

UDC 616-009+616-009.6+611.08

DOI: 10.15587/2519-4798.2021.232975

MORPHOLOGICAL MANIFESTATIONS OF EXPERIMENTAL PACLITAXEL-INDUCED SCIATIC NEUROPATHY UNDER CORRECTION OF 2-ETHYL-6-METHYL-3-HYDROXYPYRIDINE SUCCINATE

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Paclitaxel is an effective chemotherapeutic agent for many cancers, but it has a number of limiting side effects that not only significantly reduce the quality of life of patients, but also limit their further treatment. Peripheral neuropathy is one of these, but there are currently no proven effective druHS for the prevention or treatment of paclitaxel-induced neuropathic pain (PINP) in particular, or chemotherapy-induced peripheral neuropathy (CIPN) in general. 2-ethyl-6-methyl-3-hydroxypyridine succinate (HS) is a derivative of succinic acid with neuroprotective, antihypoxic, membrane-protective, nootropic, sedative effects.

The aim of the study was to study the effect of the neuroprotective agent HS on the pathomorphogenesis of the sciatic nerves under conditions of paclitaxel-induced peripheral neuropathy in the experiment.

Materials and methods. The experiment was carried out on 80 white rats, which were injected intraperitoneally with paclitaxel (Actavis, Romania), previously dissolved in isotonic saline at a dose of 2 mg / kg of body weight four times every other day until a total dose of 8 mg / kg was reached. Then forty of these animals were injected intraperitoneally with 2-ethyl-6-methyl-3-hydroxypyridine succinate at a dose of 10 mg / kg (the remaining 40 rats received intraperitoneal water for injection). Morphological studies were carried out on the first, seventh, fifteenth, twenty-eighth, sixtieth, ninetieth and one hundred and twentieth days after the last injection of the drug. We investigated the pharmacological potential of HS in the prevention and treatment of CIPN at the level of sciatic nerve (SN) morphology.

Results. The maximum value of the average profile area of myelinated nerve fibers with the use of HS is significantly lower than with uncorrected flow, and is $(78.12 \pm 2.24) \mu\text{m}^2$ compared to $(94.04 \pm 1.03) \mu\text{m}^2$ ($p < 0.001$). The introduction of HS provides a stable content of the value of the ratio of the areas of the axial cylinder and the fiber within 0.39 ± 0.01 (first day) - 0.44 ± 0.01 (ninetieth day), and a rapid recovery of the indicator value to normal values during the final 30 days of the experiment. the maximum value of the index of the profile area of the myelin sheath with the introduction of HS is 1.4 times less than with an uncorrected flow, and is, respectively, $(49.01 \pm 1.59) \mu\text{m}^2$ and $(69.77 \pm 1.87) \mu\text{m}^2$ ($p < 0.001$). HS provides a more intensive restoration of the indicator of the area of the myelin sheath during the 90th – 120th day of the experiment.

Conclusions. Our results allow us to conclude that the introduction of HS creates a protective effect against paclitaxel-induced peripheral neuropathy (PIP) by acting on both the axial cylinder and the myelin sheath of the heart failure. Due to the known pathophysiological mechanisms of the development of neuropathy, this method can be a promising therapeutic agent for the prevention and treatment of PIPN

Keywords: paclitaxel, paclitaxel-induced peripheral neuropathy, sciatic nerve, 2-ethyl-6-methyl-3-hydroxypyridine succinate

How to cite:

Ostrovskiy, M. (2021). Morphological manifestations of experimental paclitaxel-induced sciatic neuropathy under correction of 2-ethyl-6-methyl-3-hydroxypyridine succinate. ScienceRise: Medical Science, 3 (42), 20–26. doi: <http://doi.org/10.15587/2519-4798.2021.232975>

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1. Introduction

Chemotherapy-induced peripheral neuropathy (CIPN) is a common and dose-limiting side effect of some widely used anticancer drugs, including taxanes (paclitaxel), Vinca alkaloids, platinum salts, epothilones, and thalidomide. This is important clinically because neurotoxicity can be a dose-limiting side effect, leading to early treatment discontinuation. Extensive clinical trials have shown the advantage of survival with taxanes (paclitaxel) in adjuvant therapy for breast cancer, ovarian cancer, lung cancer and other nosologies. Unfortunately, taxanes often cause PIPN, which manifests

itself primarily as a distal sensory neuropathy and is characterized by pain, paresthesias, and reduced functional ability. Up to 80 % of patients treated with taxanes report neuropathy [1].

