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CISPLATIN AND DEXAMETHASONE SEPARATE AND COMBINED ACTION ON LIPID PEROXIDATION IN NUCLEAR FRACTIONS OF RAT BRAIN AND KIDNEY CELLS

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ABSTRACT

It is well known, that antitumor drug cisplatin is an antineoplastic drug which widely used in chemotherapy. However, the usage has been limited due to cisplatin-caused various side effects. It has been established that toxic effects of cisplatin are the result of oxidative stress. Oxidative stress is the result of the excessive formation of reactive oxygen species, that can be induced by cisplatin.

The reactive oxygen species in turn can interact with DNA, lipids and proteins, leading to lipid peroxidation and DNA damage.

Dexamethasone is being used in chemotherapy practice as concomitant agent to mitigate the side effects of antitumor drug cisplatin. It is known that both cisplatin and dexamethasone are capable of stimulating the production of reactive oxygen species, which in turn target various biomolecules, including lipids.

The aim of this study was to evaluate the quantitative alterations in lipid peroxidation products within the nuclei of cells from various rat tissues following the separate and combined administration of cisplatin and dexamethasone.

The amount of lipid peroxidation products was determined using a spectrophotometric method, following extraction with a heptane- isopropanol mixture. When administered individually, cisplatin and dexamethasone increase the formation of lipid peroxidation products in the examined tissues of rats to varying degrees.

As a result, these alterations led to corresponding changes in the oxidation index values of the analyzed nuclear preparations. During the combined administration of cisplatin and dexamethasone, some antagonistic effects were observed in the actions of these agents. Contrary to the expected synergistic enhancement of lipid peroxidation processes, a reduction in cisplatin's effect by dexamethasone was observed.

Thus, it is hypothesized that such antagonistic effect of dexamethasone together with its anti-inflammatory and immunomodulatory properties allows to mitigate the side effects of cisplatin.

KEYWORDS: *cisplatin, dexamethasone, lipid peroxidation, unsaturated fatty acids, diene conjugates, triene conjugates, oxidative index.*

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INTRODUCTION

Cisplatin (cis-diaminedichloroplatinum [II]) is an antineoplastic drug widely used in chemotherapy for the treatment of various human cancers [*Aldossary S, 2019; Jadon A et al., 2019; Tchounwou P et al., 2021; Dasari S et al., 2022*]. However, its usage has been limited due to cisplatin-caused various side effects [*Hashem R et al., 2015; Stone J, DeAngelis L, 2016; Tchounwou P et al., 2021; Dasari S et al., 2022*]. It has been shown that toxic effects of cisplatin are the result of excessive formation of reactive oxygen species [*Hashem R et al.,2015; Yang H et al., 2018; Aldossary S, 2019; Jadon A et al., 2019; Dasari S et al., 2022*]. Cisplatin induces the formation of reactive oxygen species, which in turn can interact with DNA, lipids and proteins, leading to lipid peroxidation and DNA damage [*Habtemariam S, 2019; Sidharta B et al.,2022; Singh R, Manna P, 2022*]. The induction of oxidative stress and the formation of reactive oxygen species are considered additional mechanism of cisplatin action) [*Yang H et al., 2018; Aldossary S, 2019; Jadon A et al., 2019; Sidharta B et al., 2022*]. Cisplatin increases the production of free oxygen radicals and decreases the antioxidants, thereby disturbing the oxidant/antioxidant balance [*Gaschler M, Stockwell B, 2017; Singh R, Manna P, 2022*]. It is well known that in normal physiological conditions the presence of reactive oxygen species is vital for the normal functioning of cells. To promote normal cellular physiological function and survival, redox homeostasis regulation is maintained in the cell [*Gaschler M, Stockwell B, 2017*]. A high accumulation of reactive oxygen species is mostly due to an imbalance in its production and its elimination process. This imbalance arises from either increased production of oxidants, decreased levels of antioxidants, or both [*Gaschler M, Stockwell B, 2017; Sidharta B et al., 2022*]. Cisplatin disrupts the oxidant/antioxidant balance by inducing reactive oxygen species formation and reducing antioxidant levels. Disturbed redox homeostasis leads to oxidative damage to biomolecules, such as proteins, lipids, and nucleic acids [*Gaschler M, Stockwell B, 2017; Sidharta B et al., 2022*]. These damages result in harmful effects on cells. In contrast, modulation of reactive oxygen species levels contributes to the regulation of cell survival, death, differentiation, and proliferation [*Mirzaei S et al., 2021*].

