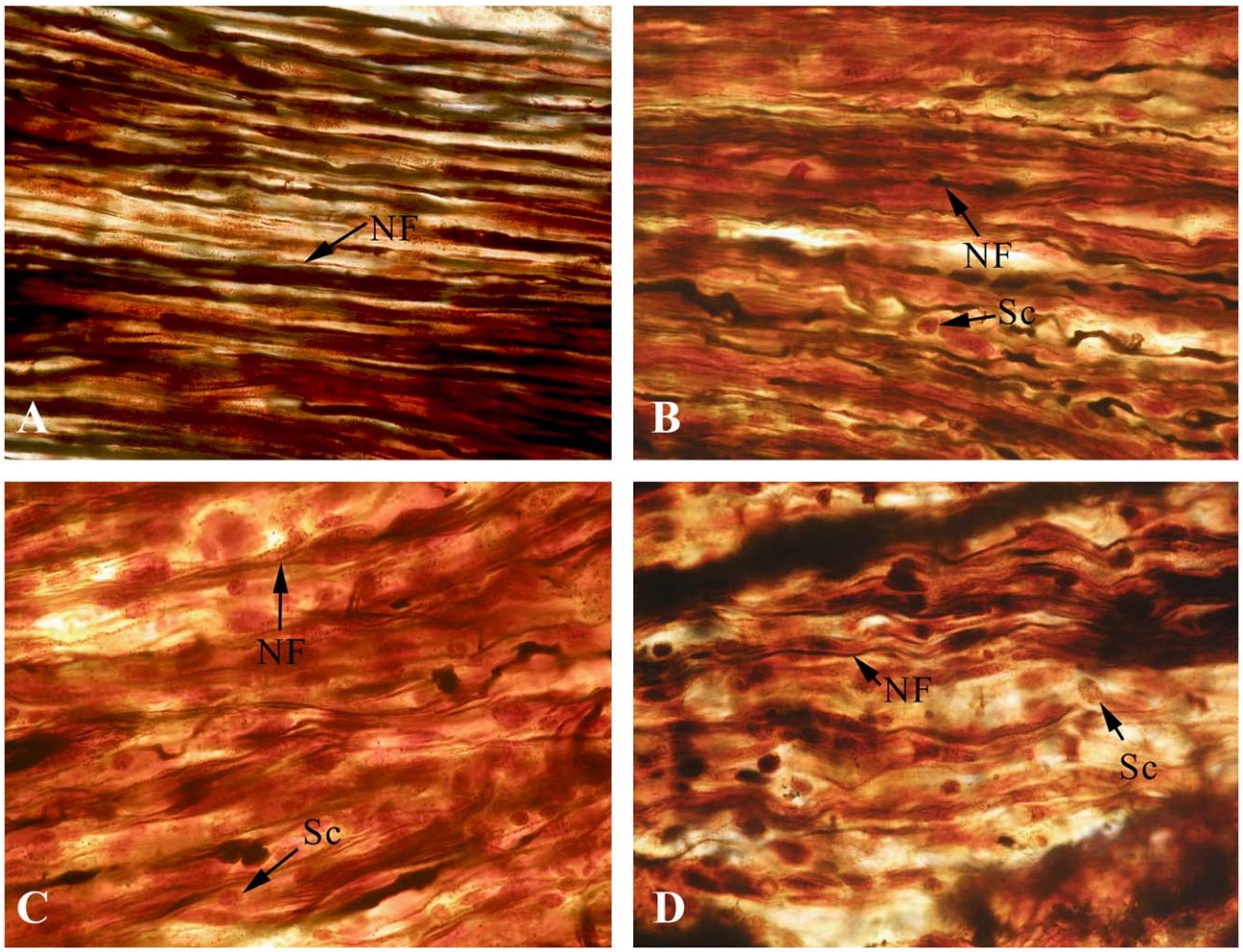

Fig. 3. Histological structure of the sciatic nerve of control and experimental animals. The regenerated nerve fibers and Schwann cells are preserved. (A) intact sciatic nerve (group 4); (B) crushed sciatic nerve (group 3); (C) HSV-I infected mice with crushed nerve (group 1); (D) HSV-I infected mice, crushed nerve, and acyclovir (group 2); NF, nerve fibers: Sc, Schwann cells. Silver impregnation, ×1000.

the Origin Lab version 8.0. The Kolmogorov–Smirnov indicated that the data were not normally distributed. Intergroup difference in sample data was evaluated by the nonparametric Kruskal–Wallis test.