Morphological changes due to the use of paclitaxel have been demonstrated at all levels of the peripheral and central nervous system: spinal cord, neuraxis, and peripheral nerves [2]. Considering numerous studies of potential neuroprotectors, such as vitamin E [3], B vitamins [4], amitriptyline and ketamine [5], acetyl-carnitine [1] – none of them have been proven effective for the prevention or treatment of PIPN.

Pathophysiological studies have shown that paclitaxel induces altered calcium signaling, the release of neuropeptides and growth factors, mitochondrial damage and the formation of reactive oxygen species, and can activate ion channels that mediate responses to extracellular signals [6]. Recent studies also suggest a role for matrix metalloproteinase 13 (MMP-13) in mediating neuropathy. These various changes may be secondary to paclitaxel-induced microtubule transport. Human genetic studies, although still limited, also emphasize the involvement of cytoskeletal changes in the development of paclitaxel-induced peripheral neuropathy (PIPNe) [7]. The recently discovered molecular targets obtained from these studies may provide a basis for the development of a therapy that can prevent and treat peripheral neuropathy caused by paclitaxel in patients.

It has recently been shown that spinal cord stimulation during chemotherapy may slightly impair the development of CIPN in rats, causing general inhibition of synaptic plasticity due to a specific regulation of spinal cord gene networks, and affecting γ -aminobutyric acid reuptake [8, 9]. However, this approach has not found practical application.

At the same time, metabolic drugs with antioxidant, antihypoxic and membrane-stabilizing properties are widely used for the correction of non-chemotherapeutic neuropathies. Among them is 2-ethyl-6-methyl-3-hydroxypyridine succinate, which belongs to heteroaromatic phenols, in particular to derivatives of 3-oxypyridine and succinic acid [10].

Based on data of the potential stimulatory effect on carcinogenesis, the use of HS in oncology is widely discussed. But it was found that HS also inhibits spontaneous metastasis both in monotherapy and in combination with some antitumor drugs [11, 12]. Therefore, using HS as a correction of paclitaxel-induced neuropathy, it is possible to achieve a direct effect on the known pathophysiological mechanisms of this neuropathy development, as well as the suppression of spontaneous metastasis of the underlying pathology.

The aim of the study: to study the effect of the neuroprotective medium HS on the pathomorphogenesis of sciatic nerves under conditions of paclitaxel-induced peripheral neuropathy in the experiment.

2. Materials and methods

The experiment was conducted on the basis of the Department of Histology, Cytology and Embryology of Ivano-Frankivsk National Medical University during 2017–2019. The study was performed on 80 white randomized male rats weighing 150–200 g, which were kept in a vivarium at a temperature of 21–24 °C, under normal light regime (day-night) and on a diet with access to food and water *ad libitum*. The experiment was conducted in accordance with the recommendations of ARRIVE and EU Directive 2010/63/EU on the protection of animals used for scientific purposes. The chemotherapeutic agent Paclitaxel (Actavis, Romania) was administered intraperitoneally, pre-dissolved in isotonic NaCl solution, at a dose of 2 mg/kg body weight four times a day four times to reach a dose of 8 mg/kg according to the model proposed by R. C. Polomano et al. [13].

The animals were then randomized to the experimental (40 animals weighing 175±25 g) and control (40 animals weighing 175±25 g) groups. In the experimental group, the animals were injected intraperitoneally for the next 10 days with 2-ethyl-6-methyl-3-hydroxypyridine succinate (drug “Armadin”, manufactured by Limited Liability Company Scientific and Production Firm “Microchem”, Ukraine-Spain) at a dose of 10 mg/kg of body weight, pre-dissolved in 0.5 ml of water for injection. Animals in the control group were injected intraperitoneally with water for injections in an equivalent volume for the same period.

Morphological studies were performed on the 1st, 7th, 15th, 28th, 60th, 90th and 120th days after the last administration of the drug. Cross sections of the sciatic nerve with a thickness of 1 μ m, made of blocks of nerve fragments intended for electron microscopic examination, and stained with toluidine blue were used.

In the interactive mode, the cross-sectional area of the SN nerve trunk, the number of myelin nerve fibers (MNF) and microvessels were determined. Indicators of the area of myelin nerve fiber (MNF) profiles and axial cylinders (AC), perimeters of MNV and AC profiles in internodal areas, cross-sectional area of endoneural blood vessels and their lumen were determined.

To calculate the derived parameters – the coefficients of the shape of the nerve cells profiles ($Cncp=4\pi Sncp/Pncp$) and their nuclei ($Cn=4\pi Sn/Pn^2$), the nuclear-cellular ratio $Sn/Sncp$, the ratio of the areas of AC and MNF, the cross-sectional area of the myelin sheath ($Smnf - Sac$), the coefficients of the form MNF and AC, the coefficient HS ($Sac/Smnf$), as well as statistical processing of measurement results, spreadsheets Microsoft Excel and StatPlus and Statistica 6.0 for Windows were used.

