



Temporal dynamics of hippocampal morphological changes in cisplatin-induced neurotoxicity and prophylactic and therapeutic effects of pioglitazone

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Article info

Received 06.01.2026

Received in revised form

09.02.2026

Accepted 24.02.2026

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Kulynych, H. B., Herashchenko, S. B., Polyvkan, M. I., Oliinyk, R. P., Fedorak, V. M., & Mykhailiuk, I. O. (2026). Temporal dynamics of hippocampal morphological changes in cisplatin-induced neurotoxicity and prophylactic and therapeutic effects of pioglitazone. *Regulatory Mechanisms in Biosystems*, 17(2), e26028. doi:10.15421/0226028

Cisplatin-induced neurotoxicity is widely investigated using experimental animal models to elucidate structural alterations of the central nervous system; however, temporal morphological changes in specific brain regions and the effectiveness of pharmacological correction strategies remain incompletely understood. The hippocampus represents one of the most vulnerable targets of chemotherapy-induced injury due to its high metabolic activity, pronounced synaptic plasticity, and critical dependence on the integrity of neurogliovascular complexes. The present study aimed to evaluate the temporal dynamics of hippocampal morphological and morphometric alterations under cisplatin-induced neurotoxicity and to compare the neuroprotective efficacy of pioglitazone administered in prophylactic and therapeutic regimens. The experiment was conducted on 120 sexually mature male white inbred rats. Cisplatin was administered intraperitoneally at a dose of 2 mg/kg once weekly for six weeks to induce chronic cumulative neurotoxicity. Pioglitazone was administered intragastrically either prophylactically (from day -5 to day +14 relative to the start of cisplatin treatment) or therapeutically (starting from day 3 after completion of the cisplatin course for 14 days). Morphological and morphometric analyses of the hippocampus (CA1 and CA3 regions) were performed on days 14, 28, 60, 90, and 120 using light microscopy and digital morphometry, including assessment of neuronal perikaryon area, nuclear diameter, nuclear-to-cytoplasmic ratio, and neuronal density. Results demonstrated that cisplatin induced a progressive cascade of hippocampal damage with maximal structural disorganization of the pyramidal layer on day 28, whereas prophylactic pioglitazone administration significantly preserved hippocampal cytoarchitecture and normalized key morphometric parameters more effectively than therapeutic treatment. At later observation periods, prophylactic pioglitazone ensured near-complete restoration of hippocampal structural organization, while therapeutic administration promoted partial stabilization without full morphological recovery. These findings indicate a clear temporal and mechanistic advantage of early PPAR γ -targeted intervention in limiting cisplatin-induced hippocampal injury.

Keywords: cisplatin; pioglitazone; hippocampus; neurotoxicity; morphology; PPAR γ ; prophylactic neuroprotection; morphometry.

Introduction

Cisplatin is currently used as one of the cornerstone cytostatic agents in the treatment of solid malignant tumors; however, its clinical application is substantially limited by the development of neurological complications. Historically, most attention has been focused on cisplatin-induced peripheral neuropathy, yet over the last decade convincing evidence has accumulated indicating that cisplatin also induces structural and functional disturbances within the central nervous system, which clinically manifest as cognitive and emotional disorders (Christie et al., 2012; Dietrich et al., 2015; Ongnok et al., 2020). Additional experimental and clinical data further support the involvement of central nervous system structures in chemotherapy-related neurotoxicity (Squillace et al., 2022; Was et al., 2022; Alotayk et al., 2023).

Among brain structures, the hippocampus is considered one of the most vulnerable targets of chemotherapy-induced injury. This vulnerability is related to its high metabolic activity, pronounced synaptic plasticity, extensive afferent and efferent projections, intense neurotransmitter turnover, and critical dependence of function on the integrity of neuroglio-vascular complexes (Dietrich et al., 2015; Kang et al., 2018; Barbosa-Azevedo et al., 2024). Structural susceptibility of the hippocampus to cytostatic exposure has been further confirmed in experimental studies (Csik et al., 2025). It has been demonstrated that cisplatin suppresses neurogenesis, reduces cell proliferation in the dentate gyrus, and induces degenerative changes in pyramidal neurons of the CA1 and CA3 regions (Manohar et al., 2014; Domouky et al., 2022; Altunkaya et al., 2025). Similar hippocampal alterations have been reported in independent experimental models (Oliveros et al., 2022; Qutifan et al., 2024). Experimental models employing chronic and combined administration of platinum-based cytostatics have been shown to reproduce stable neurotoxic phenotypes, inclu-

ding structural damage of nervous tissue, and are widely used to investigate mechanisms of chemotherapy-induced neurotoxicity (Carozzi et al., 2009).

The pathogenesis of cisplatin-induced central neurotoxicity is multifactorial. Key mechanisms include DNA damage, mitochondrial dysfunction, and induction of oxidative stress, which collectively trigger caspase-dependent apoptotic pathways (Lomelí-Cardona et al., 2017; Ren et al., 2017; Magdy et al., 2024). Additional evidence indicates that these processes are accompanied by long-term metabolic disturbances in neural tissue (Was et al., 2022). In parallel, activation of neuroinflammatory cascades occurs, characterized by increased expression of pro-inflammatory cytokines, dysregulation of glutamatergic neurotransmission, and impairment of neuron-glia interactions (Ren et al., 2017; Wellenberg et al., 2021; Alhowail, 2025). The role of glial and microvascular components in sustaining neurotoxicity has also been emphasized (Csik et al., 2025). The convergence of these processes results in persistent hippocampal morphological alterations that may remain long after completion of chemotherapy (Kumar et al., 2009; Domouky et al., 2022; Csik et al., 2025). Comparable delayed neurotoxic effects have been described in chronic exposure models (Umfreß et al., 2021).

