MORPHOLOGY, PHYSIOLOGY AND PATHOPHYSIOLOGY

PHARMACOLOGICAL BLOCKADE OF CANNABINOID TYPE II RECEPTORS AND MESENCHYMAL STEM CELL TRANSPLANTATION IN A MODEL OF PERIPHERAL NEUROPATHIC PAIN

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ABSTRACT

The aim of the study. *To evaluate the anti-nociceptive and reparative effects of adipose-derived mesenchymal stem cells (ADMSCs) under the pharmacological blockade of cannabinoid CB_2 receptors in a model of peripheral neuropathic pain.*
Material and methods. *In 40 male Wistar rats, modeling of peripheral neuropathy (NP) was performed by excising a sciatic nerve. On day 7 of the study, ADMSCs (1×10^6 cells/kg) were transplanted into the area of sciatic nerve injury without additional influences or after administration of the CB_2 receptor antagonist AM630, as well as after incubation with AM630. Within 90 days, nociceptive sensitivity was studied, as well as a detailed analysis of gait using CatWalk XT (Noldus, Netherlands). On day 21 and day 90, histostructure of the distal segment of the sciatic nerve was assessed.*
Results. *Pharmacological blockade of CB_2 receptors both on the ADMSCs and in the soft tissues surrounding the site of sciatic nerve injury led to a decrease in withdrawal threshold and withdrawal latency from day 28 of the study compared with the group of rats with NP and transplantation of ADMSCs only. Local injection of AM630 before transplantation of ADMSCs contributed to the development of NP-induced gait disturbances and increase of the number of damaged nerve fibers in the distal segment of sciatic nerve. Transplantation of ADMSCs pretreated with AM630 did not significantly affect the rate of recovery of gait parameters, and decreased the number of damaged nerve fibers by day 90 of study.*
Conclusion. *Blockade of CB_2 receptors, both on the membranes of MSCs and in the area of damage to the peripheral nerve, has a negative effect on the development of the anti-nociceptive and reparative effects of MSCs.*

Key words: *mesenchymal stem cells, neuropathic pain, sciatic nerve, cannabinoid receptors, pharmacological blockade of CB_2 receptors*

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ФАРМАКОЛОГИЧЕСКАЯ БЛОКАДА КАННАБИНОИДНЫХ РЕЦЕПТОРОВ II ТИПА ПРИ ТРАНСПЛАНТАЦИИ МЕЗЕНХИМАЛЬНЫХ СТВОЛОВЫХ КЛЕТОК В МОДЕЛИ ПЕРИФЕРИЧЕСКОЙ НЕЙРОПАТИЧЕСКОЙ БОЛИ

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РЕЗЮМЕ

Цель исследования. *Оценить антиноцицептивный и репаративный эффекты мезенхимальных стволовых клеток жировой ткани (МСК ЖТ) на фоне фармакологической блокады каннабиноидных рецепторов CB_2 в модели периферической нейропатической боли.*

Материал и методы. *У 40 крыс-самцов Wistar осуществили моделирование периферической нейропатии (НП) путём иссечения участка седалищного нерва. На 7-е сутки исследования проведена трансплантация МСК ЖТ (1×10^6 клеток/кг) в область повреждения седалищного нерва без дополнительных воздействий, а также после локального введения антагониста CB_2-рецептора AM630 и предварительной инкубации с AM630. В течение 90 суток регистрировали ноцицептивную чувствительность и анализировали походку крыс с помощью CatWalk XT (Noldus, Нидерланды). На 21-е и 90-е сутки проведена оценка гистоструктуры дистального сегмента седалищного нерва после аксотомии.*

Результаты. *Фармакологическая блокада CB_2-рецепторов как на мембранах МСК ЖТ, так и в мягких тканях, окружающих место повреждения седалищного нерва, приводила к снижению порога и латентного периода ноцицептивной реакции с 28-х суток исследования по сравнению с группой крыс с НП и группой животных после трансплантации только МСК ЖТ. После локального введения AM630 перед трансплантацией МСК ЖТ отмечены ухудшение параметров походки, вызванные НП, и увеличение доли повреждённых нервных волокон в дистальном сегменте седалищного нерва. Трансплантация МСК, преинкубированных с AM630, не оказывала существенного влияния на скорость восстановления параметров походки, к 90-м суткам исследования сопровождалась снижением числа повреждённых нервных волокон.*

Заключение. *Блокада CB_2-рецепторов как на мембранах МСК, так в зоне повреждения периферического нерва сопровождается снижением антиноцицептивного эффекта МСК при их локальной трансплантации и подавляет репаративное действие МСК.*

Ключевые слова: *мезенхимальные стволовые клетки, нейропатическая боль, седалищный нерв, каннабиноидные рецепторы, фармакологическая блокада CB_2-рецепторов*

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INTRODUCTION

Chronic pain syndromes of neurogenic origin, associated with damage or dysfunction of peripheral parts of the somatosensory nervous system [1], occur in 7–20 % of the European population [2] and in 30–50 % of the world population [3]. Cell therapy using adipose-derived mesenchymal stem cells (ADMSC) appears promising for peripheral nerve injuries and related pain syndromes [4, 5]; their antinociceptive effect with local transplantation has been evidenced in models of peripheral neuropathy of various etiologies [6–11]. The ability of MSC to alleviate neuropathic pain is currently associated mainly with the suppression of local inflammatory response by secretion of a number of paracrine factors [5], but the mechanisms of activation of these processes are not fully disclosed.

Cannabinoid CB_2 receptors are involved in modulating the transduction, transmission and processing of nociceptive signals at both the peripheral and central levels of the somatosensory nervous system [12] and serve as a target for pain management. MSCs are able to produce CB_2 receptor ligands [13], which may be one of the mechanisms of their analgesic action. Conversely, CB_2 receptors are present in MSCs and are involved in sustaining their viability and metabolic activity [13–16], indicating a possible interaction between MSCs and endogenous cannabinoids in the transplantation area. It is essential to study the changes of MSC effects under the conditions of blockade of these receptors in order to understand the role of CB_2-receptors in analgesic and reparative action of MSC at local injection into the area of peripheral nerve injury.