RESULTS

The presence of the inflammatory process in the brain of the infected animals was confirmed histologically. Infiltration by lymphocytes and monocytes was observed in the corpus callosum, deep cortex and hippocampus (mainly in CA1 and CA3 sites) (Fig. 1).

The results of the histological study revealed significant differences in the structure of the sciatic nerve between the animals of the control and experimental groups. In the groups 4 and 5 (control 2 and 3), nerve fibers had a normal structure. Samples of the distal nerve segment that were prepared for SEM have been analyzed first. Endoneural tubes were not affected, fascicles were surrounded by perineurium. Microvessels were observed in epi-, peri-, and endoneurium.

In experimental groups and control 1, changes of fascicles (increased thickness, loss of nerve fibers, loss of endoneural tubes) were noted distal to the nerve crushing site. Moreover, the degree of destructive changes in the experimental groups exceeded that in control 1, that is, in the conditions of nerve trauma without concomitant HSV-I infection.

Assessment of the sciatic nerve structure revealed the features of these disorders. At the site of crushing in the upper third of the nerve formation of regenerative neuroma was observed. The preserved general architecture of the sciatic nerve, that is, individual fascicles, endoneurium, and epineurium were found in the distal stump of the nerve in experimental groups 1, 2 and control 1 (Fig. 2).

Endoneurium in the nerve fascicles was represented by a thin layer of fibroblasts, with basement membranes surrounding the individual nerve fibers. Preservation of the ultrastructural organization of basement membranes and endoneurium in the distal stump of the damaged nerve is important for its effective regeneration and growth of the nerve fibers to the denervated muscles.

However, in groups 1 and 2 destruction and increased diameter of endoneural tubes was established. These changes indicated progressive impairment of the injured

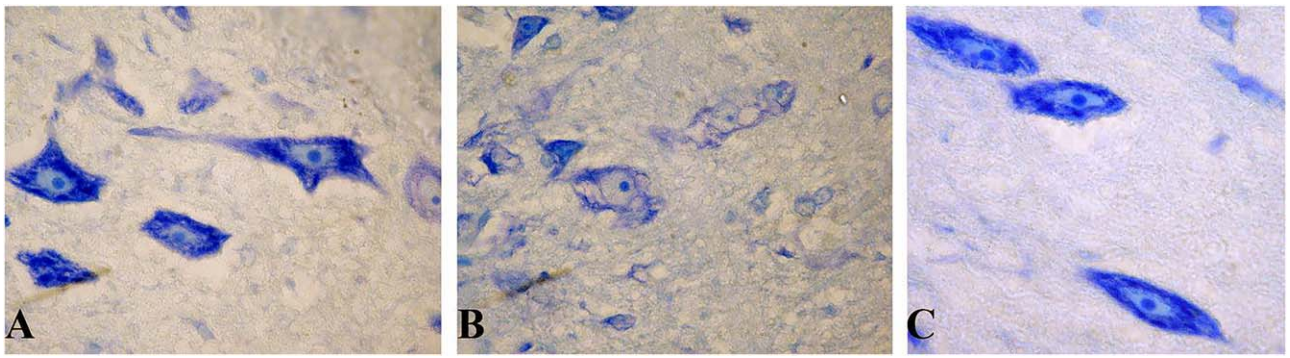

Fig. 4. Motor neurons in the anterior horn of the spinal cord of control and experimental animals. (A) intact spinal cord (group 4); (B,C) injured and intact neuron of HSV-infected animals. Nissl staining, ×1000.