The primary targets of reactive oxygen species are lipids, which undergo oxidation upon interaction with oxidants. This process, known as lipid peroxidation, leads to the formation of lipoperoxyl radicals and lipid hydroperoxides [*Casares C et al., 2012; Hauck A, Bernlohr D, 2016; Mirzaei S et al., 2021*]. As highly reactive compounds, lipid peroxides can further propagate reactive oxygen species generation or degrade into reactive compounds that are capable of cross-linking DNA and proteins. Lipid hydroperoxides have been recognized as key mediators of cellular disease and death [*Hauck A, Bernlohr D, 2016; Mirzaei S et al., 2021*]. The products of lipid peroxidation play role in the intracellular signaling mechanisms that determine the cell's ultimate fate [*Habtemariam S, 2019*]. Reactive oxygen species generated by cisplatin could also increase lipid peroxidation, thereby altering enzymes and structural proteins, and steering the cell to an apoptotic pathway [*Casares C et al., 2012; Hauck A, Bernlohr D, 2016; Mirzaei S et al., 2021*].

As already noted, the cause of side effects of cisplatin is oxidative stress. To mitigate the side effects of antitumor drug cisplatin, dexamethasone is used as a concomitant agent in chemotherapy practice [*Cook A et al., 2016; Chow R, 2018; Sverediuk Yu, Pelykh V, 2020*]. Dexamethasone is a synthetic glucocorticoid with anti-inflammatory and immunosuppressant properties [*Cook A, 2016; Chow R et al.,2018; Sverediuk Yu, Pelykh V, 2020*]. Besides, dexamethasone induced alterations in lipid peroxidation products and antioxidants content in Wistar albino rats [*Chow R et al., 2018; Sverediuk Yu, Pelykh V, 2020; Alahmar A et al., 2023*].

Thus, both the antitumor drug cisplatin and dexamethasone, used as a concomitant agent, stimulate the formation of reactive oxygen species and increase the degree of lipid peroxidation [*Casares C et al., 2012; Cook A et al., 2016; Yang H et al 2018; Chow R et al., 2018; Sverediuk Yu, Pelykh V, 2020; Tchounwou P et al., 2021; Alahmar A et al., 2023*]. From this perspective, the study of lipid peroxidation processes with separate and combined use of cisplatin and dexamethasone is of particular interest. The aim of this study is to evaluate the quantitative changes in lipid peroxidation products in the cell nuclei of various rat tissues following the separate and combined use of cisplatin and dexamethasone.

Material and methods

Experiments were conducted according to the "International Recommendations on Carrying out of Biomedical Researches with use of Animals" [*CIOMS, 1986; 2016*], to the "Human Rights and Biomedicine the Oviedo Convention" [*Roberto A, 2005*], to the European Convention for the Protection of Vertebral Animals Used for Experimental and Other Scientific Purposes [*EC, 2005*], and were approved by the National Center of Bioethics (Armenia).

The study was performed on adult albino female rats (120-150 *g* weight, 16 rats). The animals were divided into 4 groups. Group 1 served as a control group of animals without treatment (n=16). Animals in group 2 (n=16) and group 4 (n=16) received a single dose of cisplatin (8 *mg/kg*) by peritoneal injection and were decapitated 24 *hours* after administration. Group 3 was treated with dexamethasone (4 *mg/kg*, peritoneal injection) and decapitated 4 hours after administration. Animals in group 4 (n=16) received the same single dose of dexamethasone within 20 *hours* after the cisplatin injection (4 *hours* before decapitation). All animals were euthanized by decapitation at an appropriate time following inhalation ether anesthesia. Then, animals were sacrificed, and the brain and kidney tissues were extracted from each group of animals and used for isolation of nuclei by the method of Blobel G. and Potter V. (1966).

The nuclear fraction of brain and kidney tissues of rats was used for the quantitative assessment of primary products of lipid peroxidation. The primary products of lipid peroxidation are lipid hydro peroxides, which form conjugate double bonds in the fatty acid molecule – diene- and triene conjugates. The primary products of lipids peroxidation were estimated by the method of Volchegorsky. The principle of the method is based on the determination of the content of lipid peroxidation products in biological material by absorption of monochromatic light flux in the ultraviolet spectrum after its extraction from nuclei of brain and kidney tissues by heptane-isopropyl alcohol mixture. Heptane extracts contain mainly neutral lipids, while isopropanol extracts contain phospholipids. This method allows to identify lipoperoxidation products (conjugated dienes and trienes) in extracts of different lipid classes. In the lipid extracts of each phase, measurements were made at 220 *nm* (absorption of double bonds in unsaturated fatty acids), 232 *nm* (absorption reflects the content of conjugated dienes) and 278 *nm* (absorption depends on the content of ketodienes and conjugated trienes). The amount of unsaturated fatty acids, conjugated dienes and trienes is expressed in conventional units. The oxidation index was calculated as the ratio of optical densities determined at appropriate wavelengths (232*nm*, 278*nm*) and absorption of unsaturated fatty acids (220 *nm*), i.e., $D_{232/220}$; $D_{278/220}$ [*Volchegorsky I et al., 1989*].

Statistical analysis: All results were expressed as Mean ± SE from 4 independent experiments. Statistical analysis was performed using paired Student's t-test for grouped data, where $p<0.05$ was considered statistically significant (*$p< 0.05$ indicates significant differences compared with the control group). Statistical comparisons between all experimental groups were tested by analysis of variance, and $p < 0.05$ significant differences in case of intergroup comparison.

