The Pharma Innovation

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Neurophysiological outcomes of paclitaxel-induced peripheral neuropathy combined with experimental 2-ethyl-6-methyl-3-hydroxypyridine succinate correction

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Abstract
Chemotherapy-induced peripheral neuropathy is a common and potentially severe side effect of cancer drugs that target microtubules. This has important clinical significance because such neuropathy is the most frequent cause of dose reduction or treatment discontinuation in patients treated for cancer with commonly used antimitotic drugs including paclitaxel. At this day, there are no proven effective chemicals for the prevention or treatment of paclitaxel-induced peripheral neuropathy or chemotherapy-induced peripheral neuropathy in general. 2-ethyl-6-methyl-3-hydroxypyridine succinate is a molecule, derivative of succinic acid with neuroprotective, membrane protective, nootropic, antihypoxic, sedative action. We investigated the pharmacological potential of 2-ethyl-6-methyl-3-hydroxypyridine succinate in preventing paclitaxel-induced peripheral neuropathy. Paclitaxel was injected intraperitoneally to random bred male-rats at a dose of 2 mg/kg 4 times every other day which resulted in mechanical allodynia and thermal hyperalgesia. 2-ethyl-6-methyl-3-hydroxypyridine succinate was administered intraperitoneally at a dose of 10 mg/kg within 10 following days after last paclitaxel injection. The signs of paclitaxel-induced peripheral neuropathy (mechanical allodynia and thermal hyperalgesia) were measured within 150 days of experiment using the von Frey filament assay and Hot Plate test, respectively. Our results indicate that 2-ethyl-6-methyl-3-hydroxypyridine succinate administration exerts a protective effect against paclitaxel-induced neuropathy by increasing of reduced mechanical withdrawal thresholds (von Frey assay) and reaction latency (Hot Plate test) on the 7th, 14th and 30th days of experiment. 2-ethyl-6-methyl-3-hydroxypyridine succinate attenuates paclitaxel-induced neuropathic pain in rodents and could be a promising therapeutic outcome for the management of this intractable disease in humans.

Introduction
Problem statement and analysis of the resent research
One of the common side effect of anticancer chemotherapy is neurotoxicity, which mainly manifests as peripheral neuropathy. With a probability up to 90% many drugs of this group can cause chemotherapy-induced peripheral neuropathy (CIPN). CIPN is the most frequent cause of dose reduction or treatment discontinuation in patients [13].

Among all the chemotherapeutic agents used to treat malignancies, paclitaxel causes acute and chronic CIPN in 80-97% of patients. The maximum duration of this neuropathy is 4.75 years. Clinical signs of peripheral neuropathy induced by paclitaxel are manifested in the form of mainly sensory disorders (peripheral mechanical allodynia, paresthesia mainly in the distal extremities of the type of gloves and socks), thermal hyperalgesia, numbness, tingling, burning, burning and less frequently motor disorders (distal muscle weakness and myalgia) [14, 17]. Pathophysiological mechanisms underlying the development of CIPN have not yet been fully understood, but in the occurrence of Paclitaxel neurotoxicity the following pathophysiological mechanisms probably play a major role: demyelination and degeneration of axons, impaired ante- and retrograde transport, oxidative stress, mitochondrial dysfunction and immune-mediated processes [11, 14, 16]. The molecular processes underlying the onset of paclitaxel-induced neuropathy remain poorly understood and need detailed consideration.

Currently, there are no effective methods for prevention or treatment of CIPN approved by the FDA and in recent years, a number of drugs with previously proven or potential neuroprotective properties have been proposed to correct the neurotoxic effect of taxanes.

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Acetyl-L-carnitine, amifostine, amitriptyline, glutamate, glutamine, glutathione, ketamine, omega-3 fatty acids, retinoic acid, vitamin E, vitamins B have been tested as potential neuroprotectors. Meta-analyzes of the use of all these agents have proven to be ineffective in preventing or treating paclitaxel-induced neuropathy, and the chasing for effective neuroprotection strategies continues [12, 18].