THE AIM

Assessment of antinociceptive and reparative effects of adipose-derived mesenchymal stem cells against pharmacological blockade of cannabinoid CB_2 receptors in a model of peripheral neuropathic pain.

MATERIALS AND METHODS

The study was conducted on 40 male Wistar rats weighing 180–200 g. The animals were kept in the vivarium of the Institute of Physiology, National Academy of Sciences of Belarus with free access to water and food and 12/12 h day/night cycle. Animals were divided into 4 groups by simple randomization method (using random number table), in each group $n = 10$:

1) rats with peripheral neuropathy (NP) model without treatment (NP group);

2) rats with NP model and transplantation of allogeneic ADMSC to the area of sciatic nerve injury (NP + ADMSC group);

3) rats with NP model and ADMSC transplantation under pharmacological blockade of CB_2 receptors in soft tissues of the sciatic nerve injury area (NP + AM630 + ADMSC group);

4) rats with NP model and ADMSC transplantation, which underwent pharmacological blockade of CB_2 receptors when pre-incubated with selective antagonist (NP + pre-AM630-ADMSC group).

All manipulations involving experimental animals were undertaken in compliance with the principles of bioethics as set out in the European Convention for the Protection of Vertebrates Animals used for Experimental and other Scientific Purposes. The study minutes were approved by the Bioethics Commission of the Institute of Physiology, National Academy of Sciences of Belarus (Minutes No. 1 dated February 02, 2023).

Surgical manipulations. NP was modelled on the left hind limb of rats by axotomy of a 0.5 cm section of the sciatic nerve according to the previously described method [8]. The surgery was performed under general anaesthesia induced by intravenous injection of sodium thiopental (JSC 'Sintez', Russia) at a dose of 20 mg/kg with local anaesthesia (lidocaine hydrochloride (JSC 'Borisov Medical Preparations Plant', Republic of Belarus), 0.1 ml intramuscularly).

ADMSC transplantation. On day 7 after NP modelling, experimental groups were injected with ADMSCs in the amount of 1×10^6 cells/kg. ADMSCs isolated from visceral fat of intact rats were cultured in a CO_2 incubator (37 °C; 5% CO_2) until the 3rd passage according to the previously described method [17]. Using flow cytofluorimeter FACSCanto II (Becton Dickinson, USA) the phenotype of ADMSC was analysed by the presence of characteristic markers CD29, CD44 and CD90 and absence of hematopoietic marker CD45. Cell suspension in phosphate-buffered saline (PBS, phosphate-buffered saline) buffer (pH = 7.2; Sigma-Aldrich, Germany) was injected intramuscularly with an insulin syringe with an integrated 30 G needle in four injections around the area of surgical excision of the nerve site according to an imaginary dial pattern at 3, 6, 9, and 12 hours.

CB_2 receptor pharmacological blockade. To achieve blockade of CB_2 receptors on ADMSC's membranes, cells were incubated with the selective antagonist AM630 (Sigma-Aldrich, Germany) for 24 h (2 μM). Pharmacological blockade of CB_2-receptors in soft tissues of the sciatic nerve injury area was performed by intramuscular injection of AM630 (100 μg/kg) 15 min before ADMSC transplantation. The AM630 antagonist was diluted in a solvent consisting of sterile PBS buffer (pH = 7.4; Sigma-Aldrich, Germany) and 0.2 % dimethyl sulfoxide (NeoFroxx GmbH, Germany).

An assessment of nociceptive sensitivity. The Randall – Selitto algesimeter (Panlab, Spain) was used to determine nociceptive sensitivity to a mechanical stimulus (mechanical withdrawal threshold (MWT)), and Hot Plate' algesimeter (Panlab, Spain) was used to determine nociceptive sensitivity to a thermal stimulus (thermal withdrawal latency (TWL)) [18]. Measurements were performed three times with an interval of 5–7 min. Noci-

ceptive sensitivity was assessed on days 0, 7, 14, 21, 28, 60 and 90 of the study.

Analysis of gait parameters. Detailed analysis of gait was performed using the software and hardware complex CatWalk XT 10.6 (Noldus, Netherlands). The system enables qualitative and quantitative assessment of gait parameters during the animal's free movement on a glass platform. The intensity of green light at the point of contact between the paws and the surface of the glass podium illuminated with green LED lighting was recorded with a high-speed video camera with a wide-angle lens Gevicam GP-3360 (GEViCAM Inc., USA) located under the corridor. Paw prints were analysed and gait parameters were calculated using the software of this hardware-software complex. Prior to the study, the animals were adapted to the device and run recording conditions. Runs of each animal were recorded in a dark, ventilated room 3 times each with a maximum step variability of 60 % and a run time of no more than 5.00 s. The analysis included static and dynamic parameters, which reflect the degree of tonic pain sensations in this model and also indirectly demonstrate the functional state of the sciatic nerve. Dynamic parameters included:

Stand Time – duration of the paw stand phase on the ground;

Swing Time – duration of the paw swing phase in the air;

Duty Cycle – working cycle of the paw, the ratio of the duration of the paw stand phase to the duration of the full step cycle.

Static gait parameters:

Print Length – the length of the print;

Print Width – the width of the print;

Print Area – the area of the print;

Max contact area – the area of the print at the most intensive contact of the paw with the platform;

Max intensity – the maximum intensity of the paw print;

Mean intensity – the average intensity of the paw print;

Sciatic functional index (SFI).

These parameters were selected based on previous studies [6–7, 19]. To exclude the effects of run speed and body weight of animals influencing gait parameters, data were calculated as a percentage of the contralateral hind paw, except for SFI.