Results

The process of lipid peroxidation begins with the attack of free radicals on polyunsaturated fatty acids, leading to the formation of lipid radicals. This, in turn, can initiate new radicals, starting a chain reaction [*Mirzaei S, 2021; Hauck A, Bernlohr D., 2016; Casares C et al., 2018*]. Since unsaturated fatty acids are the raw material for lipid peroxidation, our research began with their quantitative evaluation in heptane and isopropanol phases, extracted from nuclei of brain and kidney tissues of rats included in four experimental groups. As mentioned earlier, heptane dissolves neutral lipids, and isopropyl alcohol mainly dissolves phospholipids [*Volchegorsky I et al., 1989*].

Quantification of Unsaturated Fatty Acids in Brain and Kidney Tissues of Rats: Table 1 shows the amount of unsaturated fatty acids (in conventional units) in heptane and isopropanol phases, extracted from the nuclear fractions of brain and kidney tissues of rats at baseline (Group 1), after treatment with cisplatin alone (Group 2), dexamethasone alone (Group 3) and after the combined treatment with cisplatin and dexamethasone (Group 4). The obtained data presented in table 1 indicate that compared to baseline there were no changes in

the amount of unsaturated fatty acids in the heptane phase from rat brain nuclei after separate use of cisplatin and dexamethasone, as well as after their combined use. However, when comparing the data between the experimental groups, a statistically significant change in the amount of unsaturated fatty acids was found in experimental group with the combined use of cisplatin and dexamethasone compared to separate treatment with cisplatin and dexamethasone. A 12 % decrease was recorded after the combined use of cisplatin and dexamethasone ($p<0.05$), compared with experimental group of cisplatin alone injection. Meanwhile, about 8% change in the amount of unsaturated fatty acids was not statistically significant when compared with dexamethasone only group. In the case of comparing the experimental groups with separate use of cisplatin and dexamethasone statistically significant changes of unsaturated fatty acids quantity were not revealed.

Marginal increase of 11% and 10% ($*p<0.05$) in quantity of unsaturated fatty acids in heptane phase from rat kidney nuclei were observed after separate treatment with cisplatin and dexamethasone, in comparing to baseline.

In the case of intergroup comparison, statistically significant differences were also recorded between the experimental groups of animals that received the combined injection of cisplatin and dexamethasone, in comparison to both separate cisplatin (decrease by 12.7%; $\#p<0.05$) and dexamethasone (decrease by 12%; $\#p<0.05$) treated groups. Statistically significant changes in the amount of unsaturated fatty acids were not revealed in the case of comparing the experimental groups with separate treatment with cisplatin and dexamethasone.

In comparison to the control Group 1 (baseline), the changes in the amount of unsaturated fatty acids in the isopropanol phase from rat brain nuclei were 31%, 20% and 63% ($*p<0.05$), respectively after the separate treatment with cisplatin and dexamethasone and in the case of their combined use (Table 1).

The intergroup comparison also showed statistically significant changes between the different experimental groups. In the group of animals treated with a combination of cisplatin and dexamethasone, statistically significant changes were recorded in the amount of unsaturated fatty acids compared to experimental groups separately with cisplatin (increase by 25%; $p<0.05$) and dexamethasone (increase by 37%; $\#p<0.05$). At the same time, no statistically significant changes in the amount of unsaturated fatty acids were observed when comparing the experimental groups that received separate treatment of either drug.

In isopropanol phase extracted from rat kidney nuclei, the changes in the amount of unsaturated fatty acids were 26%, 35% and 67% ($*p<0.05$) after separate treatment with cisplatin, separate treatment with dexamethasone and their combined use, respectively when comparing with control Group 1 (Table 1).

Additionally, statistically significant differences were recorded in the experimental group of animals that received combined treatment with cisplatin and dexamethasone, compared to the groups treated separately with cisplatin (increase by 32.7%; $p<0.05$) and dexamethasone (increase by 24%; $\#p<0.05$) treated groups. Comparison of experimental groups of rats treated with cisplatin alone or dexamethasone alone did not reveal statistically significant change in the amount of unsaturated fatty acids.

TABLE 1

Unsaturated fatty acids in heptane and in isopropanol phases

Groups	Research Conditions	heptane phase		isopropanol phase	
		Brain	Kidney	Brain	Kidney
Group 1 (n=16)	Baseline without treatment	28.00±0.36	9.00±0.08	15.30±0.85	4.34±0.15
Group 2 (n=16)	Cisplatin alone treatment	29.00±0.92	10.00±0.02*	20.00±0.9*	5.47±0.22*
Group 3 (n=16)	Dexamethasone alone treatment	27.80±1.25	9.90±0.46*	18.30±0.50*	5.87±0.28*
Group 4 (n=16)	Cisplatin and dexamethasone combined treatment	25.50±0.86	8.73±0.42	25.00±0.6*	7.26±0.09*

NOTE: *Statistical significance between baseline and each experimental group *-p<0.05*

The results of quantitative evaluation of diene conjugates is provided in table 2. These data indicate significant changes in the amount of diene conjugates in both the heptane and isopropanol phases of nuclear fractions in comparison to the control group (Table 2). The amount of diene conjugates in the heptane phase, extracted from the nuclear fractions of brain tissue nuclei of rats increased by about 200% after the separate treatment with cisplatin (*p<0.05), while treatment with dexamethasone alone increased the quantity of conjugated dienes by only 11% (*p<0.05). In case of combined treatment of these drugs the amount of conjugated dienes increased by 10% compared to baseline (*p<0.05) (Table 2).