Considering the inertia and partial irreversibility of central nervous system injury, together with potential dysfunction of the hippocampus and interconnected subcortical structures, there is a growing need to develop more effective pathogenetically grounded strategies for the prevention and pharmacological correction of chemotherapy-induced neurotoxicity. Current approaches increasingly emphasize suppression of neuroinflammation and modulation of metabolic mechanisms rather than purely symptomatic treatment (Ongnok et al., 2020; Was et al., 2022). In this context, the PPAR γ receptor agonist pioglitazone has attracted considerable attention due to its ability to at-

tenuate neuroinflammation, enhance antioxidant defense, and restore mitochondrial-dependent energy metabolism in nervous tissue.

In preclinical models, pioglitazone has demonstrated pronounced neuroprotective properties, including reduction of microglial activation, preservation of neuronal structure, and improvement of cognitive performance (Heneka et al., 2005; Xing et al., 2007; Alsaud et al., 2023). Moreover, PPAR γ activation is associated with stimulation of neural stem cell proliferation and maintenance of neuroplastic processes, which are critical for hippocampal recovery following toxic injury (Morales-García et al., 2011; Acharya et al., 2015). Nevertheless, the morphological efficacy of pioglitazone specifically under conditions of cisplatin-induced hippocampal injury, as well as differences between prophylactic and therapeutic administration regimens, remain insufficiently elucidated.

Therefore, a detailed morphological analysis of intrahippocampal changes under cisplatin-induced neurotoxicity and their pharmacological modulation by pioglitazone represents a relevant and timely research objective.

The aim of the study was to evaluate morphological changes in the rat hippocampus under cisplatin-induced neurotoxicity and to determine the neuroprotective effect of pioglitazone under prophylactic and therapeutic administration in temporal dynamics.

Materials and methods

The study was performed on 120 sexually mature male white inbred rats weighing 180–220 g, housed under standard vivarium conditions at Ivano-Frankivsk National Medical University (temperature 21 ± 2 °C, relative humidity 55–60%, 12/12 h light/dark cycle) with free access to food and water. Prior to the experiment, all animals underwent a 7-day acclimatization period. Euthanasia was performed by inhalational overdose of ether anesthesia. Housing and all experimental procedures involving laboratory animals were conducted in accordance with bioethical principles and current legislation, namely the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 2005), the Law of Ukraine “On the Protection of Animals from Cruel Treatment” (2006, Article 26), and the General Ethical Principles of Experiments on Animals adopted by the VI National Congress on Bioethics of Ukraine (Kyiv, 2019). The study protocol was approved by the Bioethics Committee of Ivano-Frankivsk National Medical University (Protocol No. 146/24, dated 26 September 2024). All experimental animals were alive until the completion of the scheduled observation periods. The conducted experimental procedures complied with internationally accepted ethical standards for laboratory animal research.

The experimental work was conducted from April to October 2025. The experimental phase started on April 2, 2025, and was completed after reaching the maximum observation period of 120 days.

Animals were randomly assigned to four experimental groups, with 30 animals in each group:

- 1) intact control – animals without administration of any drugs;
- 2) cisplatin – intraperitoneal administration of cisplatin at a dose of 2 mg/kg once weekly for six weeks (cumulative dose 12 mg/kg);
- 3) cisplatin + pioglitazone (prophylactic) – intragastric administration of pioglitazone at a dose of 20 mg/kg from day –5 to day +14 relative to the start of cisplatin administration;
- 4) cisplatin + pioglitazone (therapeutic) – intragastric administration of pioglitazone at a dose of 20 mg/kg starting from day 3 after completion of the cisplatin course and continuing for 14 days.

The cisplatin dose of 2 mg/kg with repeated administration was selected to ensure the development of chronic cumulative exposure required to reproduce stable central neurotoxicity without inducing lethal complications or severe systemic toxicity. This regimen is widely used in preclinical models and allows reproduction of morphological and functional hippocampal alterations characteristic of clinical chemotherapy. Dose conversion was performed according to interspecies scaling principles using Km coefficients, taking into account preclinical models of cisplatin-induced central neurotoxicity. These principles are widely applied in preclinical research to ensure translational rele-

vance of experimental dosing regimens based on body surface area normalization (Reagan-Shaw et al., 2008). The selected cisplatin regimen corresponds to clinically relevant cumulative exposure when adjusted for interspecies differences in body surface area and allows modeling of delayed and persistent central neurotoxicity.

The selection of pioglitazone and its administration regimens was based on evidence of the neuroprotective properties of PPAR γ agonists. The use of both prophylactic and therapeutic approaches allowed assessment of the potential to prevent toxic injury as well as the effectiveness of correction of already established morphological changes. Pioglitazone was administered intragastrically using a metal gastric gavage needle with a rounded atraumatic tip, connected to a syringe of appropriate volume. Administration was performed slowly, at the same time of day, with the dose adjusted according to individual body weight.

A tablet formulation of pioglitazone was used for preparation of the suspension. Tablets were crushed into a homogeneous powder immediately before use and suspended in distilled water to obtain a stable suspension. The pioglitazone concentration was calculated individually for each animal based on body weight, and the administered volume did not exceed 1 mL per 100 g of body weight. The suspension was thoroughly mixed before each administration to ensure uniform distribution of the active substance and reproducibility of dosing. This approach is widely applied in preclinical studies and does not affect the bioavailability of the active compound.

Animals were withdrawn from the experiment on days 1, 7, 14, 28, 60, 90, and 120 from the onset of cisplatin-induced neurotoxicity modeling.

In the prophylactic and therapeutic pioglitazone groups, morphological examinations were performed on days 14, 28, 60, 90, and 120 of the experiment.

After euthanasia, the hippocampus was dissected and fixed in 10% neutral buffered formalin, followed by standard paraffin processing. Serial sections up to 2 μ m thick were obtained using a rotary microtome (HS 2205 Zeedo, China). Paraffin sections were stained with hematoxylin and eosin, mounted under coverslips, and examined by light microscopy.

Morphological and morphometric changes in the hippocampus (CA1 and CA3 regions) were assessed by light microscopy using digital morphometry. Morphometric evaluation in CA1 and CA3 included measurement of perikaryon area, nuclear diameter, nuclear-to-cytoplasmic ratio, and neuronal density per field of view. For each animal, at least 10 fields of view from three non-consecutive sections were analyzed. Measurements were performed digitally using ImageJ software.