Assessment of the sciatic nerve histostructure. On days 21 and 90 of the study, a distal segment of the sciatic nerve was sampled for histological examination. The samples were fixed in 10 % neutral buffered formalin. Sections 5 μm thick were stained with hematoxylin and eosin according to standard techniques. The obtained preparations were viewed and digitized using an Optec BK 5000 light microscope with a digital camera (Optec, China) at a magnification of × 400. The percentage of normal and damaged nerve fibres was counted on transverse sections of the sciatic nerve [20]. Morphometric parameters were assessed in the field of view of a microscope at × 400 magnification (area 67072.0 μm^2) in at least five fields of view.

Statistics. Statistical processing of data was performed using Statistica 10 software (StatSoft Inc., USA). Data were checked for normality of distribution by the Shapiro – Wilk test. In normal characteristic distribution, results are presented as mean ± standard deviation (M ± SD); in an abnormal distribution, results are presented as median and quartiles (Me (Q25; Q75)). Differences in nociceptive sensitivity and gait parameters were assessed by repeated measures analysis of variance with posterior comparisons using the least significant difference method. Morphometric data were compared by the Kruskal – Wallis criterion followed by a posteriori comparisons. The conclusion about statistical significance of differences was made at $p < 0.05$.

RESULTS

Alterations in nociceptive sensitivity. On day 7 after NP modelling, the development of mechanical and thermal hyperalgesia was observed. This was evidenced by a 35.5 % decrease in ipsilateral limb MWT (from 136.0 ± 1.9 to 87.7 ± 2.0 g) and 34.3 % decrease in TWL (from 18.1 ± 0.6 to 11.9 ± 0.4 s) relative to baseline values ($p < 0.001$) (Fig. 1a, b). There was no tendency for MWT and TWL to recover to their original values over the course of the study. The MWT of the contralateral healthy limb did not change statistically significantly throughout the study ($p > 0.05$ compared to day 0; Fig. 1a).

A single intramuscular injection of ADMSC into the area of sciatic nerve injury resulted in a 32.3 % increase in the MWT of the ipsilateral extremity by day 14 (from 85.4 ± 2.0 to 113.0 ± 1.9 g; $p < 0.001$ by day 7; Fig. 1a), TWL by 17.1 % (from 11.7 ± 0.5 to 13.7 ± 0.5 s; $p < 0.001$ by day 7; Fig. 1b). By day 21 of the study, the MWT of the ipsilateral limb had already increased to 129.4 ± 2.0 g ($p < 0.001$ by day 7; $p > 0.05$ by day 0; Fig. 1a) and the TWL to 16.2 ± 0.5 s (38.5 % higher; $p < 0.001$ by day 7; $p > 0.05$ by day 0; Fig. 1b). Further up to and including day 90, MWT and TWL were not statistically significantly different from baseline values ($p > 0.05$; Fig. 1a, b). Administration of ADMSC to rats with NP also statistically significantly increased their MWT and MWT when compared to untreated animals already on day 14 day of the study: MWT was higher by 26.2 % ($p < 0.001$), and TWL – by 20.2 % ($p < 0.001$). From day 21 onwards, the increase in MWT relative to the untreated NP group was 53.5 % ($p < 0.001$), TWL was 54.3 % ($p < 0.001$).

Antagonist AM630 administration at a dose of 100 μg/kg to the area of nerve injury in rats with NP on day 7 of the study did not lead to statistically significant changes in MWT of the ipsilateral extremity, as well as TWL after 15 min (Table 1).

ADMSC injection 15 min after pharmacological blockade of CB_2 receptors in the area of sciatic nerve injury resulted in a 21.9 % increase in MWT on day 14 of the experiment relative to the values on day 7

(from 94.5 ± 2.1 to 115.2 ± 2.6 g; $p < 0.001$). And these values were not statistically significantly different from the NP + ADMSC group ($p > 0.05$), but were 18.7 % higher than in the NP group without treatment ($p < 0.001$). By day 21, MWT tended to decrease compared to the NP + ADMSC group and tended to increase by 33.3 % compared to the NP group without treatment ($p < 0.001$). On day 28, MWT increased to 120.4 ± 4.1 g, which was 9.3 % lower than the values of the NP + ADMSC group ($p < 0.001$) and 33.2 % higher than those of untreated rats ($p < 0.001$; Fig. 1a). Thereafter, a decreasing trend in MWT was observed (Fig. 1a). An increase in TWL by day 14 of the study by 21.5 % was observed in relation to the values obtained on day 7: from 10.7 ± 0.3 to 13.0 ± 0.3 s ($p < 0.001$; no statistically significant differences with the NP + ADMSC group ($p > 0.05$); 14.0 % higher in comparison with the PN group without treatment ($p < 0.05$) (Fig. 1b). By day 28, TWL increased to 13.1 ± 0.2 s, and the index was 22.0 % lower than in the NP + ADMSC group ($p < 0.001$). By day 90 of the study, we observed a marked decrease in TWL to 11.5 ± 0.3 s ($p > 0.05$ compared to NP without treatment; $p < 0.001$ compared to NP + WL MSCs; Fig. 1b).