Due to the fact that the distribution of metrics in some variation series differed from normal, the significance of differences between groups was assessed using the nonparametric Mann-Whitney test.

3. Research results

PIPNe is characterized by significant violations of the sciatic nerve myeloarchitectonics. The endoneurium swelling of varying severity, polymorphic changes in the metric parameters of myelin nerve fibers (area of fiber profiles and their axial cylinders, the thickness of the myelin sheath) are determined in the dynamics of the experiment. Often there are significant violations of the structure of the myelin sheath – the formation of interlamellar vacuoles, dissociation of myelin plates in some areas of the tibial and fibula portions of the sciatic nerve. Quite often fibers with sharply thickened myelin sheath with disturbance of congruence of external and internal contours, the phenomena of atrophy of axial cylinders are visualized. In general, experimental PIPNe is considered as a slowly progressive disease accompanied by polymorphic changes of axial cylinders and myelin sheath, which deepen to the 60th day of the experiment with a tendency to slow and incomplete recovery by the 120th day of the experiment.

When using HS to correct pathological changes caused by the toxic effects of paclitaxel, during the first 2 months of the disease quite polymorphic changes, mani-

fested by swelling or deformation of myelin nerve fibers, edema or, conversely, atrophy of axial cylinders, manifested by swelling or deformation of myelin nerve fibers, edema or, conversely, atrophy of the axial cylinders, uneven thickening, sometimes defibering, loss of congruence of the outer and inner contours of the myelin sheath, the formation of interlamellar vacuoles are determined (Fig. 1). Usually the violation of the structure and tincto-

rial properties of the fibers is determined against the background of the endoneurium swelling of varying severity. Starting from the 60th day of the experiment, the microcirculation is gradually normalized, the severity of endoneuria swelling decreases, the diameter of the axial cylinders decreases, the degree of fibers and axons (dendrites) deformation decreases, and the myelin sheath looks more orderly.