For functional assessment of hippocampal status, conditioned reflex tests were employed to evaluate learning and memory processes. To allow direct comparison of functional and structural alterations, behavioral testing and morphological analysis of the hippocampus were performed at identical experimental time points.

The passive avoidance test was used to assess retention and retrieval of contextual memory. The method is based on the formation of a conditioned avoidance reflex using negative reinforcement. The primary outcome measure was the latency to enter the dark compartment during the memory retention test.

The conditioned food reflex test was applied to evaluate the ability to form and retain associative memory. The time required for conditioned reflex acquisition and the stability of its reproduction were recorded at predefined observation periods.

Data are presented as mean \pm standard deviation ($x \pm SD$). To assess the effects of two independent factors (group and observation time) and their interaction, two-way analysis of variance (two-way ANOVA) was applied, followed by post hoc Tukey testing when significant differences were detected. In the absence of normal data distribution, the nonparametric Kruskal–Wallis test with Dunn’s post hoc analysis was used. For pairwise comparisons between individual groups within the same observation period, the Mann–Whitney U test was applied. Differences were considered statistically significant at $P < 0.05$.

Results

Morphological analysis of the rat hippocampus demonstrated that the pattern and severity of structural changes varied depending on the experimental conditions and observation time points. The most informative time points for assessing toxic injury and the effectiveness of pharmacological correction were days 14 and 28, which corresponded to the phase of progressive destruction and the peak of neurotoxicity.

Thus, at early time points after cisplatin administration (days 1 and 7), initial morphological signs of toxic injury developed in the hippocampus. On day 1, edematous-dystrophic changes predominated, manifested by pericellular edema, moderate cytoplasmic vacuolization of pyramidal neurons, and focal rarefaction of the neuropil, without pronounced disruption of laminar organization. By day 7, morphological alterations became more pronounced: individual neurons with signs of nuclear hyperchromasia and pyknosis appeared, irregularity of cellular rows in the CA1–CA3 regions was observed, and glial reactivity increased. Collectively, these changes corresponded to a transition from the early reactive phase to the initial destructive stage of cisplatin-induced neurotoxicity.

At all time points of the experiment, the hippocampal cytoarchitecture of intact rats remained preserved, and no morphological abnormalities were detected. Microphotographs of the hippocampus of intact rats clearly demonstrated the typical histoarchitecture of the pyramidal layer. Cellular organization was characterized by dense and

uniform neuronal arrangement, without signs of thinning or disorganization. Pyramidal neurons exhibited homogeneous perikaryal contours; the cytoplasm was moderately basophilic, without vacuolization or features of hydropic dystrophy.

Neuronal nuclei were round or slightly oval, with a distinct nuclear membrane and evenly distributed euchromatin. In most cells, prominent nucleoli were visualized, indicating preserved synthetic activity. No signs of pyknosis, karyorrhexis, or karyolysis were detected. The neuropil appeared homogeneous and compact, without areas of rarefaction; intercellular spaces were not expanded. Pericellular edema and tissue loosening were absent. Glial cells were represented by single elements with thin, non-hypertrophied nuclei, corresponding to the physiological state of the neuroglial microenvironment.

Overall, the morphological pattern corresponded to the normal structural state of the hippocampus in intact animals, with preserved integrity of neurogliovascular complexes and no signs of toxic injury.

On days 14 and 28 after cisplatin administration, progressive dystrophic and destructive changes were observed in the rat hippocampus. On day 14, these alterations markedly exceeded early edematous reactions and were characterized by loss of uniform laminar organization of the pyramidal layer in the CA1–CA3 regions, fragmentation of neuronal rows, and focal areas of neuropil rarefaction. By day 28, hippocampal morphological changes reached maximal severity, with sharp disorganization of the pyramidal layer, almost complete loss of cellular rows, and extensive areas of neuronal depletion (Fig. 1).