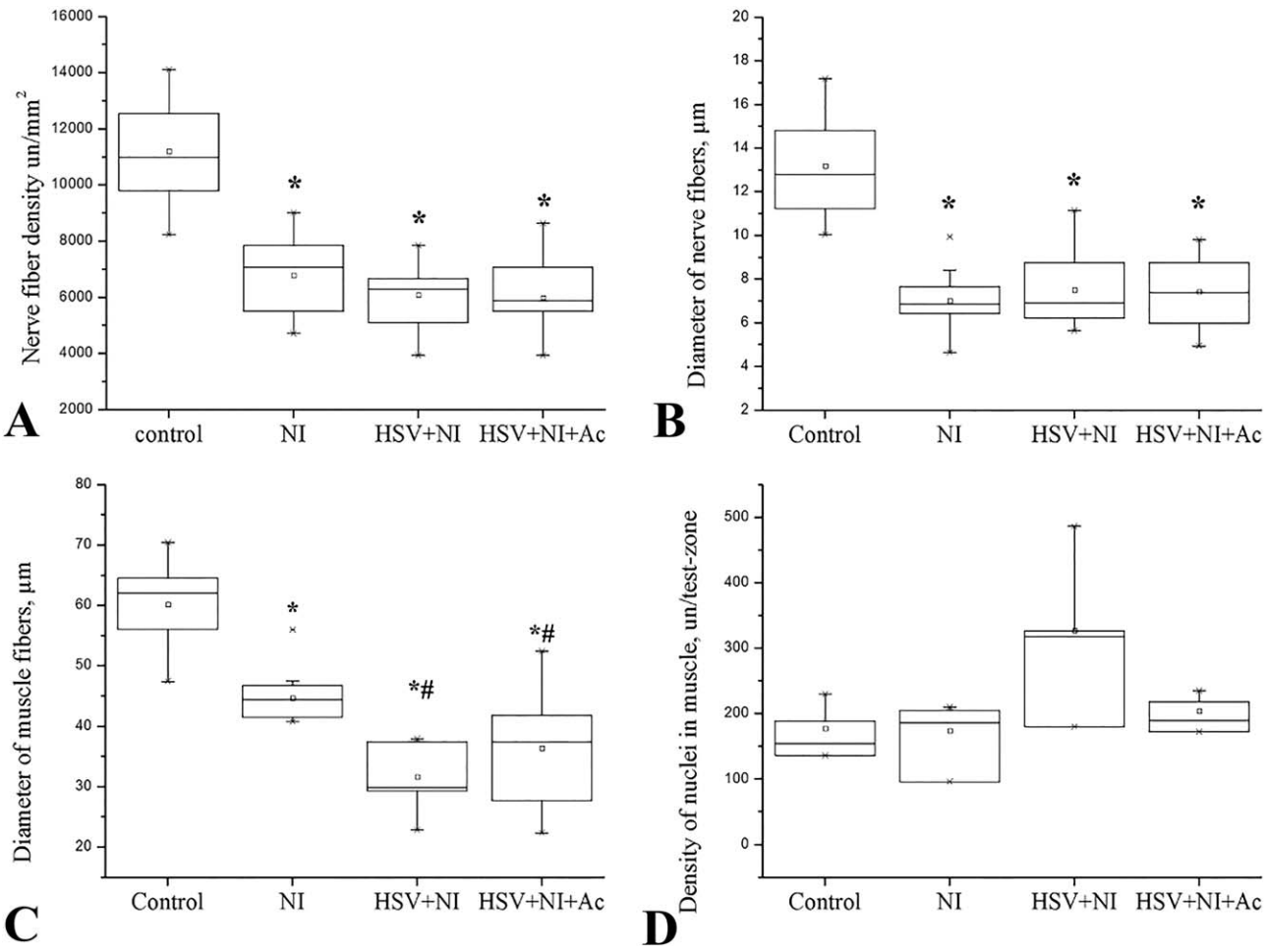

Fig. 5. Morphometric analysis of the sciatic nerve and m.gastrocnemius of control and experimental animals. (A) The density of nerve fibers in the distal stump of the sciatic nerve; (B) Changes in the diameter of nerve fibers; (C) Changes in the diameter of muscle fibers; (D) Myonuclei density in the field of view of the histological slide of m.gastrocnemius; * - parameters differ from control (group 4) ($P < 0.05$); # - parameters differ from NI (group 3) ($P < 0.05$); NI—nerve injury (group 3); HSV + NI (group 1); HSV + NI +Ac (group 2).

nerve structure in the presence of concomitant viral neuroinfection, and also indicated that in the condition of the herpetic neuroinfection not only the central nervous system but also the peripheral nerves were damaged as well.