Measurement of Conjugated Dienes in Brain and Kidney Tissues of Rats: Table 2 shows the amount of conjugated dienes (in conventional units) in heptane and isopropanol phases, phases extracted from the nuclear fraction of brain and kidney tissues of rats at baseline (Group 1), after the treatment with cisplatin alone (Group 2), after the treatment with dexamethasone alone (Group 3) and after the combined treatment with cisplatin and dexamethasone (Group 4).

The intergroup comparison also revealed statistically significant changes between the different experimental groups. Specifically, in the group of animals treated with a combination of cisplatin and dexamethasone, a statistically significant decrease of 63% (p<0.05) in the amount of conjugated dienes was recorded, compared to experimental group injected with cisplatin alone. There was no statistically significant change in the quantity of conjugated dienes between the experimental groups of animals treated with a combination of cisplatin and dexamethasone and dexamethasone alone).

At the same time, there was also a statistically significant difference observed in the amount of conjugated dienes between experimental groups of animals injected with cisplatin and dexamethasone alone. Dexamethasone reduced diene conjugates by 63%; #p<0.05 compared to cisplatin treated group. Conversely, compared with the dexamethasone treated group, the cisplatin alone injection increased the quantity of conjugated dienes by about 170 %; #p<0.05. Changes in the quantity of conjugated dienes compared with the control group is also recorded in the heptane phase obtained from rat kidney nuclei. Compared to the control group, an increase in the amount of diene conjugates was recorded as a result of both alone and combined use of cisplatin and dexamethasone. The observed changes were 330%, 113% and 30%, respectively (*p<0.05). In the case of intergroup comparison, statistically significant changes of the quantity of conjugated dienes were also recorded between the different experimental groups. The combined use of these drugs resulted in a decrease in the amount of conjugated dienes in both experimental groups received a dose of cisplatin alone (by 70%) and dexamethasone alone (by 38.7%; #p<0.05).

A change in the quantity of conjugated dienes was also recorded in the heptane phases of kidney nuclei of rats receiving cisplatin alone and dexamethasone alone injections. Compared to cisplatin treated group, dexamethasone reduced the amount of lipid peroxidation product by 50.5% (#p<0.05), and compared to dexamethasone, a dramatic two-fold increase (102%; #p<0.05) in the amount of conjugated dienes induced by cisplatin was recorded. In the isopropanol phase extracted from rat brain nuclei changes of the amount of conjugated dienes were recorded in all three experimen-

Table 2

Conjugated dienes in heptane and in isopropanol phases

Groups	Research Conditions	heptane phase		isopropanol phase	
		Brain	Kidney	Brain	Kidney
Group 1 (n=16)	Baseline without treatment	4.00±0.07	1.12±0.10	3.00±0.27	1.52 ±0.05
Group 2 (n=16)	Cisplatin alone treatment	12.00±0.37*	4.81±0.24*	7.10±0.32*	2.80±0.10*
Group 3 (n=16)	Dexamethasone alone treatment	4.45±0.12*	2.38±0.11*	6.70±0.30*	2.33±0.03*
Group 4 (n=16)	Cisplatin and dexamethasone combined treatment	4.40±0.11*	1.46±0.07*	11.4±0.12*	3.30±0.08*

Note: *Statistical significance between baseline and each experimental group *-p<0.05*

tal groups compared to the control group. These changes were by 137%, 123% and 280% (*p<0.05), respectively after the cisplatin alone treatment, after the dexamethasone alone treatment and after the combined use of these drugs.

We also revealed statistically significant changes between the different experimental groups. In case of co-injection of cisplatin and dexamethasone, in the isopropanol phase of rat brain nuclei the amount of conjugated dienes was increased both compared to the group with a separate injection of cisplatin (increase by 60%; #p<0.05) and after exposure to dexamethasone alone (70% increase; #p<0.05). At the same time, there were no statistically significant quantitative changes observed in quantity of conjugated dienes between the experimental groups of animals with cisplatin and dexamethasone alone treatment groups. The amount of conjugated dienes in the isopropanol phase of the nuclear fraction obtained from rat kidney was changed compared to control after both single and combined drug exposure. Quantitative changes in conjugated dienes compared to control were 84% after cisplatin exposure, 53% increase (*p<0.05) after dexamethasone exposure, and an increase by 117% after the combined cisplatin and dexamethasone exposure (*p<0.05).

