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Lipid peroxidation level and histological changes in rat liver after the cisplatin and dexamethasone separate and combined action

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Cisplatin is known to exhibit pro-oxidative properties, which are responsible for various toxicities caused by this drug, including hepatotoxicity. Dexamethasone, which is known as anti-inflammatory and immunomodulatory drug, is used with cisplatin to mitigate its side effects. However, dexamethasone, like other glucocorticoids, can induce oxidative stress and lipid peroxidation processes. In addition, it is known that dexamethasone causes liver damage and hepatotoxicity.

The aim of this study was to clarify how dexamethasone, having a similar effect to cisplatin, alleviates the side effects caused by this antitumor drug.

Our studies have shown that cisplatin and dexamethasone increase the formation of lipid peroxidation products conjugated dienes and trienes of rat's liver to varying degrees extent in the case of separate and combined injection. In addition, cisplatin and dexamethasone were shown to increase the amount of lipid peroxidation marker malondialdehyde (MDA), in the rat liver tissue homogenate after separate and combined administration. These changes, as well as a decrease in the activity of the antioxidant enzyme catalase, confirm the pro-oxidative nature of cisplatin and dexamethasone. Moreover, the histopathological studies also testify to their hepatotoxic effect.

However, contrary to the expected synergistic enhancement of both lipid peroxidation processes, and histological changes, a reduction in cisplatin effect by dexamethasone was observed.

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

Keywords: Catalase, Hepatotoxicity, Histological changes, Malondialdehyde, Reactive oxygen spaces

Currently the most effective in the field antitumor drug cisplatin (cis-diaminedichloroplatinum) used in chemotherapeutic practice often causes undesirable side effects that affect various organ systems and disrupt their normal functioning[1,2]. Cisplatin-induced side effects manifest in the form of various toxicities such as nephro, neuro, cardio and other toxicities[1,2]. Cisplatin also induced hepatotoxicity, which is another important dose-limiting side effect of cisplatin-based chemotherapy[3]. Studies have shown that the reactive oxygen spaces (ROS) formation and oxidative stress are at the root of antitumor drug cisplatin-induced toxicities[4]. Cisplatin induces the formation of ROS, which in turn can interact with DNA, lipids and proteins, leading to protein carbonylation, lipid peroxidation and DNA damage[5-7]. The induction of oxidative stress and the formation of ROS are considered as novel mechanism of cisplatin action[2,8].

It is well known that in normal physiological conditions the presence of ROS is vital for the normal functioning of cells[9]. Moreover, a certain physiological amount of ROS is maintained by balancing the processes of their generation and destruction, that is, due to the oxidant/antioxidant balance. This imbalance arises from either increased production of oxidants, decreased levels of antioxidants, or both[6,10]. Cisplatin disrupts the oxidant/antioxidant balance by both inducing ROS formation and reducing antioxidant levels[7,10].

The primary targets of ROS are lipids, which undergo oxidation with lipoperoxyl radicals and lipid hydro peroxides formation upon interaction with oxidants[6,11]. Lipid hydro peroxides have been recognized as key mediators of cellular disease and death[10,11]. The products of lipid peroxidation play role in the intracellular signaling mechanisms that

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determine the cell's ultimate fate[5]. Cisplatin can increase lipid peroxidation, thereby altering enzymes and structural proteins, and steering the cell to an apoptotic pathway[10]. Lipid peroxide products are unstable compounds that break down to form a number of aldehydes, including malondialdehyde[9,12]. The amount of malondialdehyde expresses the intensity and level of oxidative stress and the resulting lipid peroxidation. That's why the MDA is considered an oxidative stress and lipid peroxidation marker[9,12]. Malondialdehyde, in turn, disrupts the functions of various biomacromolecules by direct binding to them or cross-linking them to each other through Schiff bases[9,12].