Given that the level of preservation of the above stromal structures also plays an important role in the directed regeneration of nerve fibers into the distal stump of the nerve through the neuroma, the impairment and loss of

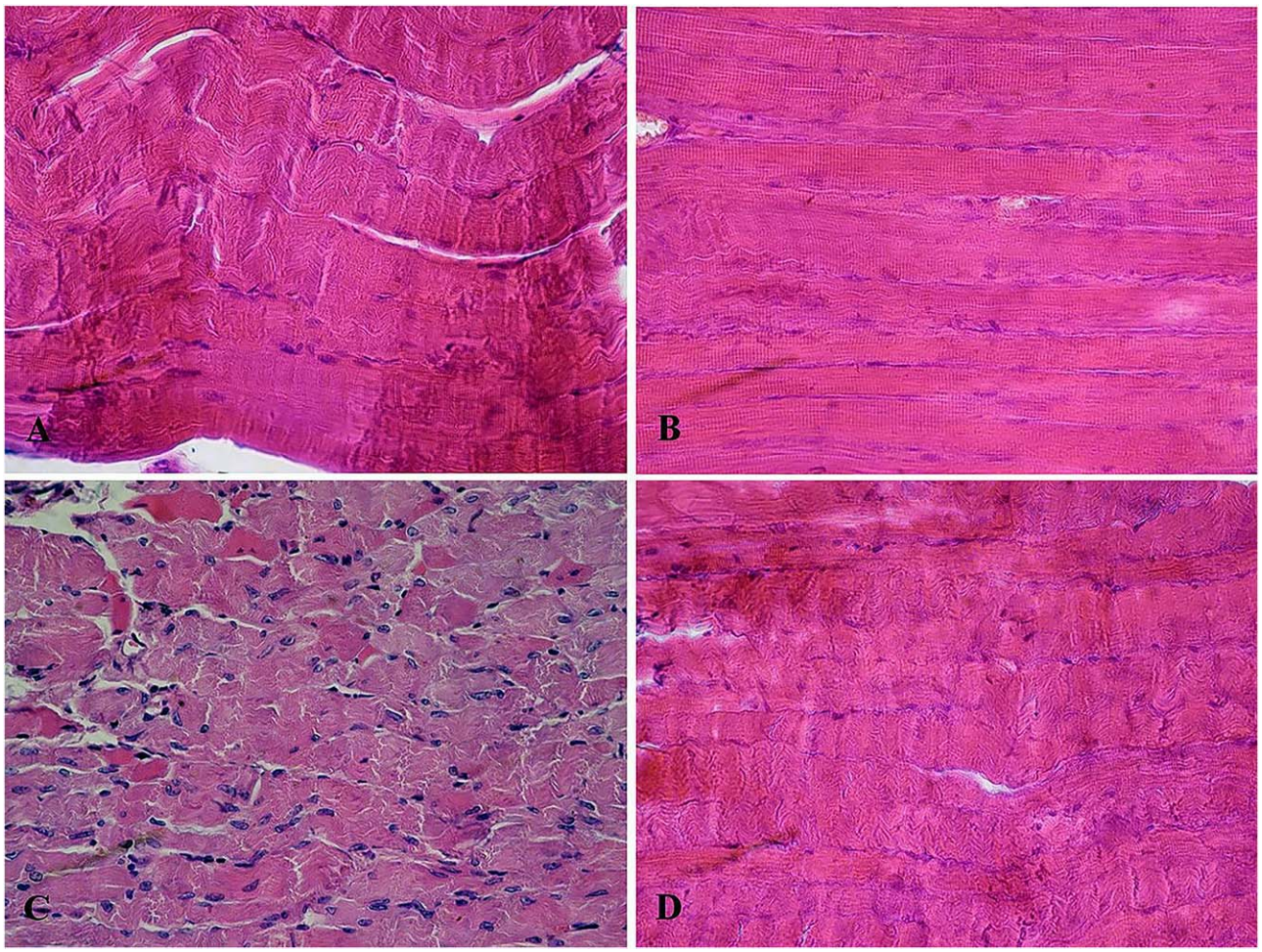

Fig. 6. Histological structure of m.gastrocnemius of control and experimental animals. (A) muscle in mice with intact sciatic nerve (group 4), the structural organization of the intact muscle is preserved; (B) muscle in mice with crushed sciatic nerve (group 3); (C) HSV-I infected mice with crushed nerve (group 1), loss of transverse striation and decrease in the diameter of muscle fibers in the presence of HSV-infection; (D) HSV-I infected mice, crushed nerve, and acyclovir (group 2).

these morphological structures in the presence of the infectious process reduce the likelihood of satisfactory recovery. However, in all experimental groups clusters of nerve fibers were recorded in the distal stump.