Statistically significant quantitative changes in conjugated dienes were also revealed between different experimental groups. When these drugs are used combined, a statistically significant increase in the amount of conjugated dienes was recorded both after exposure to cisplatin alone (18% increase; #p<0.05) and compared with the group receiving dexamethasone alone (42% increase; #p<0.05). At the same time, there were quantitative changes noted in the quantity of conjugated dienes between the experimental groups of animals with cisplatin and dexamethasone alone. Compared to cisplatin alone treated group, dexamethasone reduced the amount of conjugated dienes by 17% (#p<0.05), and vice versa, compared to dexamethasone injected experimental group, the quantity of this lipid peroxidation product increased by 20%; #p<0.05.

Evaluation of Conjugated Trienes in Brain and Kidney Tissues of Rats: Table 3 shows the amount of conjugated trienes (in conventional units) in heptane and isopropanol phases extracted from the nuclear fraction of brain and kidney tissues of rats at baseline (Group 1), after the treatment with cisplatin alone (Group 2), after the treatment with dexamethasone alone (Group 3) and after the combined treatment with cisplatin and dexamethasone (Group 4).

Quantitative analysis data for conjugated trienes extracted from the nuclear fractions of brain and kidney tissues of rats presented in indicate that the quantity of conjugated trienes in heptane phases of both nuclear fractions of brain and kidney tissues was increased significantly in all experimental groups when compared with control group (Table 3). Thus, after the separate action of cisplatin and dexamethasone in nuclear fraction of rat brain tissue the quantities of triene conjugates increased respectively by 153% and by 21% compared to the baseline (*p<0.05). After the combined use of these drugs the quantity of conjugated trienes in heptane phase from nuclear fraction of brain tissue increased by 43% compared to baseline (*p<0.05). The quantitative changes of conjugated trienes were also revealed between different experimental groups. In the group with combined use of cisplatin

TABLE 3

Conjugated trienes in heptane and in isopropanol phases

Groups	Research Conditions	heptane phase		isopropanol phase	
		Brain	Kidney	Brain	Kidney
Group 1 (n=16)	Baseline without treatment	1.83±0.08	0.56±0.03	2.15±0.10	1.40±±0.07
Group 2 (n=16)	Cisplatin alone treatment	4.63±0.21*	1.73±0.04*	5.08±0.24*	2.10±0.10*
Group 3 (n=16)	Dexamethasone alone treatment	2.22±0.10*	1.32±0.06*	4.00±0.18*	1.78±0.08*
Group 4 (n=16)	Cisplatin and dexamethasone combined treatment	2.61±0.12*	1.10±0.04*	6.50±0.30*	2.90±0.03*

NOTE: *Statistical significance between baseline and each experimental group *-p<0.05*

and dexamethasone, the amount of conjugated trienes in the heptane phase of rat brain nuclei was decreased compared to the group injected with cisplatin alone (about 44% decrease; $p<0.05$). Compared to the group injected with dexamethasone alone the amount of triene conjugates increased by only 18%, #$p<0.05$. We also observed changes in the content of conjugated trienes when comparing the experimental groups treated separately with cisplatin and dexamethasone.

Compared to the experimental group injected with cisplatin, dexamethasone reduced the amount of conjugated dienes by 52%, #$p<0.05$. Conversely, compared to dexamethasone-treated group, cisplatin exhibited a stimulatory effect, increasing the amount of conjugated trienes by 108.6%, $p<0.0$.

The quantity of triene conjugates in heptane phase from nuclear fraction of kidney cells increased in comparison to baseline after the cisplatin and dexamethasone alone and combined treatment by 209%, 136% and 96% respectively (*$p<0.05$). The intergroup comparison showed statistically significant changes in amount of conjugated triene between different experimental groups. The amount of conjugated trienes in the heptane phase of rat kidney nuclei from experimental group of animals with combined use of cisplatin and dexamethasone was decreased by 36% ($p<0.05$) when compared with cisplatin only injected experimental group, and was decreased by only 17%, (#$p<0.05$), when compared to experimental group of animals with dexamethasone alone treatment. Significant changes in the amount of triene conjugates in the heptane phase obtained from rat kidney nuclear fraction was also observed when comparing experimental groups of animals with cisplatin and dexamethasone alone treatments. Compared to cisplatin only treated experimental group, dexamethasone reduced the amount of conjugated trienes by 23.7%, #$p<0.05$ and conversely, compared to dexamethasone, in the cisplatin injected group the amount of conjugated trienes was increased by 31% #$p<0.05$. The quantity of conjugated trienes was estimated also in isopropanol phase, extracted from brain nuclear fraction of all experimental groups. In comparison with baseline the quantity of conjugated trienes was increased after the cisplatin alone, dexamethasone alone and combined use by 136%, 86% and 202%, respectively, (*$p<0.05$).