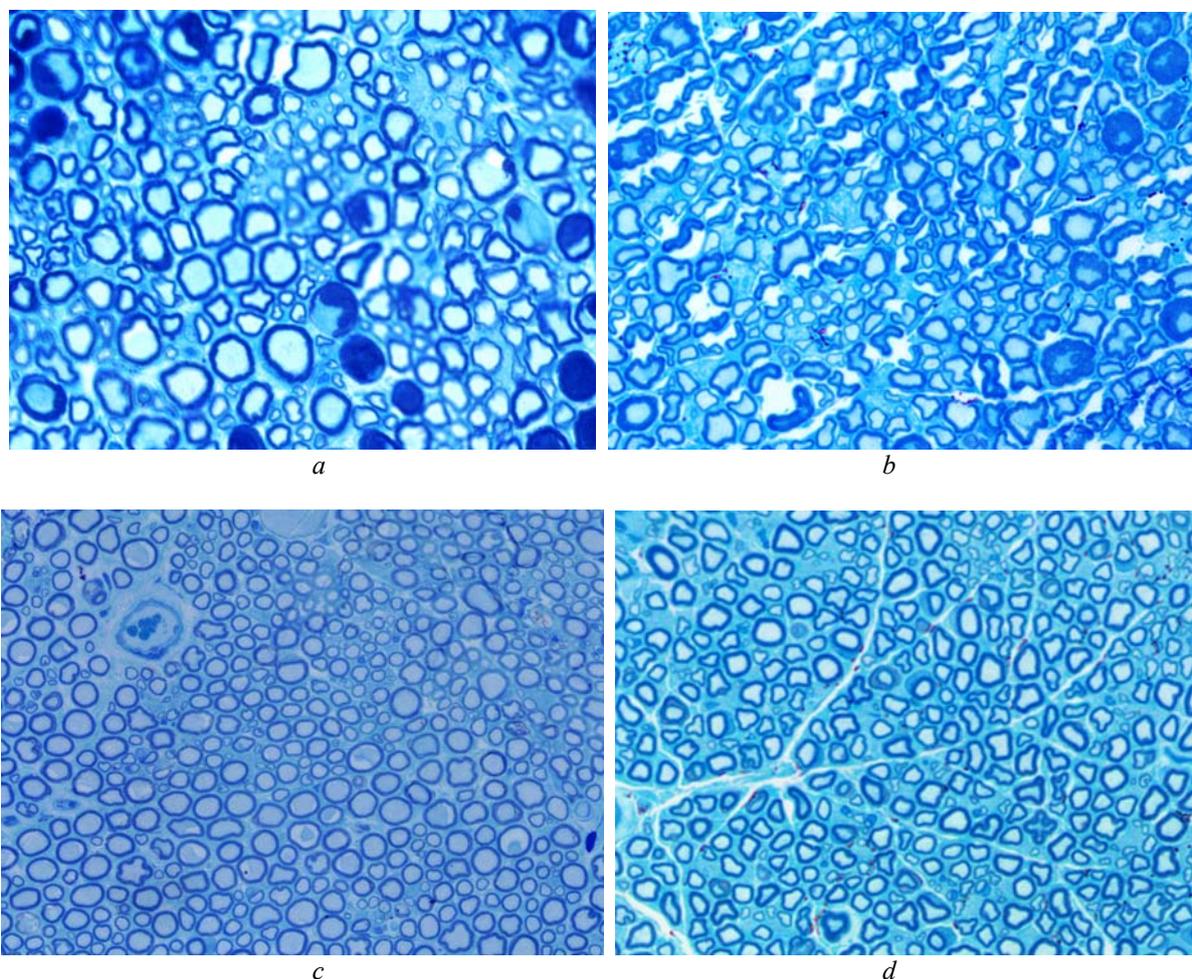


Fig. 1. Cross sections of the sciatic nerves of white rats: *a* – on the 1st day; *b* – on the 28th day; *c* – on the 90th day; *d* – on the 120th day of paclitaxel-induced neuropathy under conditions of HS correction. Microphotographs. Semi-thin slices. Toluidine blue coloring. Magnification: obj. $\times 40$, approx. 1.7

At the same time, it is obvious that qualitative analysis of the conductive component does not allow detailed determination in the nature of morphological changes, establishing general patterns of neuropathy and morphological basis of conduction disorders of motor and sensory myelin nerve fibers which are primarily due to differences in the sensitivity of fibers of different diameters and the degree of myelination to the toxic effects of paclitaxel and specific features of metabolism of motor and sensitive neurons.

In this regard, we conducted a detailed metric indicators analysis of the whole set of myelin nerve fibers, which constitute the conductive component of the tibia and fibula portions of the sciatic nerve (Table 1). On the 1st day after the last drug administration a significant

increase in the average value of the MNF profile area is observed, which is $(81.42 \pm 2.26) \mu\text{m}^2$, which is almost 1.8 times higher than normal – $(45.34 \pm 1, 18) \mu\text{m}^2$ ($p < 0.001$). During the next 4 weeks (up to the 28th day) the value of the studied indicator increases from $(77.58 \pm 3.12) \mu\text{m}^2$ to $(80.41 \pm 2.13) \mu\text{m}^2$, which significantly exceeds the norm ($p < 0.001$), but does not differ on the 7th, 15th, 28th day. The highest values of the MNF profile area mean value reaches on the 60th day of the experiment and equals $(94.04 \pm 1.03) \mu\text{m}^2$, which is almost 2.1 times higher than the norm ($p < 0.001$). The gradual decrease in the average value of the MNF profile area is determined on the 90th and 120th day and is, respectively, $(84.38 \pm 3.39) \mu\text{m}^2$ and $(67.51 \pm 1.74) \mu\text{m}^2$, but almost 1.5 times higher than normal.

Table 1

Morphometric parameters of MNF of rat SN in the dynamics of paclitaxel-induced neuropathy ($M \pm m$, $n=40$)