ADMSC injection preincubated with AM630 increased the MWT of the ipsilateral extremity on day 14 of the study by 18.3 % relative to the values obtained on day 7 (from 95.9 ± 1.4 to 113.4 ± 2.0 g; $p < 0.001$; Fig. 1a) and TWL by 13.3 % relative to the values obtained on day 7 (from 10.5 ± 0.4 to 11.9 ± 0.3 s; $p < 0.001$). Meanwhile, MWT was not significantly different from the values in the NP + ADMSC group ($p > 0.05$) and in the NP + AM630 + ADMSC group

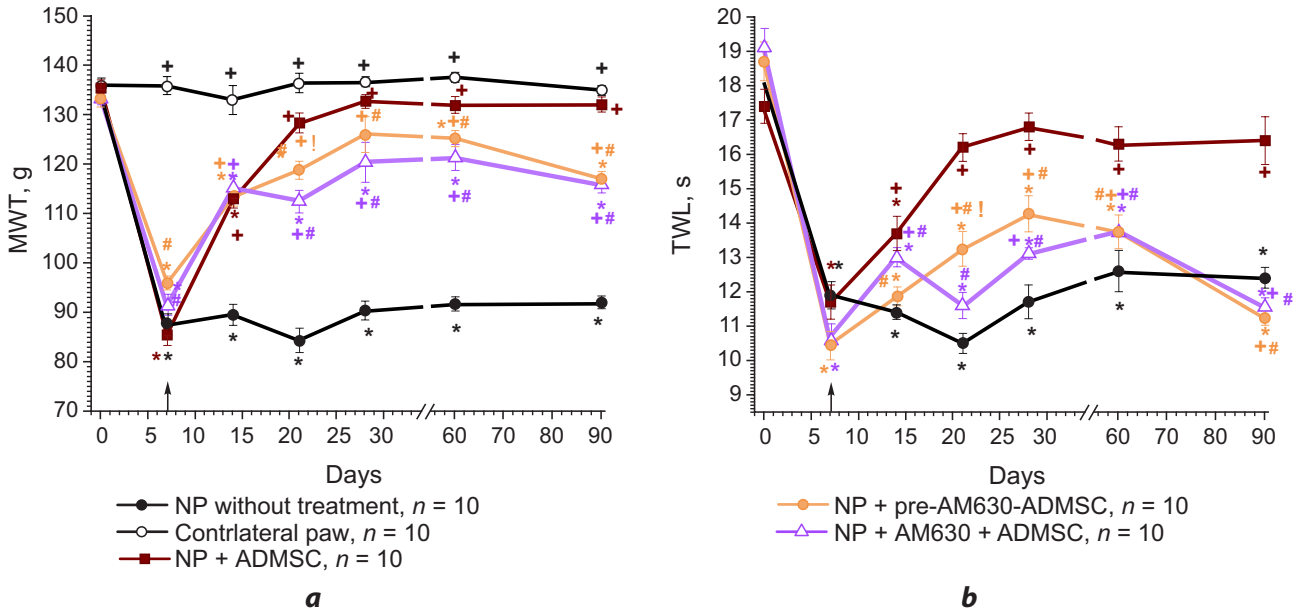


FIG. 1.

*Dynamics of nociceptive sensitivity to mechanical (**a**) and thermal (**b**) stimuli in rats with NP, ADMSC transplantation on the background of pharmacological blockade of CB_2-receptors by AM630 antagonist: arrow indicates the time of ADMSC transplantation; * – $p < 0.05$ compared to day 0; + – $p < 0.05$ compared to NP without treatment; # – $p < 0.05$ compared to NP + ADMSC; ! – $p < 0.05$ compared to NP + AM630 + ADMSC*

TABLE 1

NOCICEPTIVE SENSITIVITY PARAMETERS IN RATS WITH PN AFTER ADMINISTRATION OF AM630 ANTAGONIST TO THE SITE OF SCIATIC NERVE INJURY

Parameters	MWT (left paw), g	MWT (right paw), g	TWL, s
Day 0	133.2 ± 1.3	132.5 ± 1.0	19.1 ± 0.6
Day 7	91.2 ± 1.8*	133.9 ± 0.9	10.6 ± 0.3*
15 min after AM630 injection	94.5 ± 2.1*	131.6 ± 1.3	10.7 ± 0.3*

Note. * – $p < 0.05$ compared to day 0.

($p > 0.05$), whilst TWL was 13.1 % lower than the values of the NP + ADMSC group and was not statistically significantly different from the group with NP without treatment ($p > 0.05$). By day 21, both parameters in this group were lower than those in the rats that had only received ADMSC by 8.3 % ($p < 0.05$) and 18.5 % ($p < 0.001$), respectively, but higher than those in the NP + AM630 + ADMSC group by 5.6 % and 13.8 %, respectively ($p < 0.05$). By day 90, a decrease in MWT to 117.0 ± 1.5 g ($p < 0.001$ to NP group without treatment; $p < 0.001$ to NP + ADMSC group) and TWL to 11.3 ± 0.2 s ($p > 0.05$ to NP without treatment; $p < 0.001$ to PN + ADMSC) were observed.

By comparing both methods of pharmacological blockade of CB_2-receptors it was revealed that after transplantation of ADMSC preincubated with AM630 there was observed an increase of MWT relative to NP + AM630 + ADMSC group on day 21 of the study by 5,6 % ($p < 0.05$), and also a decrease of TWL on day 14 by 8.5 % ($p < 0.05$) with the subsequent increase on day 21 of the study by 13.8 % ($p < 0.02$). At later terms of the study no statistically significant differences in the studied indices between these groups were revealed. Overall, ADMSC transplantation following local injection of AM630 resulted in a more pronounced reduction in the antinociceptive action of ADMSC.

Dynamic gait parameters. In the untreated NP group, a reduction in the duration of the ipsilateral paw stand duration to 91.0 % ($p < 0.001$) and of the duty cycle to 95.4 % ($p < 0.001$) was observed from day 7 of the experiment, with no statistically significant differences in the swing duration ($p > 0.05$; Fig. 2c). By day 21 of the study, there was an adaptive recovery of the above parameters (Fig. 2a, b).