The method of silver impregnation allowed a more detailed identification of nerve fibers in samples and a morphometric analysis. Regeneration of nerve fibers was found in all samples from experimental groups (Fig. 3). The figure illustrates a significantly smaller number of nerve fibers in experimental groups (group 1, 2, 3) compared to sham-operated group (group 4). A dramatic increase in the number of activated Schwann cells in infected HSV mice is noteworthy (Fig. 3C). We assumed this to be due to systemic changes in the murine nervous system and performed an additional analysis of cross-sections of their spinal cord. Cytopathological changes of individual motor neurons in the anterior horns were detected, although intact neurons with the intensely colored Nissl substance were observed (Fig. 4). The results of the morphometric study have not shown any statistically significant difference between the experimental groups at the level of nerve regeneration. The number of regenerated nerve fibers in the distal nerve stump did not exceed 60.5% of the sham-operated group (Fig. 5A). The HSV administrated groups revealed only a tendency of decrease in this index.

The results of the morphometric analysis showed a significant decrease in the diameter of the nerve fibers in the experimental groups and control 1 compared with controls 2 and 3 (Fig. 5B). The diameter of the nerve fibers in groups 1–3 was less than $\sim$ 55% of groups 4 and 5 ($P \leq 0.05$), indicating an incomplete recovery of the nerve. There being no differences in qualitative and quantitative data on nerve and muscle between the control groups 4 and 5, the study results in the figures and table are given only for control 4.

Impaired regeneration of the nerve affected the state of the denervated muscles. As is known, the loss of neuro-muscular interactions leads to atrophy of skeletal muscles. At the same time, the restoration of impaired neuro-muscular interactions in the presence of concomitant neuroinfection has not been considered before. According to the results of histological examination of the *m.gastrocnemius* in the experimental groups, 1–3