Термін досліджу	Area of the MNF profile (μm^2)	Area of the AC of MNF profile (μm^2)	MS area (μm^2)	The ratio of the areas of the AC and MNF profiles	The coefficient of the MNF shape	The coefficient of the AC of MNF shape
Norm	45.34 \pm 1.18	20.46 \pm 0.81	25.71 \pm 1.54	0.49 \pm 0.03	0.74 \pm 0.04	0.71 \pm 0.05
1 st day	81.42 \pm 2.26 p1<0.001	32.78 \pm 2.15 p1<0.001	51.35 \pm 2.03 p1<0.001	0.40 \pm 0.01 p1<0.05	0.83 \pm 0.01 p1<0.05	0.84 \pm 0.01 p1<0.05
7 th day	77.58 \pm 3.12 p1<0.001 p2>0.05	32.31 \pm 1.54 p1<0.001 p2>0.05	47.22 \pm 2.83 p1<0.001 p2>0.05	0.41 \pm 0.01 p1<0.05 p2>0.05	0.87 \pm 0.01 p1<0.01 p2<0.001	0.86 \pm 0.01 p1<0.01 p2<0.01
15 th day	78.51 \pm 3.64 p1<0.001 p2>0.05	33.02 \pm 1.51 p1<0.001 p2>0.05	47.95 \pm 1.24 p1<0.001 p2>0.05	0.42 \pm 0.01 p1<0.05 p2>0.05	0.86 \pm 0.01 p1<0.01 p2>0.05	0.85 \pm 0.01 p1<0.01 p2>0.05
28 th day	80.41 \pm 2.13 p1<0.001 p2>0.05	32.09 \pm 0.84 p1<0.001 p2>0.05	51.15 \pm 1.87 p1<0.001 p2>0.05	0.41 \pm 0.01 p1<0.05 p2>0.05	0.85 \pm 0.01 p1<0.05 p2>0.05	0.84 \pm 0.01 p1<0.01 p2>0.05
60 th day	94.04 \pm 1.03 p1<0.001 p2<0.001	29.18 \pm 1.78 p1<0.001 p2>0.05	69.77 \pm 1.87 p1<0.001 p2<0.001	0.33 \pm 0.01 p1<0.001 p2<0.001	0.84 \pm 0.01 p1<0.05 p2>0.05	0.84 \pm 0.01 p1<0.05 p2>0.05
90 th day	84.38 \pm 3.39 p1<0.001 p2<0.001	36.23 \pm 1.14 p1<0.001 p2<0.001	51.27 \pm 2.48 p1<0.001 p2<0.001	0.39 \pm 0.01 p1<0.01 p2<0.001	0.83 \pm 0.01 p1<0.05 p2>0.05	0.82 \pm 0.01 p1<0.05 p2>0.05
120 th day	67.51 \pm 1.74 p1<0.001 p2<0.001	29.28 \pm 0.96 p1<0.001 p2<0.001	37.06 \pm 1.03 p1<0.001 p2<0.001	0.42 \pm 0.01 p1<0.05 p2>0.05	0.83 \pm 0.01 p1<0.05 p2>0.05	0.81 \pm 0.01 p1<0.05 p2>0.05

Note: p1 – the difference between the reliability of the indicator compared to the control; p2 – the difference in the reliability of the indicator compared to the previous study period

A similar dynamics of changes in the average values of the cross-sectional area of the axial cylinders of myelin nerve fibers is observed during the 7th – 60th day of the experiment. In contrast to the cross-sectional area of the nerve fiber, the average value of the area of the axial cylinders profile reaches maximum values on the 90th day of the experiment, and is (36.23 \pm 1.14) μm^2 . A significant decrease in this parameter is observed on the 120th day, it equals (29.28 \pm 0.96) μm^2 . However, its value is more than 1.4 times higher than the norm and equals (20.46 \pm 0.81) μm^2 ($p < 0.001$).

The thickness of the myelin sheath is an extremely important parameter that provides the speed of the nerve impulse through the myelin nerve fibers. The study of the area of the myelin sheath in the dynamics of PIPN shows a significant increase during the 1st – 60th day and a gradual decrease during the 90th – 120th day of the experiment. However, even on the 120th day, the value of this indicator exceeds the norm by more than 1.4 times.

The study of HS (the ratio of the axial cylinder and myelin fiber profiles areas) indicates the asynchronous course of pathological processes in the axons and dendrites of nerve fibers and schwannocytes that surround them. In particular, a steady decrease in the value of the studied parameter during the 1st – 60th day of the experiment indicates a more significant swelling of the myelin sheath, which reaches its maximum severity on the 60th day of the experiment (the lowest value of HS – 0.33 \pm 0.01). On the 90th – 120th day of the experiment, a gradual increase in the average values of the axial cylinders and nerve fibers areas ratio is determined, which probably indicates a gradual synchronization of changes in the axial cylinders and myelin sheath.

Analysis of the average values of the myelin nerve fibers and axons (dendrites) shape shows its stable growth throughout the experiment, which indicates the dominance of the processes of the axial cylinders swelling.

Analysis of the metric parameters of myelin nerve fibers in the dynamics of the experiment with the use of HS as a neuroprotector indicates that the drug has a fairly pronounced effect on the course of paclitaxel-induced neuropathy (Table 2). The introduction of HS significantly reduces the severity of the pathological process that develops in the myelin nerve fibers under the influence of chemotherapy. It is noteworthy that the maximum value of the average profile area of myelin nerve fibers when using HS is much lower than in the uncorrected course, and is (78.12 \pm 2.24) μm^2 compared to (94.04 \pm 1.03) μm^2 ($p < 0.001$). Achieving the peak value of this indicator is determined on the 28th day, compared with the 60th day in the absence of correction. During the following terms, there is a pronounced tendency to reduce the average profile area of myelin nerve fibers. On the 120th day of the experiment, the value of the indicator is (52.33 \pm 1.89) μm^2 , which is significantly less than in the experiments without correction – (67.51 \pm 1.74) μm^2 ($p < 0.05$), however exceeds the norm by 1.2 times.