After ADMSC injection at a dose of 1×10^6 cells/kg into the area of sciatic nerve transection, the recovery of the stand duration (up to 98.4 %) and duty cycle (up to 101.9 %) was observed by day 14 (Fig. 2a, b),

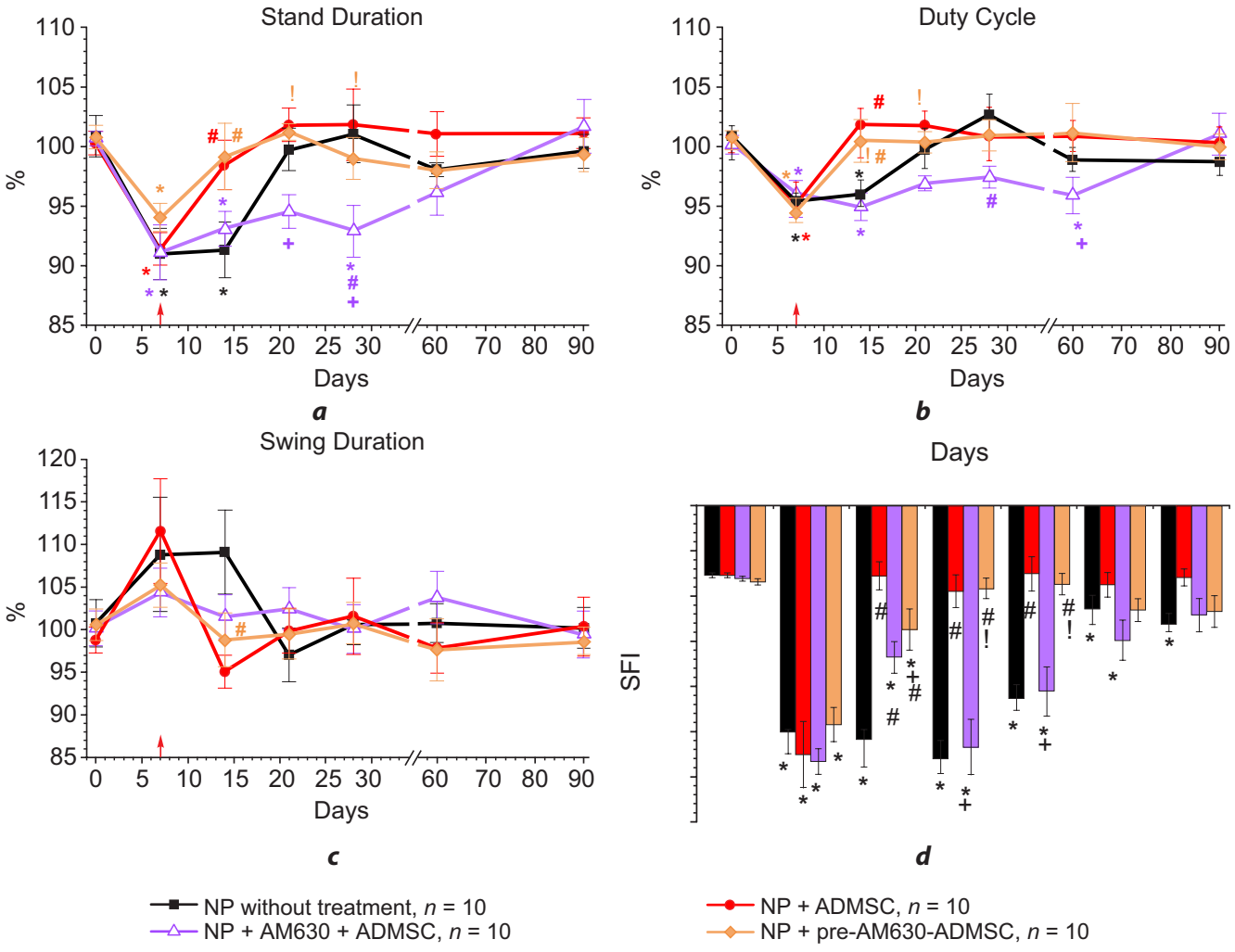


FIG. 2.

*Gait dynamic parameters (**a**–**c**) and functional sciatic index (**d**) changes in rats after NP modelling, ADMSC transplantation on the background of pharmacological blockade of CB_2 receptors by AM630 antagonist: arrow indicates the time of transplantation; * – $p < 0.05$ to the values before NP modelling; # – $p < 0.05$ to the NP group without treatment; + – $p < 0.05$ to NP + ADMSC; ! – $p < 0.05$ to the NP + AM630 + ADMSC group*

and no further changes were revealed. After ADMSC injection against the background of pharmacological blockade of CB_2-receptors in the area of sciatic nerve transection, the recovery of the stand duration was fixed by day 60 of the study (Fig. 2a), and of the duty cycle – by day 90 of the study (Fig. 2b). On day 28 of the experiment, there was a decrease in the stand duration of the ispilateral paw compared to the NP group without treatment (by 8.1 %; $p < 0.005$) and duty cycle (by 5.1 %; $p < 0.01$). Compared to the NP + ADMSC group, the stand duration of the ipsilateral paw was shorter on day 21 (7.1 %; $p < 0.05$) and day 28 (8.8 %; $p < 0.002$) of the study; the duty cycle was shorter on day 14 (6.8 %; $p < 0.005$) and day 60 (4.9 %; $p < 0.02$) of the study. Pre-AM630-MSC injection of ADMSCs resulted in restoration of the stand duration to 99.2 % by day 14 ($p > 0.05$ by day 0; $p < 0.05$ for the NP group without treatment) and of the duty cycle to 100.5 % ($p > 0.05$ by day 0; $p < 0.05$ for the NP group without treatment). No statistically significant differences in dynamic parameters from the NP + ADMSC group were revealed, and the pattern of changes in these gait parameters in these two groups was similar.