Quantitative changes of conjugated trienes between different experimental groups were also observed. In experimental group of animals co-injected with cisplatin and dexamethasone an increase in the quantity of conjugated trienes was recorded in both, in comparison with the experimental group receiving a dose of cisplatin alone (by 28%; #$p<0.05$) and in comparison with the group of rats injected with dexamethasone alone (by 62.5%; #$p<0.05$).

Significant changes were also observed in the amount of triene conjugates in the isopropanol phase obtained from rat brain nuclear fraction, when comparing experimental groups of animals with cisplatin and dexamethasone alone treatments. In comparison with the group of rats injected with dexamethasone alone cisplatin increased the amount of conjugated trienes by 27% (#$p<0.05$), and vice versa, in comparison to cisplatin, 21.3% decrease was recorded.

Quantitative analysis of triene conjugates in the isopropanol phase, extracted from the nuclear fractions of kidney tissues of all experimental groups of rats is presented in. The obtained data indicate that the quantity of triene conjugates was increased after the cisplatin and dexamethasone alone and combined treatment in comparison to the baseline by 50%, 27% and 107%, respectively. There were statistically significant differences observed in quantity of conjugated trienes between different experimental groups. In case of combined use of these drugs, an increase in the quantity of conjugated trienes was recorded in both, compared to the experimental group receiving a dose of cisplatin alone (by 38%) and compared to the group of rats injected with dexamethasone alone (by 63%).

Statistically significant changes in the amount of conjugated trienes in the isopropanol phase obtained from rat brain nuclear fraction was also observed when comparing the experimental groups of animals with separate injections of cisplatin and dexamethasone. Compared to the experimental group of rats injected with cisplatin, dexamethasone reduced the amount of conjugated trienes by 15%, and conversely, compared to dexamethasone-treated rats, the amount of conjugated trienes increased by 18% in the cisplatin injected group.

Oxidation Index of Conjugated Dienes and Trienes in Rat Brain and Kidney Nuclei: Oxidation

index calculated for conjugated dienes (*D232/D220*) and trienes (*D278/D220*) in the heptane and isopropanol phases extracted from nuclei of rat brain and kidney cells at baseline, after treatment with cisplatin alone, after treatment with dexamethasone alone and after the cisplatin and dexamethasone combined treatment (Cisplatin+Dexamethasone) (Table 4).

Based on the results of quantitative assessment of lipid peroxidation products in the investigated experimental groups the oxidation index for diene and triene conjugates from both heptane and isopropanol phases were evaluated.

The oxidation index was calculated as the ratio of optical densities recorded at wavelengths of 232 *nm* and 278 *nm* and optical absorption values at 220 *nm* (unsaturated fatty acids) [*Volchegorsky I et al.,1989*]. The results are shown in table 4. Assuming the values recorded in the control group as 100%, the changes in the oxidative index values expressed in % after exposure to cisplatin and dexamethasone separate and combined action were calculated.

Thus, oxidative index values calculated for conjugated dienes and trienes increased to varying extents as a result of both cisplatin and dexamethasone separate and combined exposure. The greatest changes in oxidation index values of conjugated dienes and conjugated trienes were recorded in the heptane phase extracted from rat brain and kidney nuclei after exposure to cisplatin. Thus, the oxidation index value of conjugated dienes increased by 190% in the heptane phase of the brain nuclei, and by 231% in the heptane phase of kidney nuclei.

The changes in oxidation index values calculated for conjugated trienes in the heptane phase of the studied nuclear fractions after exposure to cisplatin were 146% in brain nuclei and 179% in kidney nuclei. As a result of exposure to dexamethasone, the changes in oxidation index values in the heptane phase obtained from rat brain nuclei amount to 12% for conjugated dienes and 23% for conjugated trienes. After exposure to dexamethasone in the heptane phase obtained from kidney nuclei, the oxidation index value of conjugated dienes increased by 94%, and for conjugated trienes by 115%. Following the combined use of cisplatin and dexamethasone, oxidation index values for conjugated dienes and trienes changed as follows. In heptane phases obtained from rat brain and kidney nuclear fractions, oxidation index values for conjugated dienes increased by 20% and by 35%, respectively and for conjugated trienes, by 57% and by 103%, respectively.

Oxidation index values calculated for both conjugated dienes and conjugated trienes increased by 81% after exposure to cisplatin in the isopropanol phase obtained from rat brain nuclei. After exposure to dexamethasone in the same isopropanol phase, the oxidation index value of conjugated dienes increased by 87% and for conjugated trienes, by 56%. As a result of the combined treatment with cisplatin and dexamethasone the oxidation index values calculated for conjugated dienes and conjugated trienes in the isopropanol phase of the rat brain nuclei increased by 133% and 86%, re-

TABLE 4

Value of oxidation index for conjugated dienes (D_{232}/D_{220}) and trienes ($D_{278}/_{D220}$) in the heptane and isopropanol phases