Recently, metabolic drugs with antioxidant, antihypoxic and membrane stabilizing properties have become widespread in the clinic. Among them, 2-ethyl-6-methyl-3-hydroxypyridine succinate was widely used. This preparation relates to heteroaromatic phenols, 3-oxypyridine and succinic acid derivatives. It has already acquired application in neurology, cardiology, endocrinology, surgery clinic [3, 7]. The use of 2-ethyl-6-methyl-3-hydroxypyridine succinate in patients with peripheral neuropathy in diabetes mellitus of the second type and metabolic syndrome leads to a significant improvement in the objective indicators of clinical diabetic neuropathy [1, 8].

We have hypothesized the use of paclitaxel as a correction for the neurotoxic effects. From our point of view, such a strategy will allow a direct impact on known pathophysiological mechanisms of development of this complication and, as a consequence, will lead to the prevention of peripheral neuropathy induced by paclitaxel.

The aim of the study: To investigate the effect of the neuroprotective agent 2-ethyl-6-methyl-3-hydroxypyridine succinate on the qualitative indicators of paclitaxel-induced peripheral neuropathy in the experiment.

Material and Methods
Prior to the experiment, a bioethics commission approved a protocol for future research. In accordance with the requirements of the GLP and The European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes, all procedures relating to the keeping, humane treatment and use of animals in research were agreed.

The experiment was performed on 80 white randomized 150-200 g male rats that were kept under the same standard vivarium conditions at constant temperature, under normal light regime (day-night), and on a standard diet. Paclitaxel (Actavis, Romania) was administered intraperitoneally, pre-dissolved in an isotonic saline at a dose of 2 mg / kg body weight four times a day to achieve a dose of 8 mg / kg according to the model proposed by R.C. Polomano et al. [10].

Subsequently, the animals were randomly assigned to the experimental (40 animals) and control (40 animals) groups. In the experimental group, animals were injected intraperitoneally with 2-ethyl-6-methyl-3-hydroxypyridine succinate (Armadine, manufactured by LLC Microchem, Ukraine-Spain) at a dose of 10 mg / kg body weight over the next 10 days, pre-dissolving 0.5 ml of water for injection. Animals of the control group were intraperitoneally injected with water for injection in an equivalent volume for a similar period.

Neurophysiological studies were performed on the 1st, 7th, 15th, 27th, 60th, 90th, 120th and 150th days after the last drug administration. The marker of PNIH, mechanical allodynia, was defined as the retraction of the hind paw of experimental animals in response to irritation by von Frey monofilaments using the up-and-down method. Animals were alternately housed in a plastic cage with a mesh bottom surface for 30 minutes prior to the test to minimize the effect of stress on the

results. Then a series of calibrated von Frey monofilaments with increasing force from 8 g to 180 g consistently exerted pressure on the middle plantar of the hind paw of the animal at an angle of 90 degrees 1.0-1.5 with until the filament bends. Between studies withstood an adaptation interval of 10 s. The test began with the use of a thread with a force of 26 g. The positive response was defined as a clear pulling or shaking of the paw in response to irritation. When receiving a positive response, the following smaller caliber monofilament was used, and when receiving a negative response, the following larger caliber monofilament was used. Mechanical pain threshold was defined as the lowest pressure force that caused a positive response in the subjects [2, 9].

The basic physiological method for studying thermal hyperalgesia is the "Hot Plate" test. In its execution, the experimental animals were placed alternately on a plate heated to 55 ± 1 °C. The stopwatch measured the time from the moment the animal was placed on the plate to the end point of the test: licking the pads of the front and / or hind paws or bouncing. This indicator was a latent time of pain reaction. The maximum time of finding animals on the plate is 35 seconds [4, 5, 6].

In order to assess the motor manifestations of peripheral neuropathy caused by paclitaxel, measurements were made of the length of stay of the animals on the drum of the Rotarod unit. The rat was placed on a 6 cm diameter drum rotating at an initial speed of 4 rpm. In order to stay and not fall, the subjects had to constantly run forward. After the animal remained for 5 s, the rotational speed was progressively increased from 4 rpm to 40 rpm for 300 s. The time to fall was measured simultaneously with the onset of the acceleration stopwatch. Three separate measurements were performed, separated by 10-minute intervals. If the animal clung to the drum and performed a complete passive revolution, the stopwatch and the measurement stopped. The arithmetic mean of the three measurements formed the mean time to fall. Three days before the control measurements, the animals were adapted to stay on the drum for 3 min at a speed of 4 rpm [15].