Sciatic functional index. This index in the untreated NP group decreased 2.3-fold, by 229.9 % (from –7.65 ± 0.30 to –24.99 ± 2.45; $p < 0.001$ compared to the data on day 0) by day 7 of the study (Fig. 2d). The tendency to recovery of this index was observed only from the day 60 of the study, but by day 90 of the study no recovery of SFI to the baseline level was observed ($p < 0.01$) (Fig. 2d). After ADMSC transplantation, recovery of SFI to –7.75 ± 1.57 was observed by day 14 of the study. Further, no statistically significant changes in SFI were observed throughout the study compared to the values on day 0. If AM630 was administered 15 min before ADMSC transplantation, a partial recovery of SFI was observed by day 14 of the study (to –16.75 ± 1.74; $p < 0.002$ by day 0; $p < 0.05$ to the untreated NP group). From day 21 of the study, the index decreased again to the level of the NP group without treatment (to –26.69 ± 3.05; $p < 0.001$ by day 0; $p > 0.05$ to the NP group without treatment). SFI in this group returned to baseline only by day 90 of the study (to 12.10 ± 1.85), but was not statistically significantly different from NP without treatment. Compared to the NP + ADMSC group, SFI in this group was statistically significantly decreased on day 14 ($p < 0.001$), day 21 ($p < 0.001$) and day 28 ($p < 0.001$) of the experiment. After transplantation of ADMSC preincubated with AM630, SFI was fully recovered by day 21 (to –9.16 ± 1.12; $p > 0.05$ by day 0; $p < 0.001$ to the NP group without treatment). Compared to the NP + ADMSC group, SFI in this group was lower on day 14 of the study (by 76.4 %; $p < 0.005$).

Static gait parameters. From day 28 onwards, the untreated NP group observed a decrease in the print length of the injured paw compared to day 0 to 93.6 % ($p < 0.02$; Fig. 3a), and in the print width to 96.1 % ($p < 0.001$; Fig. 3b). The print area of the ipsilateral extremity decreased to 84.5 % of the contralateral paw ($p < 0.001$; Fig. 3c), the max contact area decreased to 84.7 %, ($p < 0.005$; Fig. 3f), the max intensity decreased to 91.5 % ($p < 0.001$; Fig. 3d), and the mean intensity decreased to 98.3 % ($p < 0.01$; Fig. 3e). No tendency to recovery of static gait parameters in animals of this group was revealed up to day 90 of observation inclusive.

No statistically significant changes in static parameters were observed after ADMSC transplantation throughout the study. Relative to the NP group without treatment, an increase in print area and max contact area, as well as intensity parameters were observed on day 28 ($p < 0.005$) and day 60 ($p < 0.02$) day of the experiment. When ADMSCs were transplanted 15 min after AM630 injection from day 21 of the experiment, we observed a decrease in print area to 90.2 % of the contralateral paw ($p < 0.002$ by day 0; Fig. 3c). Compared to the NP group without treatment, there was a reduction in print width to 93.8 % ($p < 0.001$), max intensity to 102.5 % ($p < 0.001$), and mean intensity to 100.3 % ($p < 0.005$; Fig. 3b, d, e) on day 21 of the study. Relative to the NP + ADMSC group, print area was lower on day 21 ($p < 0.05$), day 28 ($p < 0.05$) and day 60 ($p < 0.005$) of the experiment; max contact area was lower on days 28 and 60 of the experiment ($p < 0.05$). No other gait parameters were different when compared with the NP + ADMSC group. No statistically significant changes in static gait parameters were observed after transplantation of pre-AM630-ADMSCs against the values before NP modelling, as well as against the group of NP + ADMSCs.

Changes in the histologic structure of the sciatic nerve. The content of normal and damaged nerve fibres of the distal segment of the sciatic nerve of the experimental groups was assessed by the state of the myelin sheath and the location of the axial cylinder in the nerve fibre. Normal nerve fibres were clearly differentiated axial cylinders around which a uniformly stained myelin sheath with clear boundaries was observed. Damaged nerve fibres were characterized by swelling, vacuolated degeneration of myelin sheath, blurring of nerve fibre boundaries, with the axial cylinder displaced to the periphery or undetectable on histological sections (Fig. 4).

The contents of normal and damaged nerve fibres in the distal segment of the sciatic nerve in the NP group without treatment on day 21 of the experiment were 13 [11.75; 14] % and 87 [86; 87.5] %, respectively (Table 2; Fig. 4c).

After ADMSC transplantation, the proportion of preserved nerve fibres was statistically significantly higher in the distal segment of the sciatic nerve on day 21 of the study compared to the NP group without treatment ($p = 0.002$; Fig. 4a).

After ADMSC injection against the background of pharmacological blockade of CB_2-receptors in the soft tissues of the sciatic nerve transection area on day 21 no statistically significant changes in the content of normal and damaged nerve fibres were observed in comparison with the NP group without treatment ($p = 0.326$), but their number was significantly lower in comparison with the NP + ADMSC group ($p = 0.004$).

By day 90 of the study, 74 [72; 74] % damaged nerve fibres and 13 [11; 14] % undamaged nerve fibres were ob-