Experimental groups	Research Conditions	Brain nuclei		Kidney nuclei	
		D_{232}/D_{220}	D_{278}/D_{220}	D_{232}/D_{220}	D_{278}/D_{220}
Heptane phase					
Group 1 (n=16)	Baseline without treatment	0.143	0.065	0.124	0.062
Group 2 (n=16)	Cisplatin alone treatment	0.414	0.160	0.410	0.173
Group 3 (n=16)	Dexamethasone alone treatment	0.160	0.080	0.240	0.133
Group 4 (n=16)	Cisplatin and dexamethasone combined treatment	0.172	0.102	0.167	0.126
Isopropanol phase					
Group 1 (n=16)	Baseline without treatment	0.196	0.140	0.350	0.322
Group 2 (n=16)	Cisplatin alone treatment	0.355	0.254	0.512	0.384
Group 3 (n=16)	Dexamethasone alone treatment	0.366	0.219	0.397	0.303
Group 4 (n=16)	Cisplatin and dexamethasone combined treatment	0.456	0.260	0.455	0.400

spectively. In the isopropanol phase obtained from rat kidney nuclei, after exposure to cisplatin, oxidation index values of conjugated dienes and for conjugated trienes increased by 46% and by 19%, respectively.

Dexamethasone increased the oxidation index value of conjugated dienes by 13%, while the value for conjugated trienes remained unchanged. In the studied isopropanol phase, the combined treatment of animals with cisplatin and dexamethasone increased the oxidation index value calculated for conjugated dienes by 30% and the oxidation index value of conjugated trienes by 24%.

Discussion

Steroids play various roles in cancer treatment. First, they sometimes are being used as part of the treatment for the cancer itself, such as in some lymphomas and multiple myeloma. Second, they are very effective in reducing nausea and vomiting related to chemotherapy. Although steroids, especially dexamethasone, do not interfere with the cytotoxic action of cisplatin in some cell lines [*Wagenblast J et al., 2010*], it is well known for their frequent use in preventing side effects of chemotherapy, such as nausea, vomiting, and pain. It is also recognized for enhancing the anti-tumor activity of cancer chemotherapeutic agents as a chemosensitizer and for inhibiting tumor growth as an anticancer agent in certain cancers [*Wagenblast J et al., 2010*].

As previously mentioned, both cisplatin and dexamethasone function as pro-oxidants, stimulating oxidative stress and promoting the generation of reactive oxygen species [*Casares C et al., 2012; Chow R et al., 2018; Sverediuk Yu, Pelykh V, 2020; Mirzaei S et al., 2021*]. It is also well-established that the unwanted side effects and toxicities of cisplatin are primarily due to the oxidative stress induced by this drug [*Casares C et al., 2012; Aldossary S, 2019; Jadon A et al., 2019; Mirzaei S, 2021; Dasari S et al., 2022*]. To increase the effectiveness of cisplatin and mitigate its toxicity, various drugs are used in chemotherapeutic practice, among which dexamethasone has an important role. Despite the fact that dexamethasone is a pro-oxidant, due to its anti-inflammatory and immunomodulatory properties, it is used to alleviate the undesirable side effects of cisplatin [*Cook A et al., 2016; Chow R et al., 2018*].

It is known that both, the generation and functioning of reactive oxygen species occur at distinct subcellular sites. In addition to the cytosol, they are formed in the cell membrane and various organelles: mitochondria, nucleus, etc. [*Paardekooper L et al., 2019*]. It was found that the cell viability was only affected upon reactive oxygen species induction in the nucleus and chromatin [*Provost C et al.,2010; Kreuz S, Fischle W, 2016; Paardekooper L et al., 2019*]. Oxidative stress affects chromatin global structure, mediated by the engagement of histone modifications caused by reactive oxygen species [*Kreuz S, Fischle W, 2016*]. Additionally, it is known that lipids, included in the nucleus and intranuclear structures, perform important regulatory functions [*Hakobyan N et al., 2023*]. Since lipids are the main target of reactive oxygen species, their nuclear variants may also be oxidized by them. In this case, regulation of chromatin activity by nuclear lipid peroxidation products is not excluded. According to some authors, nuclear reactive oxygen species production induced cell death [*Paardekooper L et al., 2019*].

Our research revealed the quantitative changes of unsaturated fatty acids (raw material for lipid peroxidation) and lipid peroxidation products conjugated dienes and conjugated trienes in the nuclear fraction of rat brain and kidney of rats after the separate and combined exposure of cisplatin and dexamethasone. We recorded lipid peroxidation products in the heptane and isopropanol phases extracted from the nuclear fraction of rat brain and kidney tissues. The obtained results indicate that there was no quantitative change in unsaturated fatty acids in the heptane phase extracted from the nuclear fraction of rat brain cells in all experimental groups compared with the baseline. In comparison to the baseline, no changes were observed in the quantity of unsaturated fatty acids in the heptane phase extracted from rat kidney nuclei in the experimental group with the combined use of cisplatin and dexamethasone (Table 1). However, with the separate use of these drugs, a slight increase in the amount of unsaturated fatty acids has been reported. Significant quantitative changes in unsaturated fatty acids, compared with baseline, were recorded in the isopropanol phases extracted from the nuclei of the studied tissues. Changes to varying degrees were recorded only in experimen-

tal groups with combined use of these drugs, when compared with both cisplatin and dexamethasone alone injection (Table 1).