To mitigate cisplatin-induced toxicities often in chemotherapy regimens, dexamethasone is used as a concomitant agent[13,14]. Dexamethasone is a synthetic glucocorticoid with anti-inflammatory and immunosuppressant properties[13,14]. Besides, dexamethasone induced alterations in lipid peroxidation products and antioxidants content in Wistar albino rats[14]. Dexamethasone is not only a potent glucocorticoid with several health benefits but is also associated with severe side effects, one of which is hepatotoxicity[15,16]. Dexamethasone is known to cause liver damage because it is a pro-oxidant and can induce oxidative stress. It was found that the effect of dexamethasone on the liver is dose-dependent. Low doses of dexamethasone can have beneficial effects, while high doses have harmful effects[15,16]. Thus, both the antitumor drug cisplatin and dexamethasone stimulate the formation of reactive oxygen species and increase the degree of lipid peroxidation[1,13,16,17]. In addition, both of these drugs cause hepatotoxicity[1,15]. Cisplatin as well as dexamethasone also induced morphological impairments in rat liver cells[15,18].

In light of the above it seems interesting to investigate the quantitative changes of lipid peroxidation products and the activity of antioxidant enzyme catalase in case of cisplatin and the synthetic glucocorticoid dexamethasone separate and combined use.

The aim of this study is to determine how dexamethasone, having effects similar to cisplatin, alleviates the side effects caused by this drug. To that end, the effects of cisplatin and dexamethasone on the level of lipid peroxidation, the antioxidant enzyme catalase activity and the morphological changes in normal non-cancerous liver cells should be identified.

Materials and methods

Animal care and design of experiment

The study was performed on adult Wistar albino female rats (120-150 g weight, 16 rats). Animals were placed in a controlled environment at a temperature of 25±2°C, 12 h day and night cycle, fed commercial rat feed ad-libitum, and given free access to water in the animal house of the faculty of biology at YSU.

The animals were divided into 4 groups. Group 1 served as a control group of animals without treatment. Animals in group 2 (n=4) and group 4 (n=4) received a single dose of cisplatin (8 mg/kg) by peritoneal injection and were decapitated 24 h after administration. Group 3 was treated with dexamethasone (4 mg/kg, peritoneal injection) and decapitated 4 h after administration. Animals in group 4 (n=4) received the same single dose of dexamethasone within 20 h after the cisplatin injection (4 h before decapitation). All animals were euthanized by decapitation at an appropriate time following inhalation ether anesthesia. Then, animals were sacrificed, and the liver was extracted from each group of animals and used for isolation of nuclei by the method of Blobel and Potter[19] and for histopathological examination.

Quantitative assessment of lipid peroxidation products

The nuclear fraction of liver 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. Conjugated dienes and trienes were estimated by spectrophotometric method, after its extraction with heptane-isopropyl alcohol mixture[20]. 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 of nuclei from liver tissue by heptane-isopropyl alcohol mixture. Heptane extracts contain mainly neutral lipids, while isopropanol extracts contain phospholipids. This method allows identifying 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)[20]. The amount of unsaturated fatty acids,

conjugated dienes and trienes is expressed in conventional units[20]. After recording the amount of lipid peroxidation products in the heptane and isopropanol phases, their total quantity was calculated. Then the oxidation index values for conjugated dienes and trienes were determined. For this purpose, the ratio of total amounts of conjugated dienes or trienes and unsaturated fatty acids were calculated[20].

The malondialdehyde amount estimation

The malondialdehyde amount was estimated in 10% homogenate of rat liver tissue by Mihara *et al*., method[21]. The assay was based on a condensation reaction of two molecules of thiobarbituric acid with one molecule of malondialdehyde, in which the reaction rate depends on temperature, pH value and concentration of thiobarbituric acid. The reaction mixture contains 30% trichloroacetic acid, 5 N HCl, 0.8% solution of thiobarbituric acid and biological sample 1mL each[21]. After the heating for 20 min in a boiling water bath and after cooling, the solution was centrifuged at 3000 rpm/min for 10 min and the precipitate obtained was removed[21]. The absorbance of the pink supernatant was determined at 532nm against a blank that contained all reagents without the biological sample. The malondialdehyde concentration (in nmol/mg protein) was calculated using the appropriate formula[21].

Catalase enzyme activity assay

Catalase (EC 1.11.1.6) activity in blood plasma of female rats was estimated by method[22]. The method is based on the rate of H_2O_2 degradation by the action of catalase. The activity of catalase was determined by measuring a decrease in the hydrogen peroxide (H_2O_2) concentration at 410 nm. The method of defining of catalase activity was based on developing of stabile blue colored complex in result of ammonium molybdate reaction with H_2O_2 and subsequent photometric measurement of the recovered complex[22]. The enzyme activity was expressed in μmol H_2O_2 /min mg protein per min. The protein amount was determined by spectrophotometric method[23].