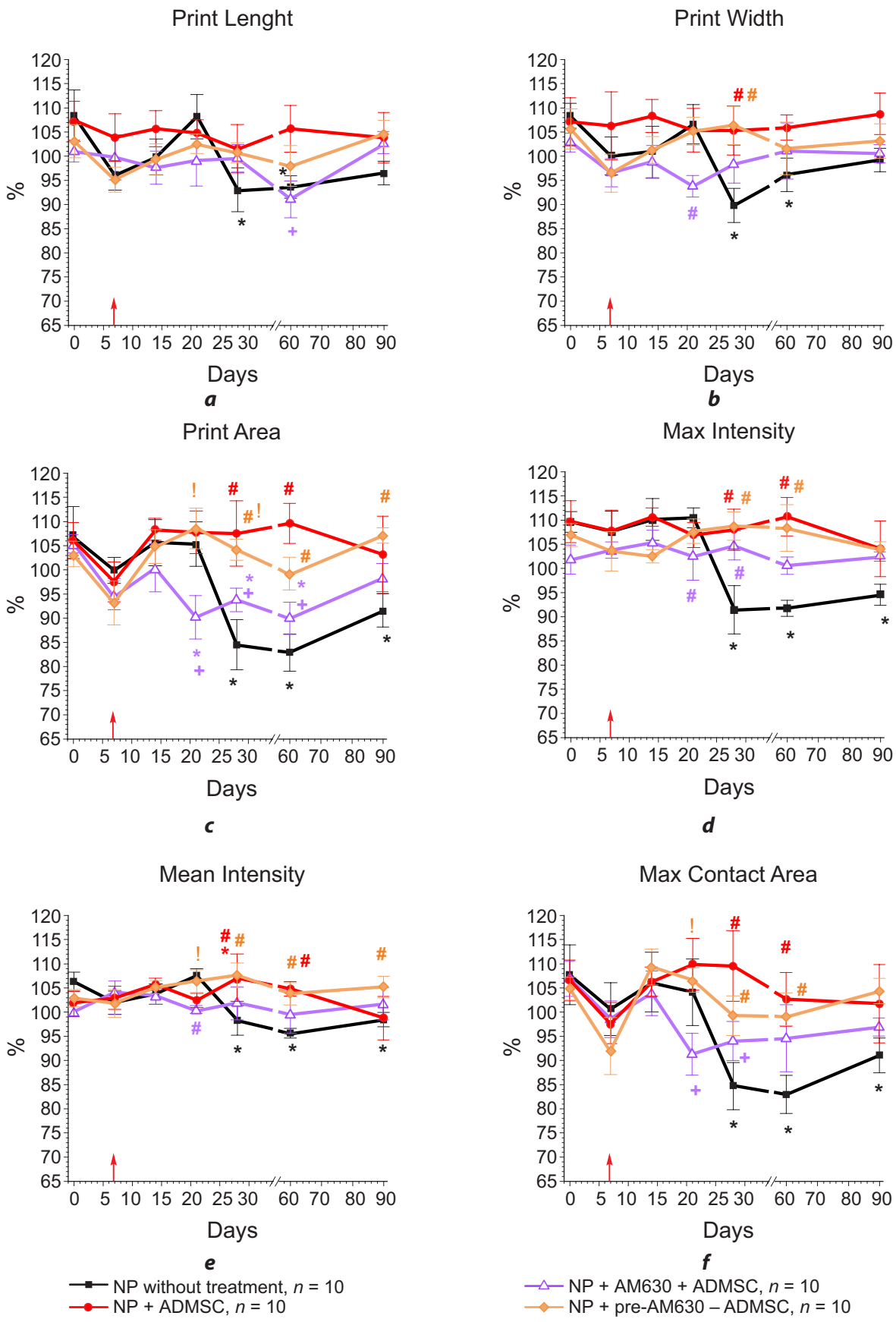
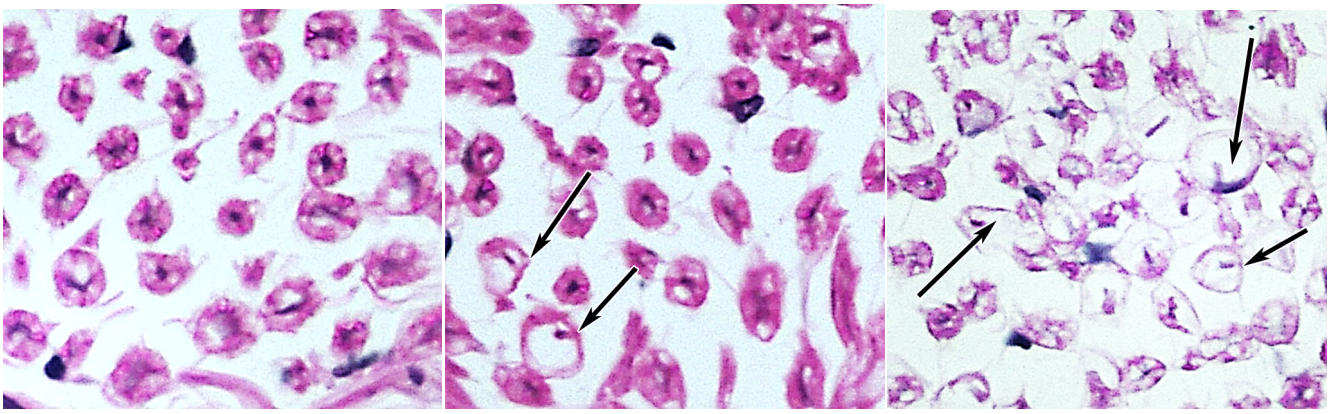


FIG. 3.

*Changes in static gait parameters: print length and print width (**a**, **b**), print area (**c**, **f**), as well as intensity parameters (**d**, **e**) – in rats after NP modelling, ADMSC transplantation on the background of pharmacological blockade of CB_2-receptors by AM630 antagonist. Arrow indicates transplantation time; * – p < 0.05 to values before NP modelling; # – p < 0.05 to NP group without treatment; + – p < 0.05 to NP + ADMSC; ! – p < 0.05 to the group of NP + AM630 + ADMSC*

served in NP without treatment (Table 2). After ADMSC transplantation, on day 90 of the study, the number of damaged nerve fibres was statistically significantly lower than that in NP without treatment ($p = 0.001$).

After ADMSC injection against the background of local administration of AM630 antagonist on day 90 a decrease in the proportion of damaged nerve fibres was observed in comparison with the NP group without treat-



a *b* *c*

FIG. 4.