HS has a more pronounced effect on the restoration of metric parameters of the axial cylinders of myelin nerve fibers. First of all, the dynamics of violations of the profile area of the axial cylinders differs significantly. Paclitaxel causes a significant long-term progressive swelling of axons (dendrites) of myelin nerve fibers, which reaches maximum values on the 90th day of the experiment and is (36.23 \pm 1.14) μm^2 . Administration of HS to experimental animals significantly reduces the severity

of neuroplasma swelling of neuronal processes, which progresses only to the 28th day, reaching a maximum value (31.35 ± 0.88) μm^2 . During the following terms of the experiment there is a steady tendency to decrease the value

of the studied parameter, the value of which on the 120th day is (24.44 ± 0.47) μm^2 , which is significantly more than normal, but significantly less than in experiments without correction – (29.28 ± 0.96) μm^2 , ($p < 0.05$).

Table 2

Morphometric parameters of rat MNF of SN in the dynamics of paclitaxel-induced neuropathy in the correction of HS (M=m, n=40)

The term of the study	Area of the MNF profile (μm^2)	Area of the AC of MNF profile (μm^2)	MS area (μm^2)	The ratio of the areas of the AC and MNF profiles	The coefficient of the MNF shape	The coefficient of the AC of MNF shape
Norm	45.34±1.18	20.46±0.81	25.71±1.54	0.49±0.03	0.74±0.04	0.71±0.05
1 st day	79.26±2.14 p1<0.001	31.42±2.06 p1<0.001	50.19±1.93 p1<0.001	0.39±0.01 p1<0.01	0.82±0.01 p1>0.05	0.79±0.01 p1>0.05
7 th day	74.40±2.95 p1<0.001 p2>0.05	31.48±1.55 p1<0.001 p2>0.05	46.14±2.76 p1<0.001 p2>0.05	0.40±0.01 p1<0.05 p2>0.05	0.85±0.01 p1<0.05 p2<0.01	0.83±0.01 p1<0.05 p2<0.05
15 th day	75.34±3.53 p1<0.001 p2>0.05	30.78±1.51 p1<0.001 p2>0.05	46.31±1.24 p1<0.001 p2>0.05	0.41±0.01 p1<0.05 p2>0.05	0.83±0.01 p1<0.05 p2>0.05	0.80±0.01 p1<0.05 p2>0.05
28 th day	78.12±2.24 p1<0.001 p2>0.05	31.35±0.88 p1<0.001 p2>0.05	49.02±1.83 p1<0.001 p2>0.05	0.40±0.01 p1<0.01 p2>0.05	0.81±0.01 p1>0.05 p2<0.05	0.78±0.01 p1>0.05 p2>0.05
60 th day	72.47±1.93 p1<0.001 p2>0.05	28.30±1.65 p1<0.001 p2>0.05	49.01±1.59 p1<0.001 p2>0.05	0.42±0.14 p1>0.05 p2>0.05	0.80±0.01 p1>0.05 p2>0.05	0.77±0.01 p1>0.05 p2>0.05
90 th day	63.17±2.11 p1<0.001 p2<0.01	27.71±0.68 p1<0.001 p2>0.05	38.28±2.23 p1<0.001 p2<0.001	0.44±0.01 p1>0.05 p2>0.05	0.78±0.01 p1>0.05 p2>0.05	0.75±0.01 p1>0.05 p2>0.05
120 th day	52.33±1.89 p1<0.01 p2<0.001	24.44±0.47 p1<0.001 p2<0.001	27.31±1.29 p1>0.05 p2<0.001	0.47±0.01 p1>0.05 p2<0.05	0.75±0.02 p1>0.05 p2>0.05	0.72±0.02 p1>0.05 p2>0.05

Note: p1 – the difference between the reliability of the indicator compared to the control; p2 – the difference in the reliability of the indicator compared to the previous study period

HS has a particularly significant effect on the area of the myelin sheath. Despite the preservation of the wavy nature of changes in the studied value with the presence of peak values on the 1st and 28th – 60th day of the experiment, the maximum value of the myelin sheath area profile with the introduction of HS is 1.4 times less than in the uncorrected course, and is, respectively, (49.01 ± 1.59) μm^2 and (69.77 ± 1.87) μm^2 ($p < 0.001$). HS provides a more intensive recovery of the myelin sheath area during the 90th – 120th days of the experiment – at the end of the experiment; the value of this indicator does not differ significantly from the norm.