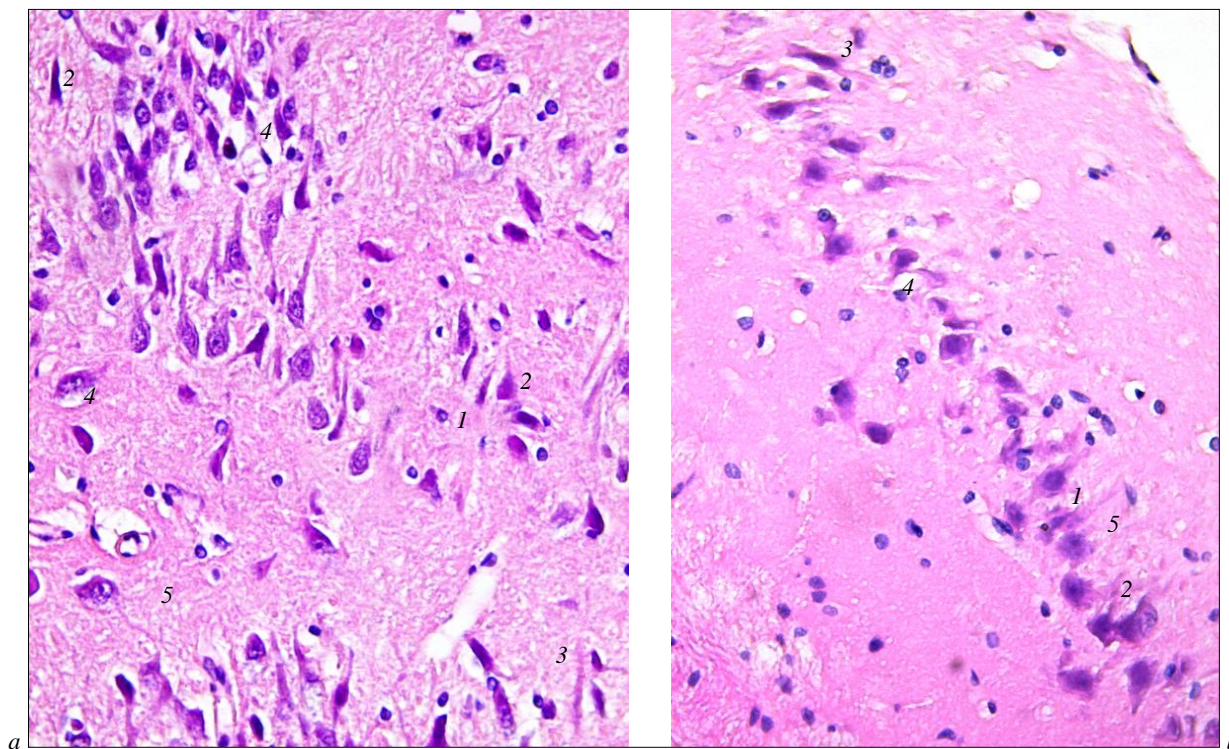


Fig. 1. Cisplatin-induced morphological changes in the rat hippocampus in temporal dynamics: *a* – day 14 after cisplatin administration: disruption of the regular arrangement of pyramidal neurons in the CA1 region, perikaryal dystrophy, nuclear pyknosis, pronounced pericellular edema, and focal rarefaction of the neuropil; *b* – day 28 after cisplatin administration: massive degeneration of pyramidal neurons in the CA1 region, marked disorganization of the pyramidal layer, homogenization of the neuropil, and severe disorganization of the neuropil; hematoxylin and eosin staining, $\times 400$: 1 – disrupted arrangement of pyramidal neurons in the CA1 region, 2 – dystrophic changes of neuronal perikarya, 3 – nuclei with signs of pyknosis, 4 – pronounced pericellular edema, 5 – focal rarefaction and disorganization of the neuropil

The morphological changes detected in the hippocampus on day 14 after cisplatin administration indicated a transition from early reactive manifestations to the phase of progressive toxic destruction. Disruption of the regular organization of the pyramidal layer, together with signs of neuronal degeneration and pericellular edema, reflected disintegration of neuronal connections and the formation of persistent damage to neuroglialvascular complexes, most pronounced in the CA1 region. By day 28, the hippocampal morphological pattern corresponded to the phase of maximal cytotoxic injury, characterized by massive disorganization of the pyramidal layer, a marked reduction in neu-

ronal density, and profound disruption of the structural integrity of neuroglialvascular complexes, indicating failure of compensatory mechanisms in the CA1–CA3 regions.

Under conditions of prophylactic pioglitazone administration, hippocampal morphological alterations demonstrated a clear time-dependent attenuation of cisplatin-induced damage. On day 14, structural changes were significantly less pronounced than in the uncorrected cisplatin group and were characterized by relative preservation of pyramidal layer regularity in the CA1 region in the majority of visual fields, without extensive areas of thinning. By day 28, no massive

destruction characteristic of cisplatin exposure was observed; although signs of neuronal dystrophy persisted, their severity was markedly reduced, with preservation of perikaryal contours and the presence of neurons exhibiting light nuclei with moderately condensed chromatin (Fig. 2).

The morphological changes observed in the hippocampus on day 14 under conditions of prophylactic pioglitazone administration indicated a substantial limitation of cisplatin-induced toxic injury. Relative preservation of the pyramidal layer organization and neuropil structure

reflected attenuation of early destructive processes and stabilization of the neuronal microenvironment. By day 28, the hippocampal morphological pattern demonstrated further structural preservation, with absence of massive pyramidal layer disorganization and maintenance of neuronal density in the CA1–CA3 regions. Although moderate dystrophic changes persisted in a subset of neurons, the overall histological picture indicated effective limitation of cytotoxic injury and preservation of the structural integrity of neuro-glial-vascular complexes compared with uncorrected cisplatin exposure.