Histopathological examination

For histological examination, the liver section of rats from all experimental groups were removed and fixed with 10% formalin solution according to the protocol[24]. The fixed material was subjected to normal histological processing and enclosed in paraffin embedding. Further, about 5-6 μm thick paraffin sections were cut and stained with hematoxylin-eosin and impregnated with silver nitrate. Hematoxylin-eosin and silver nitrate stained preparates were observed under a light microscope in a blind manner (Nikon Labophot, Japan) used to examine the morphological alterations in rat liver.

Statistical analysis of data

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 experimental groups were performed using Statgraphics Centurion 19 Software (Stagraphics Technologies, Inc., USA). Statistical comparisons between all experimental groups were tested by analysis of variance (ANOVA), and $^{\#}P < 0.05$ significant differences in case of intergroup comparison.

Results

Our research we began with quantitative evaluation of total unsaturated fatty acids extracted from nuclei of liver tissue of rats of all experimental groups. The obtained data indicate that compared to baseline statistically significant difference of the total quantity of unsaturated fatty acids was obtained in experimental group with cisplatin alone treatment (by 24%, $^*P <$ 0.05), after dexamethasone alone action by 18%, $^*P <$ 0.05. There were no changes in the amount of unsaturated fatty acids in experimental group with combined use of cisplatin and dexamethasone (increase by 7% is not statistically significant). The statistically significant change was obtained, when comparing the data between experimental groups. Thus, cisplatin separate injection increased the total amount of unsaturated fatty acids by 16% ($^{\#}P < 0.05$) in comparison to group of rats with combined use of cisplatin and dexamethasone (Table 1).

The results of quantitative evaluation of conjugated dienes reveal significant changes in the total amount of this product from nuclear fraction of liver cells in comparison to the control group (Table 1).

The total amount of conjugated dienes increased by about 184% ($^*P < 0.05$), by 53% ($^*P < 0.05$) and by 44% ($^*P <$ 0.05) respectively after the separate treatment with cisplatin, with dexamethasone alone and after the combined treatment of these drugs in comparison to baseline. The intergroup comparison also revealed statistically significant changes between the different experimental groups. There was a 97% ($^{\#}P < 0.05$) increase in the total amount of conjugated

Table 1 — The total amounts of unsaturated fatty acids, conjugated dienes and trienes (in conventional units) from the nuclear fractions of liver tissue of rats at baseline (Group 1), after treatment with cisplatin alone (Group 2), after treatment dexamethasone alone (Group 3) and after the combined treatment with cisplatin and dexamethasone (Group 4). Statistical significance between baseline and each experimental group is indicated *-$P< 0.05$

Lipid peroxidation products	Group 1 (n=4) Baseline without treatment	Group 2 (n=4) Cisplatin alone treatment	Group 3 (n=4) Dexamethasone alone treatment	Group 4 (n=4) Cisplatin and dexamethasone combined treatment
Unsaturated fatty acids	40.26±1.54	50.00±1.20*	47.56± 1.30*	43.15±1.43
Conjugated dienes	8.22±0.37	23.30±1.02*	12.56±0.46*	11.84±0.52*
Conjugated trienes	4.38±0.15	10.37±0.52*	7.08±0.22*	8.65±0.30*

Each experiment was performed 4 times. Values are represented as Mean ± SE

Table 2 — Oxidation index calculated for conjugated dienes and trienes in the nuclear fraction of rat liver cells at baseline (Group 1), after treatment with cisplatin alone (Group 2), after treatment with dexamethasone alone (Group 3) and after the cisplatin and dexamethasone combined treatment (Group 4).

Experimental groups	Group 1 (n=4) Baseline without treatment	Group 2 (n=4) Cisplatin separate treatment	Group 3 (n=4) Dexamethasone alone treatment	Group 4 (n=4) Cisplatin and dexamethasone combined treatment
Oxidation index values for conjugated dienes	0.204	0.466	0.265	0.274
Oxidation index values for conjugated trienes	0.109	0.207	0.149	0.200

Each experiment was performed 4 times.

dienes when comparing the experimental group of animals receiving cisplatin alone and the experimental group with combined injection of cisplatin and dexamethasone (Table 1).