The asynchronous nature of the metric parameters violations of myelin nerve fibers leads to significant changes in the dynamics of the average values of HS. In the uncorrected course of paclitaxel-induced neuropathy, the mean value of the studied indicator reaches a minimum value of 0.33 ± 0.01 on the 60th day of the experiment with a gradual increase to 0.42 ± 0.01 on the 120th day. In contrast, the introduction of HS provides a stable retention of the value of the ratio of the axial cylinder and the fiber areas in the range of 0.39 ± 0.01 (1st day) – 0.44 ± 0.01 (90th day), and rapid recovery values of the indicator to normal values during the final 30 days of the experiment.

Similarly, on the 120th day, the recovery of the indicators of myelin nerve fibers and axial cylinders shape is determined.

Despite the clear tendency to normalize the basic metrics of myelin nerve fibers in general, modern approaches to morphometric analysis of the peripheral nerves conductive component require the use of methods of more scrupulous analysis of myeloarchitectonics of nerve trunks. One of such methods is the study of histograms of the myelin nerve fibers distribution by the value of the fiber profile area, which are shown in Fig. 2.

Comparative statistical analysis of SN myeloarchitectonics in paclitaxel-induced neuropathy and in the conditions of HS correction clearly shows the fact that the drug has a pronounced modulating effect on the course of the pathological condition. The positive effect of HS is manifested by a gradual increase and stabilization of the proportion of myelin nerve fibers with a cross-sectional area up to $40.0 \mu\text{m}^2$, which is accompanied by a shift of the peak of the diagram to the left. Along with this, a gradual decrease in the proportion and at the end of the experiment the disappearance of fibers with a cross-sectional area of more than $60.0 \mu\text{m}^2$, which are normally almost non-existent.