It is known that cisplatin, like other anticancer drugs, is able to block all processes that contribute to the growth of tumor cells [*Plathow C, Weber W, 2008; Jadon A et al., 2019*]. Cisplatin suppresses all cellular metabolic processes, including lipid metabolism [*Plathow C, Weber W, 2008; Jadon A et al., 2019; Dasari S et al., 2022*]. Fatty acid synthase, a key enzyme in the synthesis of long-chain fatty acids and overexpressed in developing cancer cells, is a target of cisplatin [*Plathow C, Weber W, 2008; Liu H et al., 2010]*. It is also well known that this anticancer drug can not only suppress the synthesis of lipids, but also promote their cleavage, freeing fatty acids, including unsaturated ones [*Plathow C, Weber W, 2008; Dasari S et al., 2022*]. The increase of quantity of unsaturated fatty acids in the isopropanol phase extracted from the nuclei of the investigated tissues is probably a result of cleavage of various lipids. As for dexamethasone, like other glucocorticoids, it can increase the amount of fatty acids in different ways. Glucocorticoids can stimulate both lipase activity and *de novo* synthesis of fatty acids [*Sverediuk Yu, Pelykh V, 2020; Alahmar A et al., 2023*]. In addition, the unaltered levels of unsaturated fatty acids may be related to the activation of lipid peroxidation processes by cisplatin and dexamethasone. The activation of lipid peroxidation processes was evidenced by the corresponding double and triple increase in the quantity of conjugated dienes in the heptane phases extracted from the nuclear fractions of the brain and kidney of rats as a result of exposure to cisplatin (Table 2). Quantitative changes of conjugated dienes induced by dexamethasone were modest in comparison to the cisplatin effect (Table 2). The quantitative changes of conjugated dienes and trienes are more pronounced in both the heptane and isopropanol phases obtained from nuclear preparations after separate exposure to cisplatin (Tables 2, 3). In these same phases, the amounts of both conjugated dienes and trienes also increased compared to the control following exposure to dexamethasone alone treatment. However, these changes were relatively smaller compared to the effects caused by cisplatin. Naturally, this circumstance influenced the results of the intergroup data comparison. When comparing the changes in the amounts of conjugated dienes and trienes observed in the experimental group of animals treated with a combination of these prooxidants to those in the group treated solely with cisplatin, the changes were generally more modest than those compared to the group treated with dexamethasone alone (Tables 2, 3).

The advantage of the prooxidant effect of cisplatin becomes obvious, when the quantitative changes of conjugated dienes and trienes of the animals from experimental group receiving cisplatin or dexamethasone separately are compared with each other. Compared to dexamethasone, cisplatin increased the amount of conjugated dienes and trienes in the investigated preparations, while on the contrary, dexamethasone decreased these levels when compared to cisplatin (Tables 2, 3).

The calculated oxidation index values for conjugated dienes and trienes were affected to varying degrees by the separate and combined use of cisplatin and dexamethasone. The only exception was observed in the oxidation index values calculated for conjugated trienes in the isopropanol phase from kidney nuclei following treatment with the dexamethasone alone, where no change was detected. For conjugated dienes and trienes, rather high percentage changes in the oxidation index are recorded, especially in the heptane phase obtained from brain and kidney nuclei after separate exposure to cisplatin. The calculated values for the isopropanol phase after separate exposure to cisplatin were relatively low (Table 4).

Thus, both exposure to cisplatin and dexamethasone, separately and in combined use, causes profound changes in the content of lipid peroxidation products (conjugated dienes and trienes) of rat brain and kidney nuclear fractions across all experimental groups. Similar effects of cisplatin and dexamethasone when used separately was also shown by other researchers [*Cook A et al., 2016; Aldossary S, 2019; Jadon A et al., 2019; Sverediuk Yu, Pelykh V, 2020; Dasari S et al., 2022*].

CONCLUSION

Results of this study once again confirm the prooxidant nature of cisplatin and dexamethasone. Quantitative changes of conjugated dienes and tri-

enes, along with their oxidation index values, indicate that both cisplatin and dexamethasone induce the formation of oxidative stress and activate the lipid peroxidation process in nuclear fractions of rat brain and kidney. It should be noted that the enhancing effect of cisplatin is much greater than the effect of dexamethasone, when these drugs are used separately. In the case of combined treatment with cisplatin and dexamethasone there was some antagonism in the action of these drugs. Contrary to the expected synergistic enhancement of lipid peroxidation processes by both prooxidants, a reduction in the effect of cisplatin due to dexamethasone was noted.

Thus, it is hypothesized that this antagonistic effect of dexamethasone, combined with its anti-inflammatory and immunomodulatory properties, allows to mitigate the side effects of cisplatin.

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