At the same time, no change in the amount of conjugated dienes was recorded when comparing the experimental groups treated with dexamethasone alone and animals that received the combined injection of cisplatin and dexamethasone (Table 1).

Statistically significant increase in the total amount of conjugated dienes (by 86 %, $^{\#}P< 0.05$) was recorded, in the group of animals treated with cisplatin alone in comparison with experimental group injected with dexamethasone alone. On the contrary, a statistically significant reduction of conjugated dienes quantity by 46% ($^{\#}P< 0.05$) after exposure to dexamethasone was recorded when compared with the group of animals with cisplatin alone treatment (Table 1). Quantitative analysis data for conjugated trienes extracted from the nuclear fraction of liver tissue of rats indicate that the total amount of this lipid peroxidation product was increased significantly in all experimental groups when compared with control group. Thus, after the separate action of cisplatin and dexamethasone the total quantity of conjugated trienes increased respectively by 137% and by 62% (*$P< 0.05$) compared to the baseline (Table 1). The total quantity of conjugated trienes from nuclear fraction of liver cells increased by 98% (*$P< 0.05$) after the combined use of these drugs compared to baseline. The statistically significant quantitative changes of conjugated trienes were also revealed between different experimental groups. Cisplatin alone treatment increased the amount of conjugated trienes by 20%, $^{\#}P< 0.05$ compared to the group with combined use of cisplatin and dexamethasone. In the same time decrease in the total amount of conjugated trienes by 18%, $^{\#}P< 0.05$ was observed when comparing the data of group of animals treated with dexamethasone alone to the group with combined use of cisplatin and dexamethasone. Compared to the data of experimental group injected with dexamethasone alone, cisplatin exhibited a stimulatory effect, increasing the amount of conjugated trienes by 46%, $^{\#}P< 0.05$. Conversely, compared to cisplatin alone treated group, dexamethasone reduced the amount of conjugated trienes by 32%, $^{\#}P< 0.05$ (Table 1).

Based on the results of quantitative assessment of lipid peroxidation products in the investigated experimental groups the oxidation index for conjugated dienes and trienes were evaluated (Table 2).

Thus, oxidation index values calculated for conjugated dienes and trienes increased to varying extents as a result of both cisplatin and dexamethasone separate and combined exposure (Table 2). The

greatest changes in oxidation index values were recorded for conjugated dienes and trienes extracted from rat liver nuclei after exposure to cisplatin respectively by 128% and 90%. After exposure to dexamethasone, the oxidation index values for conjugated dienes increased by 30%, and for conjugated trienes increased by 37% in compare to baseline. Combined use of cisplatin and dexamethasone increased the oxidation index values for conjugated dienes and trienes respectively by 34% and by 84% (Table 2).

The results confirm that compared to the baseline statistically significant changes of MDA quantity were revealed in all experimental groups (Table 3). Thus, the amount of MDA in liver homogenate increased by 28%; *P< 0.05, by 39%, *P< 0.05 and by 23%, compared to the baseline (*P< 0.05) respectively after cisplatin separate treatment, after separate injection of dexamethasone and after the co-injection of this drugs compared to the baseline. The statistically significant quantitative change of MDA (increase by 12%, $^{\#}$ P< 0.05) was also revealed in case of comparing the results of experimental group with dexamethasone alone treatment with the experimental group co-injected with cisplatin and dexamethasone. In the same time no statistically significant changes in the amount of MDA were observed in case of comparing of results between other groups (Table 3).

In order to find out how pro-oxidant cisplatin and dexamethasone stimulate the lipid peroxidation processes the activity of the antioxidant enzyme catalase in the nuclear fractions of the investigated tissues was determined. The obtained data testify that the activity of catalase was decreased by 18% (*P< 0.05)[25], by 24.6% (*P< 0.05) and by 27% (*P< 0.05) respectively in case of cisplatin and dexamethasone separate and co-injection in compare to baseline. The comparison of data between different experimental groups of animals was not revealed statistical significance changes in catalase enzyme activity (Table 4).

Cisplatin and dexamethasone are known to induce hepatotoxicity by stimulating oxidative stress, ROS accumulation, and lipid peroxidation processes[9,13-15]. In order to visualize the hepatotoxic effect of applied pro-oxidants, histopathological studies were carried out. The histological studies revealed pathological changes in the liver tissue of rats after cisplatin exposure in compare to baseline (Fig. 1-I, b and B).