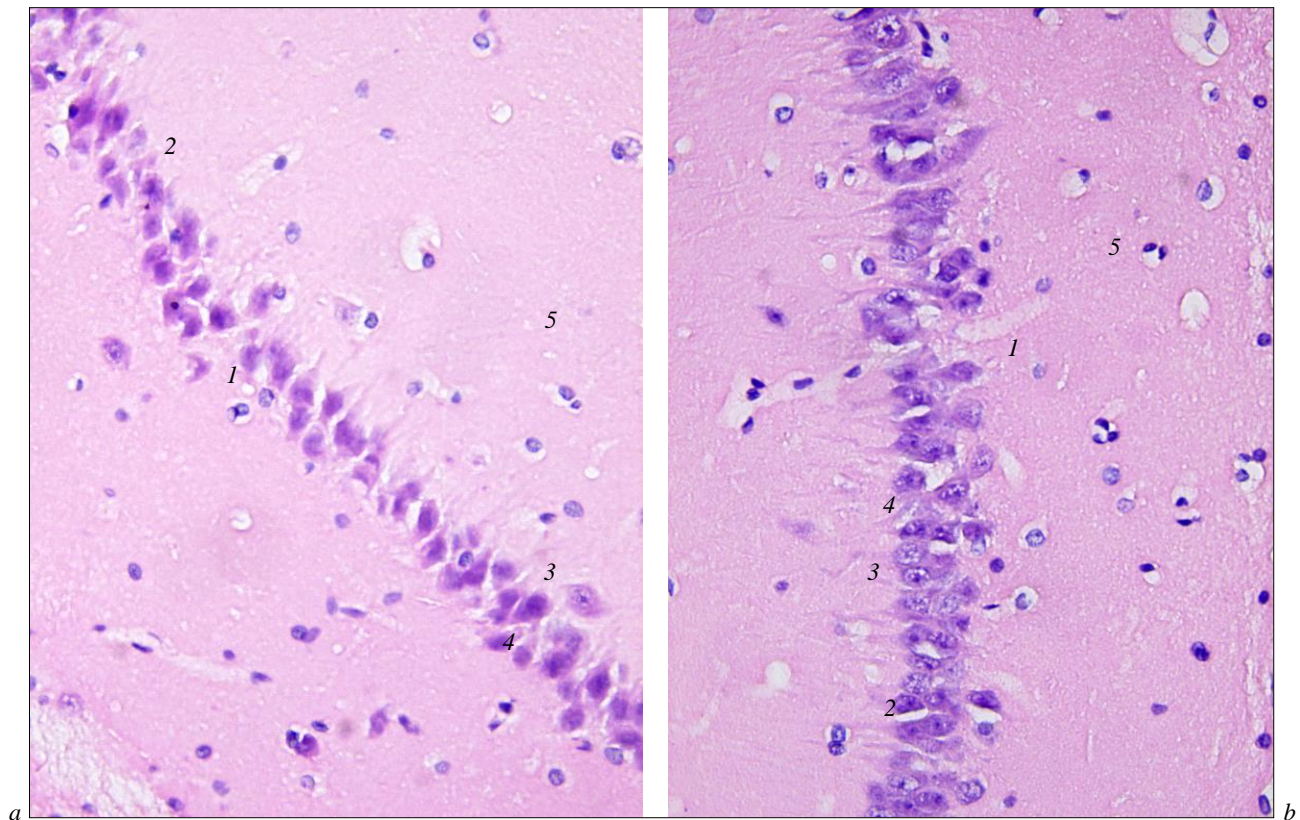


Fig. 2. Effect of prophylactic pioglitazone administration on cisplatin-induced hippocampal damage in temporal dynamics: *a* – day 14: relative preservation of pyramidal layer regularity in the CA1 region, moderate perikaryal dystrophy, and reduced severity of pericellular edema; *b* – day 28: absence of massive neuronal destruction, preservation of perikaryal contours, light neuronal nuclei with moderately condensed chromatin, and partial normalization of neuropil structure; hematoxylin and eosin staining, $\times 400$: 1 – preserved regular arrangement of pyramidal neurons in the CA1 region, 2 – moderate dystrophic changes of neuronal perikarya, 3 – neuronal nuclei with moderately condensed chromatin, 4 – reduced pericellular edema, 5 – partial normalization of the neuropil

In the therapeutic pioglitazone group, hippocampal morphological alterations demonstrated a time-dependent pattern of partial stabilization. On day 14, structural changes remained pronounced compared with intact animals; however, their pattern differed substantially from uncorrected cisplatin-induced toxicity. The pyramidal layer, predominantly in the CA1 region, exhibited disorganization, whereas areas of total neuronal loss were observed less frequently. By day 28, morphological stabilization of the hippocampus became evident. Single dystrophically altered pyramidal neurons were preserved, and no signs of massive destruction or complete disruption of the pyramidal layer were detected. In a proportion of neurons, perikaryal contours appeared more ordered, and the neuropil became denser and structurally more homogeneous (Fig. 3).

The morphological changes detected in the hippocampus on day 14 of therapeutic pioglitazone administration reflected partial limitation of the cisplatin-induced toxic process without complete stabilization of neuronal structures. Preservation of individual pyramidal neurons against the background of pyramidal layer disorganization indicated attenuation, but not arrest, of destructive changes. By day 28, the hippocampal state demonstrated a clear tendency toward morphological stabilization; however, the degree of structural recovery remained incomplete. Partial normalization of neuropil organization and pyramidal layer architecture was observed, but these changes were less pro-

nounced than those achieved under prophylactic pioglitazone administration.

For quantitative assessment of hippocampal structural changes induced by cisplatin and their pharmacological correction by pioglitazone, a morphometric analysis of neurons in the CA1 and CA3 regions was performed. The perikaryon area, nuclear diameter, nuclear-to-cytoplasmic ratio, and neuronal density per field of view were evaluated. Comparative analysis was carried out among the intact group, the cisplatin group without correction, and the groups receiving prophylactic and therapeutic pioglitazone administration. The summarized morphometric parameters are presented in Table 1. Morphometric analysis demonstrated that cisplatin administration induced statistically significant structural alterations of hippocampal neurons in the CA1 and CA3 regions, including a reduction in neuronal perikaryon area and nuclear diameter, an increase in the nuclear-to-cytoplasmic ratio, and a decrease in neuronal density compared with the control group.

Prophylactic administration of pioglitazone resulted in a more pronounced normalization of morphometric parameters than therapeutic treatment. Statistically significant differences between the prophylactic and therapeutic regimens were observed predominantly in the CA3 region, particularly with respect to neuronal perikaryon area and neuronal density, whereas in the CA1 region most parameters did not differ significantly between these regimens.