Myelin nerve fibres of rat sciatic nerve: ***a*** *– preserved nerve fibres of the distal sciatic nerve fragment of an animal from the group after injection of ADMSCs, day 21;* ***b*** *– presence of dystrophically altered nerve fibres (arrows) in the distal sciatic nerve fragment of an animal from the group of NP + preAM630-ADMSCs, day 21;* ***c*** *– presence of dystrophically altered nerve fibres (arrows) in the distal sciatic nerve fragment of the group of NP animals without treatment. Hematoxylin and eosin staining, magnification ×400*

TABLE 2

STRUCTURE OF NORMAL AND DAMAGED NERVE FIBRES OF THE DISTAL SEGMENT OF THE SCIATIC NERVE OF THE EXPERIMENTAL GROUPS ON DAYS 21 AND 90 OF THE STUDY

Group	% of normal nerve fibers	% of damaged nerve fibers
	Day 21 of the study	
Untreated NPs	13 [11.75; 14]	87 [86; 87.5]
NP + ADMSCs	86 [84; 86]*	14 [13; 16]
NP + AM630 + ADMSCs	48 [48; 48]$^{\#}$	52 [51; 52]$^{\#}$
NP + pre-AM630-ADMSCs	55 [49; 55]	45 [38; 51]
	Day 90 of the study	
Untreated NPs	26 [25; 28]	74 [72; 74]
NP + ADMSCs	61 [61; 63]*	37 [37; 39]*
NP + AM630 + ADMSCs	37 [26; 37]$^{\#}$	63 [60; 74]$^{\#}$
NP + pre-AM630-ADMSCs	65 [56.5; 65]*;$^{!}$	35 [32; 43.5]*;$^{!}$

Note. * – $p < 0.05$ to NP without treatment; $^{\#}$ – $p < 0.05$ to NP + MSC; $^{!}$ – $p < 0.05$ between preincubation and local injection (Kruskal – Wallis criterion).

ment (p = 0.008) and an increase in comparison with the NP + ADMSC group (p = 0.036). Pre-AM630-MSC transplantation resulted in a decrease in the number of damaged nerve fibres and an increase in the number of normal nerve fibres by day 90 of the study compared to NP without treatment (p = 0.042) and was comparable to the NP + ADMSC group.

DISCUSSION

ADMSC transplantation is currently positioned as an effective method to reduce the progression of pain syndrome of inflammatory [21] and neurogenic origin [6–11]. ADMSC effectiveness in suppressing nocicetptive sensitivity disorders, such as mechanical and thermal hyperlagia and allodynia has been demonstrated in experimental models of chronic ligation of the sciatic nerve [8] and infraorbital nerve [9] in rodents, as well as in models of partial [8, 10] and complete traumatic nerve injury [6, 7], a model of streptozotocin-induced diabetic polyneuropathy [11]. However, the mechanisms of ADMSC realised effects are still being studied. The endocannabinoid system is known to be involved in the inhibition of nociceptive signal transduction and transmission in both peripheral tissues and the central nervous system, and modulation of its components serves as one of the targets of pain relief in neuropathic pain. Conversely, ADMSCs express components of the endocannabinoid system, in particular, CB_1 and CB_2 receptors [14–16], the activation of which increases their secretion of such factors as vascular endothelial growth factor, transforming growth factor-beta and hepatocyte growth factor [16]. Moreover, activation of the CB_2 receptor on MSC leads to decreased production of interleukin (IL) 6, IL-8 and tumour necrosis factor α, as well as increased IL-10 [13, 15, 22]. From the evidence available in the literature, the authors suggested that CB_2 receptors mediate immunomodulatory and antinociceptive effects of ADMSCs.

The data obtained in this study indicate that pharmacological blockade of CB_2-receptors both on the membranes of MSCs and in the soft tissues surrounding the site of sciatic nerve injury, reduces the antinociceptive effect of ADMSCs at their transplantation into the site of sciatic nerve transection in rats, which was revealed by the lack of MWT and TWL recovery in the respective groups of animals, as well as worsening of mechanical and thermal hyperalgesia at the later stages of the study (Fig. 1a, b). Blockade of CB_2-receptors on MSCs significantly slowed down SFI recovery (Fig. 2d), and their deactivation in tissues in the area of sciatic nerve transection completely abolished the SFI restoring effect of MSCs (Fig. 2d). The abovementioned facts in aggregate allow to assume at least weakening of antinociceptive properties of ADMSC in response to blockade of the indicated receptor.

The histological study of the sciatic nerve distal segment revealed the protective effect of MSC, which was manifested in weakening of degenerative changes of nerve fibres. Both methods of CB_2 receptor blockade abolished the mentioned protective effect of ADMSC against the injured nerve fibres. The content of the latter on day 21 of the experiment did not differ in these groups from that in untreated animals (Table 2). In addition, at late study periods (90 days), CB_2-receptor blockade in ipsilateral soft tissues was accompanied by a higher proportion of injured nerve fibres than in the NP + pre-AM630-MSC group (Table 2). In summary, the results of this study indicate the involvement of CB_2 receptors in the mechanisms of the ADMSCs protective effects. In this case CB_2-receptor blockade applied to peripheral nerve fibres has a greater effect on the MSC reparative potential, and pharmacological inactivation of these receptors on stem cells themselves – to their antinociceptive action.

CONCLUSION

It has been experimentally confirmed that pharmacological blockade of CB_2-receptors both on ADMSC membranes and in the peripheral nerve injury area decreases antinociceptive and reparative effect of ADMSC at their local transplantation. This indicates the direct participation of these receptors in the protective effects realised by MSCs. Further study of the CB_2-receptor stimulation effect will allow to estimate the degree to which the antinociceptive and reparative effects of ADMSCs are enhanced when they have been transplanted into the site of peripheral nerve injury.

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Conflict of interest

The authors declare no conflict of interest.

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Anna-Maria V. Yerofeyeva – conducting a study, analysing data, writing a text.
Sergei V. Pinchuk – conducting a study.
Svetlana N. Rjabceva – supervision of the R&D work, text editing.
Alla Yu. Molchanova – study concept, approval of the final version of the study manuscript.