These changes manifested as various destructive alterations. When viewed under low magnification of the microscope, foci of necrosis and steatosis were identified after cisplatin alone action (Fig. 1-I, b). In addition, the accumulations of lymphocytes in the peripheral areas of the liver lobes were revealed, which indicate inflammatory foci (Fig.1-I, B). In case of dexamethasone alone injection, foci of steatosis in the liver tissue of experimental animals were observed. Accumulations of quite a large number of vacuolated hepatocytes combined with normal hepatocytes were also recorded (Fig. 1-I, c and C). In the liver of experimental animals with cisplatin alone treatment, alterations in the connective tissue support were observed.These changes were particularly evident in liver preparations treated with silver nitrate (Fig. 1-II, b and B). Thus, in the liver of rats, from experimental

Table 3 — The amount of malondialdehyde (MDA) (in nmol/mg protein) in 10% homogenate of liver tissue of rats at baseline (Group 1), after treatment with cisplatin alone (Group 2), after treatment with dexamethasone alone (Group 3) and after the combined treatment with cisplatin and dexamethasone (Group 4). Statistical significance between baseline and each experimental group is indicated *-P< 0.05

Experimental groups	Group 1 (n=4) + Baseline without treatment	Group 2 (n=4) + Cisplatin separate treatment	Group 3 (n=4) Dexamethasone alone treatment	Group 4 (n=4) Cisplatin and dexamethasone combined treatment
MDA quantity	2.65±0.06	3.40±0.17*	3.67±0.15*	3.27±0.07*

Each experiment was performed 4 times. Values are represented as Mean ± SE
+- results from article[25]

Table 4 — The catalase enzyme activity (μmol/min, mg protein) in nuclear fraction of liver of rats at baseline (Group 1), after treatment with cisplatin alone (Group 2), after treatment with dexamethasone alone (Group 3) and after the combined treatment with cisplatin and dexamethasone (Group 4). Statistical significance between baseline and each experimental group is indicated *-P< 0.05

Experimental groups	Group 1 (n=4) ++ Baseline without treatment	Group 2 (n=4) ++ Cisplatin separate treatment	Group 3 (n=4) Dexamethasone alone treatment	Group 4 (n=4) Cisplatin and dexamethasone combined treatment
Catalase enzyme activity	340.90±8.56	280.00±8.56*	257.00±6.80*	250.00±7.00*

Each experiment was performed 4 times. Values are represented as Mean ± SE
++ data taken from article[26]