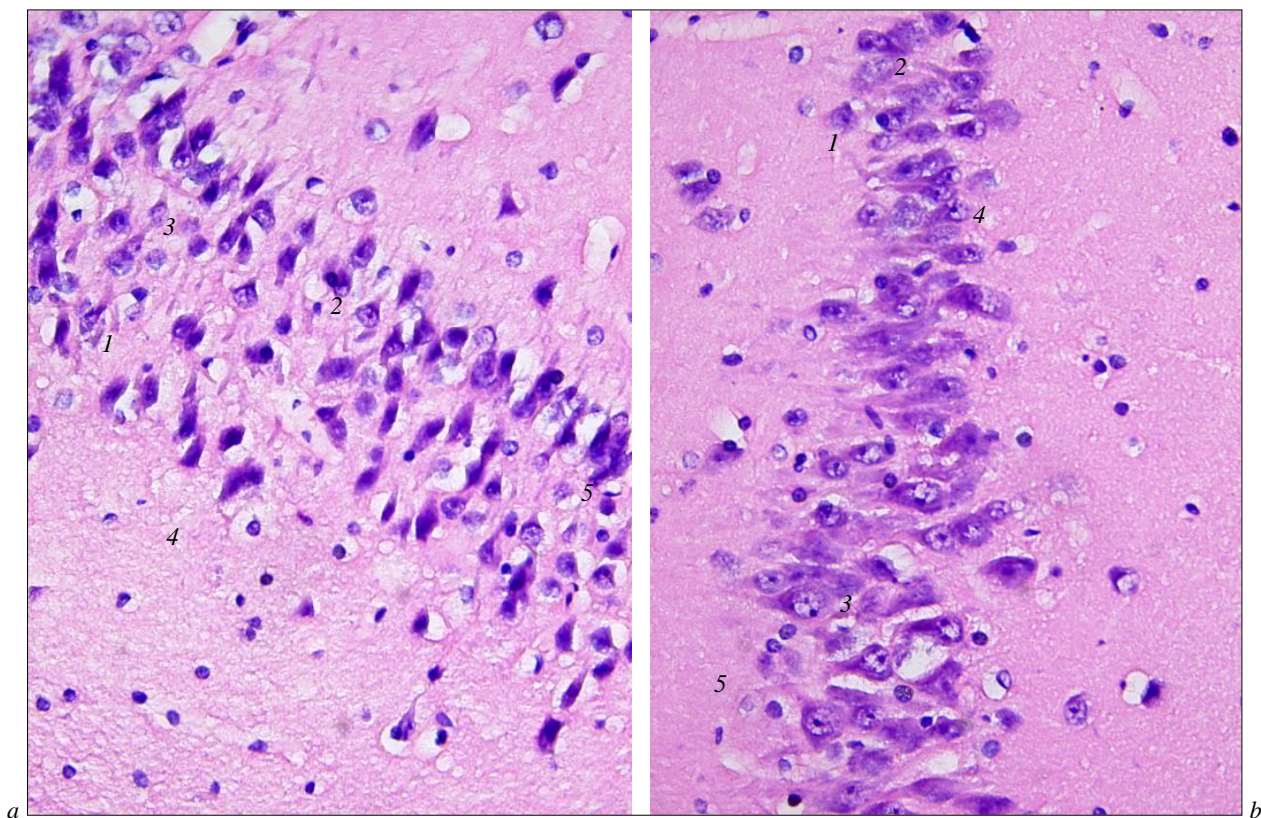


Fig. 3. Therapeutic effect of pioglitazone on cisplatin-induced hippocampal alterations in temporal dynamics: *a* – rat hippocampus, CA1 region, day 14 after completion of the cisplatin course: partial disorganization of the pyramidal layer with the presence of morphologically identifiable neurons, moderate dystrophy of neuronal perikarya, and reduced severity of neuropil destruction; *b* – rat hippocampus, CA1 region, day 28 after completion of the cisplatin course: partial preservation of the pyramidal layer, single dystrophically altered neurons, compaction of the neuropil, and an organized glial response, reflecting early manifestations of morphological stabilization; hematoxylin and eosin staining, $\times 400$: 1 – partial disorganization of the pyramidal layer in the CA1 region, 2 – morphologically preserved pyramidal neurons, 3 – moderate dystrophic changes of neuronal perikarya, 4 – reduced severity of neuropil destruction, 5 – compaction of the neuropil and organized glial response

Table 1

Effect of pioglitazone on changes in morphometric parameters of hippocampal neurons (CA1 and CA3) during the progression of cisplatin-induced neurotoxicity

Group	Perikaryon area, μm^2		Nuclear diameter, μm		Nuclear-to-cytoplasmic ratio		Neuronal density, cells/field of view	
	CA1	CA3	CA1	CA3	CA1	CA3	CA1	CA3
Intact control	245 \pm 28	355 \pm 42	11.8 \pm 1.2	13.5 \pm 1.4	0.42 \pm 0.05	0.38 \pm 0.04	28 \pm 3	16 \pm 2
Cisplatin	195 \pm 22*	285 \pm 35*	9.5 \pm 1.0*	11.2 \pm 1.2*	0.52 \pm 0.06*	0.48 \pm 0.05*	19 \pm 2*	11 \pm 1*
Cisplatin + pioglitazone (prophylaxis)	235 \pm 26#	340 \pm 38#	11.4 \pm 1.1#	13.0 \pm 1.3#	0.44 \pm 0.05#	0.40 \pm 0.04#	26 \pm 3#	15 \pm 2#
Cisplatin + pioglitazone (treatment)	225 \pm 24#	320 \pm 36@	10.8 \pm 1.1#	12.4 \pm 1.3#	0.47 \pm 0.05#	0.43 \pm 0.05#	23 \pm 3@	13 \pm 2@

Notes: data are presented as mean \pm SD; statistical analysis was performed using one-way ANOVA followed by Tukey's honestly significant difference (HSD) post hoc test for multiple comparisons; * $P < 0.05$ vs intact control; # $P < 0.05$ vs cisplatin group; @ $P < 0.05$ vs cisplatin + pioglitazone (prophylactic regimen).

Comparative analysis revealed regional differences in neuronal vulnerability to cisplatin-induced toxicity, with CA1 neurons exhibiting greater morphometric alterations, while the CA3 region demonstrated relatively higher structural stability, especially under conditions of prophylactic pioglitazone administration.

On days 60, 90, and 120 of the experiment, further positive dynamics of the hippocampal morphological state were observed in the groups receiving prophylactic and therapeutic pioglitazone compared with the early observation periods. In the prophylactic group, on day 60 a clear limitation of destructive processes was noted, with predominance of preserved pyramidal neurons, relative restoration of the regular arrangement of the pyramidal layer, and a reduction of pericellular edema. On days 90 and 120, the cytoarchitecture of the CA1 and CA3 regions in the majority of visual fields was maximally close to the intact state: neurons exhibited well-defined perikaryon contours, light nuclei with evenly distributed chromatin, and the neuropil appeared dense and well organized, without signs of progressive destruction. In the therapeutic pioglitazone group, on day 60 features of morphological stabilization predominated, with the presence of single dystrophically altered neurons and moderate disorganization of the

pyramidal layer. On days 90 and 120, a further reduction of dystrophic changes, compaction of the neuropil, and ordering of the glial response were observed, indicating activation of reparative processes. At the same time, the degree of morphological normalization remained less pronounced compared with the prophylactic regimen, with persistence of individual areas of structural remodeling of the neuronal layer. Overall, the late experimental time points confirmed a more pronounced and sustained morphoprotective effect of prophylactic pioglitazone administration, whereas the therapeutic regimen provided partial structural stabilization and limitation of secondary destruction of hippocampal neurons.