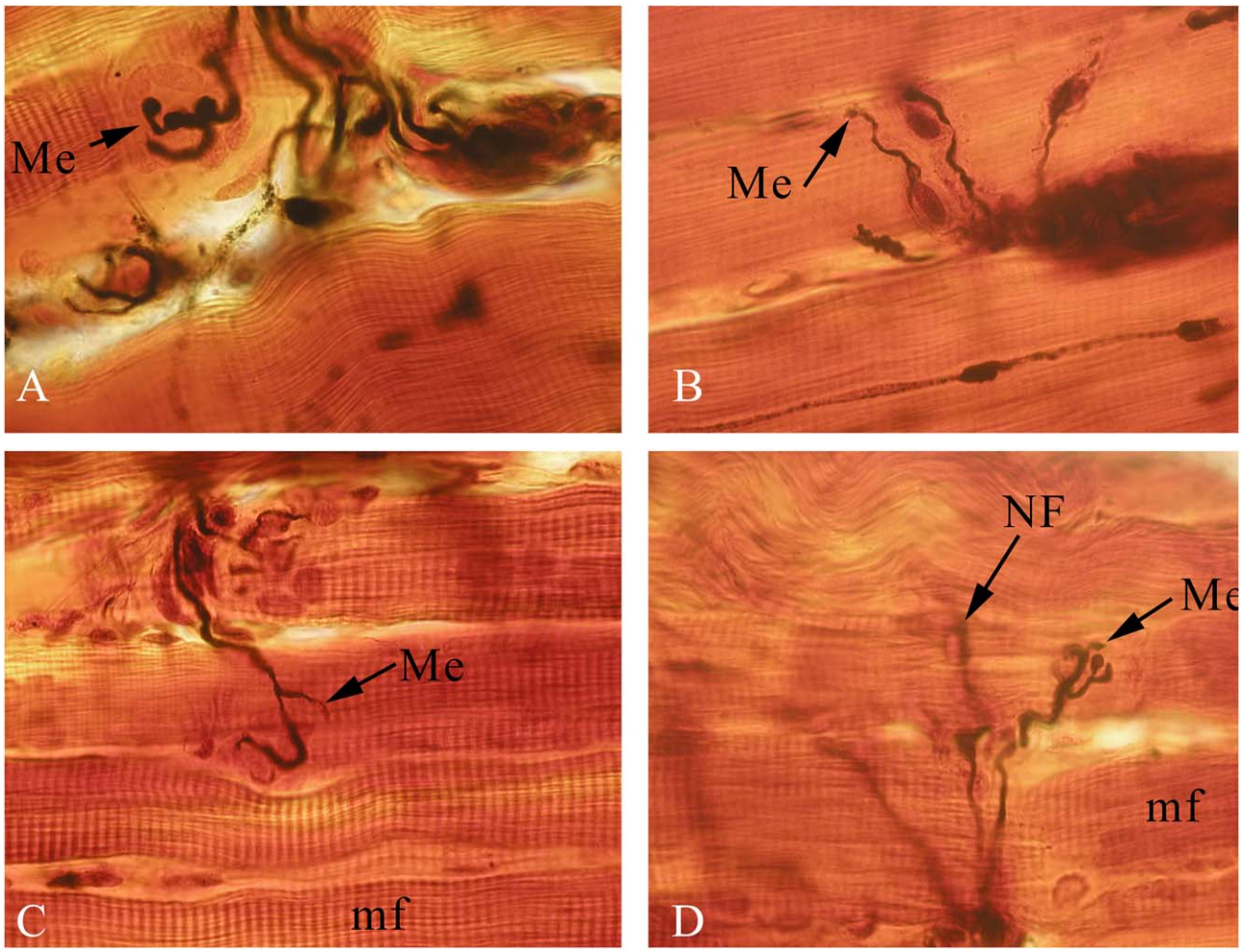

Fig. 7. Reinnervation of control and experimental animals m.gastrocnemius. The motor nerve endings are preserved. (A) intact sciatic nerve (group 4); (B) crushed sciatic nerve (group 3); (C) HSV-I infected mice with crushed nerve (group 1); (D) HSV-I infected mice, crushed nerve, and acyclovir (group 2); Me, motor end plates NF, nerve fibers; mf, muscle fibers. Silver impregnation, ×1000.

different degrees of hypotrophic changes in skeletal muscle tissue were found (Fig. 6).

Moreover, in group 1 there was a sharp increase in the density and area of nuclei in muscle fibers. These changes may be due to the compensatory reaction of the muscle to the denervation and the infectious process, possibly both, which have affected the quantitative parameters of muscle fibers (Fig. 5C,D). Thus, the diameter of muscle fibers in group 1 decreased by $\sim$ 47%, in group 2 by $\sim$ 39%, whereas in the group 3 by $\sim$ 25% ($P \leq 0.05$).

Muscle hypotrophy was characterized by the loss of muscle fibers striation and destruction of myofibrils. A higher degree of the described changes in group 1 may indicate progression of morphological disorders and decreased ability of the muscle to contract after neuroinfection. At the same time, the reduction of structural rearrangements in denervated muscles was established in the acyclovir group.

We have found motor nerve endings on single muscle fibers in silver nitrate impregnated longitudinal sections of m.gastrocnemius (Fig. 7). The emergence of these endings is a morphological sign of muscle reinnervation. This is important for understanding and explaining the causes of muscle resistance to denervation. Histological findings have not revealed any structural signs of severe muscle fibers atrophy, only the signs of hypotrophy, which was confirmed by morphometry (the muscle fibers diameter was significantly smaller than in the control group). Unfortunately, histological method only allows to state muscle fibers hypotrophy, but does not explain the mechanism of these changes. Additional biochemical study was conducted to analyze these changes.

Biochemical studies were conducted to study the molecular bases of hypotrophy in *m.gastrocnemius*.

Thus, the results of electrophoresis of the whole DNA in agarose gel in the groups 1–3, have revealed no significant changes. This suggests high resistance of skeletal muscles to denervation during the 30 days of the experiment. Figure 8 shows gel electrophoregram without signs of DNA fragmentation.

At the same time, there was a significant increase in the content of peroxidation products and oxidative modification of proteins in group 1 as compared to control