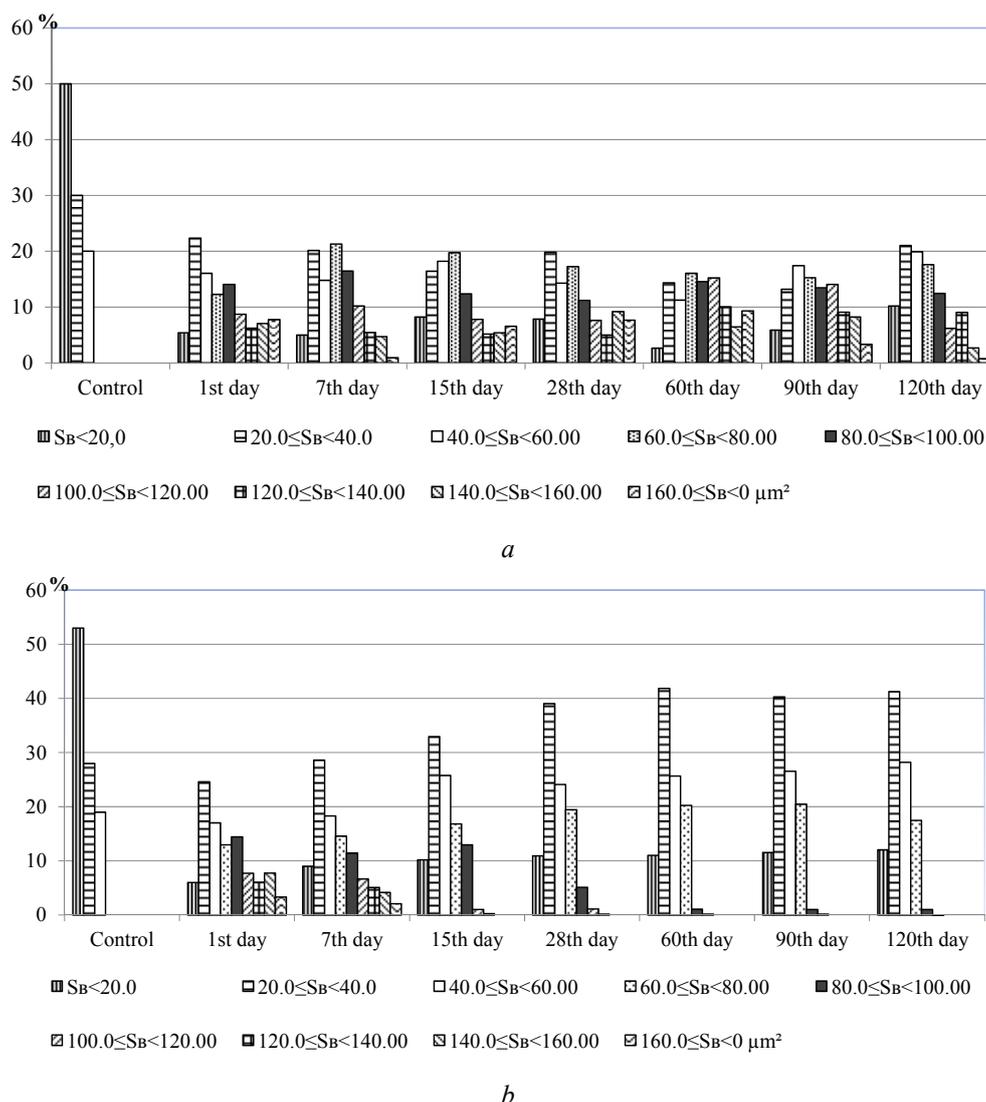


Fig. 2. Histograms of the myelin nerve fibers distribution of the rat sciatic nerve by the value of the fiber profile area: *a* – in the dynamics of paclitaxel-induced neuropathy; *b* – when correcting the HS

4. Discussion of research results

Numerous studies on the pathomorphogenesis of the nervous system toxic lesions caused by paclitaxel indicate the multifactorial nature of neuropathy induced by this cytostatic. Among the main – mitochondrial dysfunction and oxidative stress, ion transport disorders, autophagy, damage to the neurotubular apparatus [14]. The study of antioxidants as a means of neuroprotective therapy has a long history, but the use of traditional, time-tested drugs can only partially minimize the toxic effects of paclitaxel on the peripheral nervous system. Paradoxically, the use of highly effective drugs (amifostine, glutamine, acetyl L-carnitine), which in the experimental conditions showed a fairly pronounced neuroprotective effect in the early stages of neuropathy, in the clinic practice did not show high enough efficacy in long-term follow-up. Moreover, the results of a randomized placebo-controlled multicenter study SWOG S0715 [15] showed that the use of acetyl L-carnitine administration during chemotherapy caused a more pronounced neurotoxic effect of paclitaxel in patients with breast cancer compared with placebo. At the same time, studies of antioxidants, including drugs of plant origin, as neuroprotectors do not stop, but are carried out at a deeper level, taking into account their impact on the sub-

tle mechanisms of oxidative stress [14]. We were the first to use 2-ethyl-6-methyl-3-hydroxypyridine succinate as a neuroprotector in experimental paclitaxel-induced neuropathy and proved its positive effect on the metric parameters of myelin nerve fibers of sciatic nerve over a fairly long period of time (120 days). The use of this drug is not without the disadvantages inherent in antioxidant therapy in general – incomplete restoration of the structure of nerve fibers, reducing the intensity of reparative effects on the 90th – 120th days of the experiment. At the same time, we have shown that short-term administration of HS in the early period after cessation of cytostatic injections prevents the occurrence of deep degenerative lesions of MNF, reduction of demyelination, reduction of axoplasm and myelin sheaths swelling, reduction the degree of deformation of myelin nerve fibers and their axial cylinders. The obtained results of morphometric analysis are in full agreement with the data of neurophysiological studies [16] and indicate the possibility of using 2-ethyl-6-methyl-3-hydroxypyridine succinate as an effective neuroprotector in paclitaxel-induced peripheral neuropathy.

Study limitations. Our research is limited by the study of morphological and morphometric characteristics of the central and peripheral nervous systems of rats in CIPN.

Prospects for further research. Further in-depth study requires optimization of drug administration regimens, study of subtle pathomorphogenetic mechanisms of antioxidant influence on intra-axonal transport processes, the role of schwannocytes in ensuring the regenerative potential of myelin nerve fibers in paclitaxel-induced neuropathy and in the correction treatment.

5. Conclusions

1. The above data indicate a positive neuromodulatory effect of HS on the course of PIPN by reducing the maximum values of the average area of the myelin nerve fibers profile when using HS in comparison with the uncorrected course. It is $(78.12 \pm 2.24) \mu\text{m}^2$ compared to $(94.04 \pm 1.03) \mu\text{m}^2$ ($p < 0.001$). This scheme of administration provides a pronounced restorative effect on the morphogenesis of neuropathy.

2. HS provides a stable value retention of the ratio of the areas of the axial cylinder and fiber in the range of 0.39 ± 0.01 (1st day) – 0.44 ± 0.01 (90th day), and rapid

recovery of the indicator value to normal values during the final 30 days of the experiment. On the 120th day, the recovery of the indicators of the shape coefficients of myelin nerve fibers and axial cylinders is determined. However, the use of the drug does not lead to a complete recovery of metric indicators of myelin nerve fibers and requires further in-depth study.

Conflict of interests

The authors declare that they have no conflicts of interest.

Financing

None

Acknowledgement

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Received date 02.03.2021

Accepted date 15.04.2021

Published date 31.05.2021

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