Conditioned reflex testing demonstrated that cisplatin administration was accompanied by impaired formation and reproduction of conditioned reflex responses, manifested by a shortening of passive avoidance latency and a decrease in the stability of the conditioned food reflex compared with the intact group. The most pronounced functional impairments were recorded on days 14–28 of the experiment, corresponding to the period of maximal hippocampal morphological alterations. The obtained morphological data indicate that cisplatin induces a sequential cascade of hippocampal damage with a

peak of destruction on day 28. Prophylactic administration of pioglitazone provides the most pronounced morphoprotective effect, limiting both early and peak toxic changes. The therapeutic regimen reduces secondary destruction and promotes structural stabilization; however, it is inferior to the prophylactic approach in terms of the depth of the

protective effect. Under conditions of prophylactic pioglitazone administration, improvement of conditioned reflex parameters was observed compared with the cisplatin group, whereas therapeutic administration of pioglitazone ensured partial functional compensation that was less pronounced than that achieved with the prophylactic regimen (Table 2).

Table 2

Effect of pioglitazone on memory and learning parameters in rats during the progression of cisplatin-induced neurotoxicity

Experimental groups	Passive avoidance latency, s		Conditioned food reflex latency, s	
	day 14	day 28	day 14	day 28
Intact control	210 ± 25	215 ± 28	65 ± 8	62 ± 7
Cisplatin	120 ± 20*	95 ± 18*	110 ± 12*	125 ± 15*
Cisplatin + pioglitazone (prophylaxis)	185 ± 22#	195 ± 26#	75 ± 9#	78 ± 10#
Cisplatin + pioglitazone (treatment)	155 ± 24@	165 ± 25@	90 ± 11@	95 ± 13@

Notes: * $P < 0.05$ – statistically significant differences compared with the corresponding value in the control group; # $P < 0.05$ – statistically significant differences compared with the corresponding value in rats treated with cisplatin without pharmacological correction; @ $P < 0.05$ – statistically significant differences compared with the corresponding value in rats receiving pioglitazone under prophylactic or therapeutic regimens.

Analysis of conditioned reflex parameters demonstrated that cisplatin administration was associated with a significant impairment of cognitive function, manifested by a reduction in passive avoidance latency and a prolongation of the time required for acquisition of the conditioned food reflex on days 14 and 28 of the experiment compared with the intact group. The most pronounced functional deficits were observed on day 28, corresponding to the period of maximal hippocampal morphological alterations. Prophylactic administration of pioglitazone resulted in a marked improvement of behavioral performance, whereas the therapeutic regimen provided partial functional compensation that was statistically less pronounced than that achieved with prophylactic administration.

Discussion

The obtained data indicate that the effects of cisplatin are not limited to early reactive changes but are accompanied by persistent structural alterations in pyramidal neurons of the CA1 and CA3 regions of the hippocampus. These findings are consistent with experimental evidence demonstrating suppression of cell proliferation, impaired neurogenesis, and degeneration of hippocampal neurons after cisplatin administration. In particular, Manohar et al. (2014) showed that cisplatin directly decreases hippocampal cell proliferation, which may contribute to long-term morphological consequences. Similar cisplatin-associated hippocampal damage and neurobiological correlates have been reported in experimental studies and mechanistic works (Christie et al., 2012; Domouky et al., 2022; Oliveros et al., 2022; Magdy et al., 2024).

Neuroinflammation represents an important component of cisplatin-induced central neurotoxicity. Increased expression of pro-inflammatory cytokines may be accompanied by dysregulation of glutamatergic and serotonergic neurotransmission, thereby creating conditions for progression of hippocampal injury and impairment of functional integrity (Alhowail, 2025; Wellenberg et al., 2021). These observations align with data emphasizing the role of glial cells and neuro-glial interactions in chemotherapy-induced cognitive impairment, and with the concept of multicomponent injury of neuro-glio-vascular complexes during chemotherapy (Ren et al., 2017; Barbosa-Azevedo et al., 2024; Csik et al., 2025).

The pronounced destructive changes observed on days 14–28 correspond to the phase of maximal cytotoxic impact of cisplatin and are supported by evidence on mitochondrial dysfunction, oxidative stress, and DNA damage in brain tissue under cisplatin exposure (Lomeli-Cardona et al., 2017; Magdy et al., 2024). Lomeli-Cardona et al. (2017) demonstrated impairment of mitochondrial respiration and accumulation of reactive oxygen species in brain tissue, which is consistent with ultrastructural and histological features of neuronal destruction. Similar hippocampal histopathological changes in rats were described by Domouky et al. (2022), including nuclear pyknosis, neuropil disorganization, and neuronal loss in CA1 and CA3.

The impairment of conditioned reflex activity detected in passive avoidance and conditioned food reflex paradigms corresponds well to the hippocampal morphological alterations observed in CA1 and CA3.

These hippocampal subfields play a key role in contextual and associative memory formation, and their structural integrity is critical for learning and consolidation processes (Christie et al., 2012; Dietrich et al., 2015). Accordingly, chemotherapy-related hippocampal vulnerability is widely discussed as a mechanistic substrate for cognitive dysfunction (Dietrich et al., 2015; Ongnok et al., 2020; Was et al., 2022).

Similar alterations in hippocampal structure and inflammatory signaling have been described in aged animal models treated with antimetabolite chemotherapy, where increased cytokine expression was associated with changes in dendritic complexity and cognitive decline (Groves et al., 2017).

In the present study, conditioned-reflex performance reflected the integrative functional state of the hippocampus, whereas regional differences in vulnerability between CA1 and CA3 were clarified morphometrically. Improvement of conditioned reflex indices under prophylactic pioglitazone administration correlated with preservation of hippocampal cytoarchitecture and stabilization of morphometric parameters of CA1 and CA3 neurons, indicating functional realization of its neuroprotective effect. Similar functional and anti-inflammatory effects of pioglitazone in chemotherapy-related neurotoxicity models were reported previously (Alsaud et al., 2023). Moreover, early PPAR γ activation has been shown to reduce glial inflammation (Heneka et al., 2005), and inhibition of microglial activation is associated with reduced neuronal loss (Xing et al., 2007).