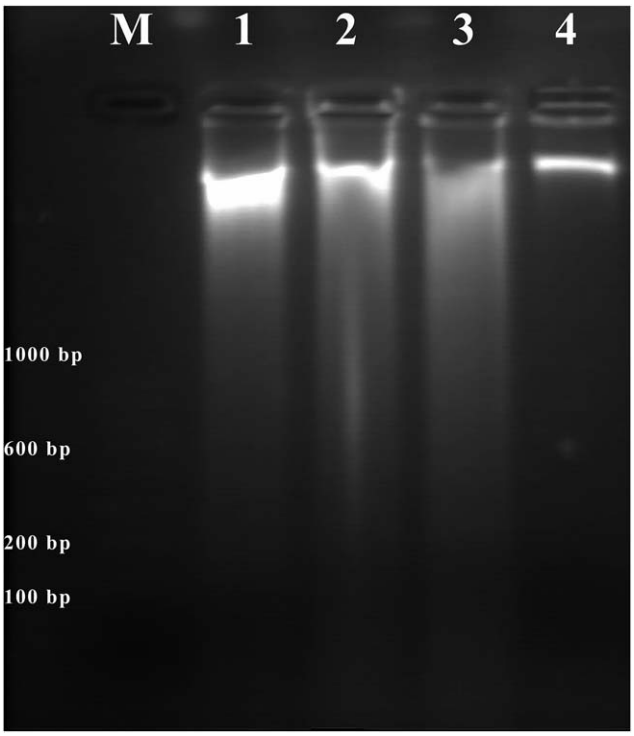

Fig. 8. Electrophoregram obtained by DNA extraction from the tissue of m.gastrocnemius of control and experimental animals. M - marker of DNA (100–1000 bp); 1—group 4 (control 2, shame-operated group); 2—group 3 (control 1); 3—group 1; 4—group 2.

(Table 1). The statistically significant increase in the level of TBA-active products ($P < 0.05$), conjugated dienes ($P < 0.05$), and carbonyl groups ($P < 0.05$) with the simultaneous decrease in the concentration of free low molecular weight SH-groups (the main component of glutathione) indicated marked metabolic disturbances and exhaustion of antioxidant defense system in the presence of transmitted viral neuroinfection.

Prolonged denervation of skeletal muscles is characterized by the development of metabolic changes, one of its components being the accumulation of peroxidation products against the background of the disturbed balance between pro- and antioxidant compounds in the cells. In this case, the level of enzymatic activity of NAD(P)H-quinone oxidoreductase, which is an important enzyme in the metabolism of quinones in the body, got back to the control values in groups 2.

Such a reaction may be considered a compensatory response of the denervated muscles to the development of the infectious process, which may be due to the excess of quinone metabolites formation in the damaged tissues. At the same time, suppression of HSV-I by acyclovir indirectly reduced the degree of metabolic disorders in the denervated muscles, which further confirms the hypothesis of the negative effect of HSV-I on the regenerative potential of the injured peripheral nerve.

DISCUSSION

The results of experimental studies have established the role of herpes neuroinfection in the development of systemic disorders of the nervous system. Unlike the central nervous system, peripheral nervous system is known to have the potential for functional recovery after limb injury. Regeneration occurs through a process of elimination of the damaged nerve fibers, which have lost contact with the neuronal soma, known as Wallerian degeneration, and subsequent activation of axons growth.

In many morphological studies features of the nerve damage and reparative recovery with the involvement of the spinal cord and spinal ganglia neurons, and Schwann cells of the injured nerve have been shown. The effect of HSV-I on the morphology of the sciatic nerve and shin muscles was not considered earlier. In experiments on the model of the crushed sciatic nerve, the dependence of the morphological disorders of the nerve and the development of metabolic changes in the denervated muscles of the shin in the presence of HSV-I in animals was demonstrated. The application of the SEM method allowed to reveal the features of structural changes in the nerve, which had not previously been described in this model. It is interesting that the crushed nerve preserves the general organization of the stroma of the distal stump.

Significant preservation of such endoneural cylinders gives reason to predict a high functional level of recovery. This may be proved by the fact that on the morphological level *m.gastrocnemius* had not suffered from severe atrophy, and a significant number of myofibers was assessed as not affected. Nerve regeneration and *m.gastrocnemius* reinnervation in the study groups 1, 2, and 3 were confirmed. This explains the cause of the partial resistance of the muscle to denervation, however, it were biochemical findings which clarified the role of HSV-I in neuro-muscular damage.

In general, damage to the sciatic nerve is accompanied by the development of metabolic changes in the skeletal muscles of the shin. These changes consist in the accumulation of peroxidation and oxidative modification products, quantified as increasing content of conjugated dienes and carbonyl groups, and simultaneous impairment of endogenous protective mechanisms. The latter was shown by a sharp decrease in the level of free SH-groups.

Taking into account that glutathione is the main carrier of free SH-groups, it can be argued that the antioxidant system is depleted in a denervated *m.gastrocnemius*. The increased enzymatic activity of NAD(P)H-quinone oxidoreductase in the tissue of the denervated muscle of infected animals by more than 20% compared to the sham-operated mice is important. Convincing evidence suggests that in addition to the canonical role of quinones in oxidation-reducing modifications, this enzyme is involved in the regeneration of antioxidants in the body cells, tocopherols in particular (Bello et al., 2003), and in the direct scavenging of the superoxide radical (Siegel et al., 2004). As demonstrated in the studies (Kapinya et al., 2003), the excessive growth of the activity of this enzyme may correlate with neurodegenerative processes.