Microsoft Excel 2016 spreadsheets and Biostat and STATISTICA for Windows programs were used for statistical processing.

Results
Administration of paclitaxel reliably elicited signs of neurotoxicity in the form of mechanical allodynia and thermal hyperalgesia in experimental animals.

When conducting the test using von Frey monofilaments in intact animals, the mechanical pain threshold was ($55,33 \pm 7,57$) g. In the animals of the control group showed significantly expressed mechanical allodynia from the 1st to the 30th day after the last injection of drugs. At day 1, the mechanical pain threshold of control animals decreased to (26.08 ± 3.39) g ($p<0.05$) compared to intact and reached the lowest point on day 7 and was triggered by monofilament with force pressure (18.11 ± 2.04) g ($p<0.001$). Starting from the next experimental period, there was a positive dynamic of mechanical allodynia: on the 14th day the pain threshold increased to ($23,25 \pm 1,80$) g ($p<0,001$), and on the 30th day it was increased to ($27,71 \pm 5.73$) g ($p<0.01$). The following terms demonstrated the dynamics of approaching the mechanical pain threshold to the baseline: on the 60th day it was ($47,60 \pm 14,66$) g, and on the 90th day was caused by monofilaments with a force of pressure ($53,00 \pm 17,60$) In the

long terms of the experiment, no manifestations of mechanical allodynia were observed: on the 120th day the pain threshold reached (62.00 ± 21.39) g, and on the 150th day was recorded at (73.33 ± 13.33) g.

Similar dynamics of neuropathy development were observed in animals of the experimental group, which were corrected for the neurotoxic effects of paclitaxel by intraperitoneal injection of 2-ethyl-6-methyl-3-hydroxypyridine succinate. As in the control group, at the initial dates after the last administration of the drug marked signs of mechanical allodynia were noted: on the 1st day the indicator was at the level $(35, 45 \pm 4,27)$. Similar to the control group, on the 7th day was the lowest point was reached: mechanical pain threshold was $(28, 33 \pm 3,66)$ g $(p<0,05)$, which is 56.43% higher in comparison with the control. From the 14th day, we observed stable positive dynamics, which significantly outstripped the dynamics of recovery in the animals of the control group. On the 14th day the threshold of pain sensitivity was $(33, 85 \pm 3,14)$ g $(p<0,05)$ - 45,59% higher in comparison with the control, and on the 30th day it reached the level $(54,91 \pm 8.28)$ g $(p<0.05)$, which is 98.16% different from the same indicator in the control group of animals. Mechanical allodynia manifestations are negligible in the experimental group starting from the 60th day and until the end of the experiment. On the 150th day, the indicator reached (86.67 ± 13.33) g compared with the control group did not differ significantly.

Estimating the length of stay of intact animals on a hot plate, it was found that the latent time of pain response was $(17, 19 \pm 0, 93)$ s. In animals of the control group on the 1st day after the last injection of drugs observed signs of thermal hyperalgesia: the indicator decreased to $(12, 43 \pm 1, 25)$ s $(p<0, 01)$, compared with intact animals. On day 7, the lowest level of latent pain time was noted (9.89 ± 0.70) s $(p<0.001)$. From the next term of the experiment, the level of this indicator begins to return to baseline. On the 14th day it was (10.29 ± 1.00) s $(p<0.001)$, and on the 30th day it was (13.00 ± 0.98) s $(p<0.01)$. Manifestations of thermal hyperalgesia reliably disappear on the 60th day of the experiment: the latent time of pain sensitivity was $(15, 62 \pm 1, 61)$ s, on the 90th day – $(15,58 \pm 1,53)$ s. In the long term of the experiment, the manifestations of thermal hyperalgesia were rarely observed.