At later observation time points (days 60, 90, and 120), persistent morphological defects in the cisplatin group indicate a prolonged nature of central neurotoxicity. Comparable long-lasting hippocampal micromorphometric alterations after chronic chemotherapy were described by Kang et al. (2018), and broader mechanistic syntheses also emphasize the persistence of chemotherapy-associated neurotoxicity and cognitive impairment (Ongnok et al., 2020; Was et al., 2022; Alotayk et al., 2023; Csik et al., 2025).

Against this background, prophylactic pioglitazone administration demonstrated a pronounced morphoprotective effect. Limitation of destructive changes during early and peak experimental periods, preservation of pyramidal layer organization, and neuropil stabilization are consistent with the anti-inflammatory properties of PPAR γ agonists. Previous studies have shown that pioglitazone reduces glial inflammation (Heneka et al., 2005) and microglial activation (Xing et al., 2007), and may also modulate oxidative stress and cognitive dysfunction in experimental settings (Kumar et al., 2009). Recent experimental evidence also supports the potential of pharmacological interventions targeting oxidative stress and neuroinflammation to attenuate cisplatin-induced neurotoxicity (Hussien & Yousef, 2022).

The preservation of hippocampal structural integrity at late stages in the prophylactic group may be related not only to suppression of inflammation and oxidative stress (Heneka et al., 2005; Kumar et al., 2009) but also to support of neuroplastic processes. In this context, PPAR γ activation has been associated with stimulation of neural stem cell proliferation (Morales-García et al., 2011), which may be relevant for hippocampal recovery after toxic injury.

Therapeutic pioglitazone administration also promoted morphological stabilization of the hippocampus; however, recovery was less

pronounced than under prophylactic use. This observation complements the concept that once a full cytotoxic and inflammatory cascade has developed, the potential for complete structural repair may be limited (Dietrich et al., 2015; Ongnok et al., 2020; Was et al., 2022). Similar differences between preventive and corrective strategies have been discussed in other chemotherapy-induced neurotoxicity models (Umfrress et al., 2021; Was et al., 2022).

To further interpret the obtained data and the likely mechanisms of pioglitazone-mediated neuroprotection, it is appropriate to consider established actions of this compound. In preclinical models, pioglitazone demonstrated neuroprotective properties, including attenuation of neuroinflammation and improvement of cognitive outcomes (Alsaud et al., 2023), reduction of glial inflammation (Heneka et al., 2005), and suppression of microglial activation-associated neuronal loss (Xing et al., 2007). In addition, PPAR γ activation is linked to neuroplasticity-related processes, including proliferation of neural stem cells (Morales-García et al., 2011), which may contribute to recovery of hippocampal structure following toxic injury. Nevertheless, the morphological efficacy of pioglitazone specifically in cisplatin-induced hippocampal damage, as well as the difference between prophylactic and therapeutic regimens, remains insufficiently elucidated.

Thus, the present results support a fundamental difference between prophylactic and therapeutic pioglitazone regimens at the morphological level. Prophylactic administration may interrupt key pathogenetic mechanisms of cisplatin neurotoxicity at early stages, whereas therapeutic administration predominantly limits secondary destruction and promotes partial structural stabilization of the hippocampus.

Conclusions

Cisplatin administered in a cumulative regimen (2 mg/kg once weekly for 6 weeks, total dose 12 mg/kg) induces pronounced and long-lasting hippocampal neurotoxicity in rats, characterized by a sequential progression of morphological alterations, ranging from early edematous-dystrophic reactions to profound destruction of the pyramidal layer in the CA1 and CA3 regions, with a peak of damage observed on day 28 of the experiment.

The detected morphological and morphometric disturbances of the hippocampus exhibit a persistent nature and remain evident even at late observation periods (up to day 120), indicating a high vulnerability of neuroglia-vascular complexes to the cumulative effects of platinum-based cytostatics.

Prophylactic administration of pioglitazone (20 mg/kg, intragastric), initiated prior to the onset of cisplatin treatment, exerted a pronounced protective effect on hippocampal neurons. This effect was manifested by a significant reduction of both early edematous-dystrophic changes and the most severe peak destructive alterations. As a result, the regular arrangement of pyramidal neurons was preserved, pericellular edema was reduced, and key morphometric parameters, including neuronal perikaryon area, nuclear diameter, and neuronal density in the CA1 and CA3 regions, remained stable or approached normal values.

Therapeutic administration of pioglitazone (starting from day 3 after completion of the cisplatin course) contributed to attenuation of secondary destruction, morphological stabilization of the tissue, and activation of reparative processes; however, in terms of the degree and durability of structural protection, it was markedly inferior to the prophylactic regimen.

At later observation time points (days 90 and 120), prophylactic pioglitazone administration resulted in the most complete restoration of normal hippocampal structure. The cytoarchitectonics of the hippocampus in these animal groups approached the condition observed in intact rats. In contrast, the therapeutic regimen led only to partial improvement, with stabilization of tissue structure accompanied by residual areas of reorganization manifested as focal disorganization or incomplete restoration of the neuronal layer.

The obtained data indicate the feasibility of early pharmacological targeting of PPAR γ -dependent mechanisms to limit cisplatin-induced central neurotoxicity and underscore the potential for further preclinical investigation of the neuroprotective properties of pioglitazone.

The results of this study expand current understanding of the pathogenesis of chemotherapy-induced neurotoxicity of the central nervous system, demonstrating a distinct temporal pattern of hippocampal morphological changes and a clear advantage of the prophylactic approach over the therapeutic strategy at the level of structural brain organization.

Future studies should focus on elucidating the molecular mechanisms underlying PPAR γ -mediated neuroprotection and on evaluating the translational potential of prophylactic strategies for preventing chemotherapy-associated cognitive impairment.

This research was conducted within the framework of the research project "Morphofunctional changes in organs and body systems under the influence of antitumor drugs and under conditions of their correction" (state registration No. 0121U111598) and received no additional external funding.

The authors declare no conflict of interest.

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