However, the role and activity of NAD(P)H-quinone oxidoreductase in the skeletal muscles tissue, during the nerve denervation, in particular, remains largely unexplored, therefore our work allows to expand the understanding of these processes.

TABLE 1. Biochemical parameters of *m. gastrocnemius* of control and experimental animals

Parameter/Group	Group 1 (HSV-I + crushed nerve)	Group 2 (HSV-I + crushed nerve + acyclovir)	Group 3 (Control 1) (crushed nerve)	Group 4 (Control 2)
TBA-active products (nmol MDA/mg of protein)	1.23 ± 0.05^{a}	1.01 ± 0.07	0.97 ± 0.04^{b}	0.80 ± 0.11
Conjugated dienes (nmol/mg of protein)	3.10 ± 0.08^{a}	2.90 ± 0.14^{a}	$2.60 \pm 0.11^{a,b}$	1.30 ± 0.05
Carbonyl groups (nmol/mg of protein)	2.65 ± 0.15^{a}	2.15 ± 0.18^{a}	2.10 ± 0.29^{a}	1.05 ± 0.08
Free low-molecular weight SH-groups (nmol/mg of protein)	4.95 ± 0.25^{a}	5.48 ± 0.16^{a}	6.10 ± 0.49^{a}	15.23 ± 1.58
Activity of NAD(P)H- quinone oxidoreductase (nmol/min/mg of protein)	2.83 ± 0.06^{a}	2.32 ± 0.11	$1.76 \pm 0.05^{b,c}$	2.25 ± 0.07

[a]Parameter differs from group 4 ($P < 0.05$).
[b]Parameter differs from group 1 ($P < 0.05$).
[c]Parameter differs from group 2 ($P < 0.05$).

The presence of HSV-I in animals with a crushed nerve has negatively affected the restoration of neuro-muscular interactions. HSV-I caused changes in endoneurium of the fascicles of the distal stump of the nerve, in which no nerve fibers were observed. Viral neuroinfection caused reactive changes in *m.gastrocnemius*, a sharp increase in the number of myonuclei, diffuse hypotrophy of myofibers, the increased amount of peroxidation products (TBA-active products, conjugated dienes).

The authors show the association of oxidative stress, peroxidation reactions and other changes in the elements of both central and peripheral nerve systems with the pathogenesis of HSV-I (Valyi-Nagy and Dermody, 2005; Schachtele et al., 2010; Hu et al., 2011; Hato et al., 2013).

An important indicator in the pathogenesis of herpetic infection is the neuro-muscular function. As demonstrated in the studies (Brun et al., 2010), it is after intragastric inoculation that HSV-I causes latent infection of the intestinal ganglia, which leads to malfunctioning of the intestinal smooth muscles. However, data on metabolic disorders in muscle tissue in the presence of herpetic lesion are scarce. Some studies using modern methods substantially prove that the virion fragments are fixed in the skeletal muscle (Gonzalez and Sanjuan, 2013) during activation of herpetic infection, which indicates the direct effect of this viral invasion on it.

In our studies, the results of changes in the nerve and skeletal muscle of infected animals with injuries and acyclovir injections remained controversial. Morphological data indicate a lower level of changes in the thickness of the nerve fascicles and the density of nuclei in muscle fibers, but morphometric indices (criteria) chosen in our study did not show a statistically significant difference. Earlier, it was demonstrated that the use of acyclovir in the experiment inhibited viral reproduction and further development of viral infection in the brain cortex and hippocampus (Gumenyuk et al., 2017). Our results are based on the assumption that the use of acyclovir can indirectly contribute to the partial preservation of tissue organs in case of systemic infection. This correlates with other studies that demonstrate acyclovir properties in mitigating oxidative stress (Müller et al., 2005). Our findings indicate the relevance of this issue, but they require additional research using other more sensitive methods (immunocytochemical detection of neurofilaments, HSV or antibodies to HSV), and therefore the work will be continued. The biochemical changes found in the gastrocnemius muscle of the infected animals were explained relying on the available data of other researchers. Our own results prove the negative impact of HSV-I on the denervated *m.gastrocnemius* status, with no statistically significant recovery of muscle metabolism occurring even with acyclovir use (only the downward trend in the levels of TBA-active products and activity of NAD(P)H- quinone oxidoreductase). The fact that the issue of metabolic changes in muscle tissue during herpes infection is not sufficiently investigated, allows us to believe that our description will expand the general understanding of these processes.

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