In the animals of the experimental group, thermal hyperalgesia was significantly less pronounced: on day 1, the indicator was (14.11 ± 1.30) s, and on day 7, (13.81 ± 0.73) s $(p<0.001)$, which is 39.60% higher than the control group. The approximation of the latency time of pain sensitivity to baseline was detected already on the 14th day - $(16, 87 \pm 1,61)$ s $(p<0,01)$, which is 63.95% higher compared to the same indicator of the control group. At day 30, the marker of thermal hyperalgesia remained close to baseline (17.15 ± 1.00) s $(p<0.01)$. At the 60th day and until the end of the experiment, the manifestations of thermal hyperalgesia in the animals of the experimental group was not observed.

Particular attention should be paid to the development of movement disorders of experimental animals. When conducting the Rotarod test, the average time to fall of intact animals was (160.25 ± 21.51) s. In the experimental control group noted unreliable changes in this indicator throughout the experiment. On the 1st day there was a decrease to $(133,25 \pm 15,07)$, on the 7th day after the last injection the indicator was $(143,56 \pm 16,65)$ s, and on the 14th day - $(138,38 \pm 22,18)$ p. At 30 days, the mean time to fall in the

control group of animals was (153.71 ± 12.56) s, and on the 60th day a decrease to (124.50 ± 19.64) s was observed. During the 90th and 120th days of the experiment, the mean time to fall was (140.75 ± 15.99) s and (141.00 ± 22.81) s, respectively, and $(144.67 \pm 17, 70)$ s.

In the animals of the experimental group, the dynamics of the development of motor disorders was not significantly different from the control. The average time to fall on the drum of the Rotarod installation in these animals was (146.85 ± 17.89) s on day 1 and (143.06 ± 15.23) s on day 7 of the experiment. The 14th day was marked by a similar indicator - (145.62 ± 15.67) s, similar to the 30th day of the experiment - (148.55 ± 13.78) s. From the 60th day until the end of the experiment, the average time to fall on the Rotarod drum was in the range 173.44 - 216.00 s, which is also not significantly different from the control group.

Discussion
The use of paclitaxel in cancer patients can induce the development of CIPN, which limits the chemotherapy regimen and decreases patients' quality of life. Despite the large number of neuroprotective agents that have been proposed for the prevention and treatment of PNIH, no effective solution has yet been found. At present, the search for effective neuroprotection strategies is crucial in the oncology clinic.

Our findings indicate the development of sensory peripheral neuropathy caused by paclitaxel, manifested as thermal hyperalgesia and mechanical allodynia. A significant decrease in both marker values was observed already on day 1 after the last injection, the lowest levels were observed on day 7 of the experiment. Starting at 14th day, there was a positive trend in the reduction of neuropathy until it disappeared from day 60 and the end of the experiment.

When using 2-ethyl-6-methyl-3-hydroxypyridine succinate to correct the neurotoxic effects of paclitaxel, a qualitative positive effect on the severity of manifestations of peripheral neuropathy was observed. When conducting the von Frey test, the mechanical pain threshold on the 7th day of the experiment was 56.43% higher, compared to the control, on the 14th day was 45.59% higher, and on the 30th day differed by 98, 16%, compared to the same indicator in the control group of animals. Evaluating the results of the test "hot plate" in animals of the experimental group, found a decrease in the manifestations of thermal hyperalgesia by 39,60%. on the 7th day of the experiment. Particularly significant is the result of the test in the period of 14 days there was a decrease in the manifestations of thermal hyperalgesia by 63, 95%. In addition, we observed a return of the latent time of pain sensitivity to baseline already on the 30th day, indicating a more intensive recovery after chemotherapy of animals of the experimental group, compared with the return to baseline at 60 days in animals of the control group.

In assessing motor disorders caused by the use of Paclitaxel, similar dynamics of the mean time to fall on the Rotatod drum were observed. No significant differences were found between the animals in the control and experimental groups.

Conclusions
The use of 2-ethyl-6-methyl-3-hydroxypyridine succinate at a dose of 10 mg / kg body weight for 10 days after the last injection of Paclitaxel significantly reduces the manifestations of peripheral neuropathy caused by the latter, 7-th, 14-th and 30- that day of the experiment. The disappearance of

manifestations of thermal hyperalgesia with the use of 2-ethyl-6-methyl-3-hydroxypyridine succinate has been observed for the 30th day of the experiment. There is no effect of 2-ethyl-6-methyl-3-hydroxypyridine succinate on the development of motor disturbances caused by Paclitaxel throughout the experiment.

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