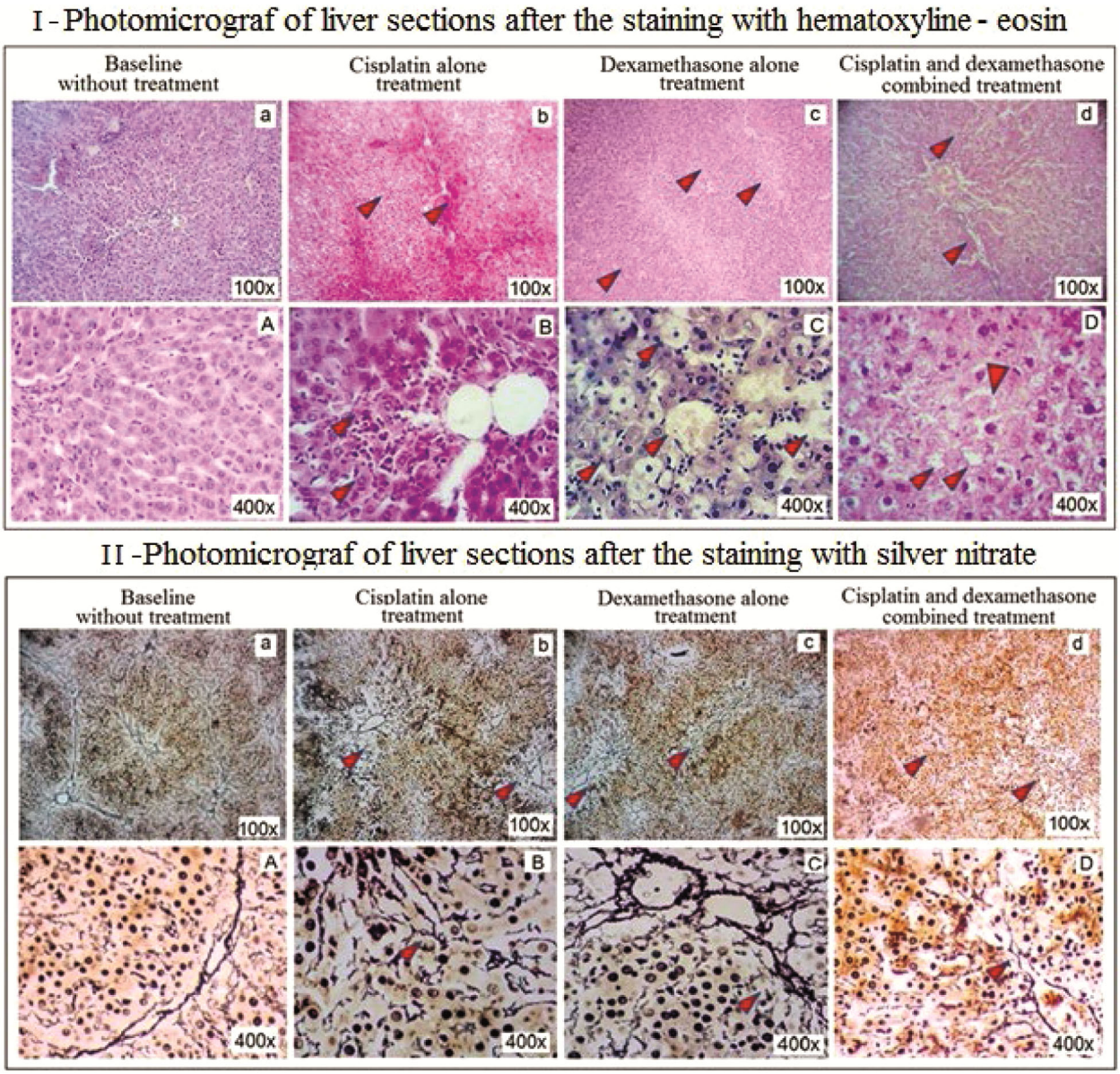

Fig. 1 — Photomicrograph of liver sections after the staining with hematoxylin-eosin (I) and with silver nitrate (II). Photomicrograph of liver sections in the control group (a and A), after administration of cisplatin (b and B), after dexamethasone injection (c and C), after combined use of cisplatin and dexamethasone (d and D). a, b, c and d – magnification:100x; A, B, C and D - magnification:400x and D). Only in this case, necrotic changes are not observed on the same scale as in the case of cisplatin (Fig. 1-I and 1-II, d and D)

group of animals with cisplatin treatment, partial destruction of collagen fibers were observed (Fig. 1-II, b and B). The similar discernible changes in the connective tissue support were revealed also in case of combined use of cisplatin and dexamethasone (Fig. 1-II, d and D).

In the same time no discernible changes in the connective tissue support were observed when comparing the livers of animals with dexamethasone injection and the livers of animals from control group (Fig. 1-II, a and A, c and C). In the liver of animals with dexamethasone alone treatment parenchymal changes manifest as focal steatosis and vacuolization of hepatocytes (Fig. 1-II, c and C).

Changes in the liver of rats receiving dexamethasone along with cisplatin are similar to those in rats receiving cisplatin alone. There are also many vacuolized hepatocytes in this slides (Fig. 1-I and 1-II, d).

In the same time no discernible changes in the connective tissue support were observed when comparing the livers of animals with dexamethasone

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Changes in the liver of rats receiving dexamethasone along with cisplatin are similar to those in rats receiving cisplatin alone. There are also many vacuolized hepatocytes in this slides (Fig. 1-I and 1-II, d and D). Only in this case, necrotic changes are not observed on the same scale as in the case of cisplatin (Fig. 1-I and 1-II, d and D).

Discussion

It is known that both, the generation and functioning of ROS are take place at distinct sub cellular sites. In addition to the cytosol, ROS are formed in the cell membrane and various organelles: mitochondria, nucleus, *etc*[27]. It was found that the cell viability was affected upon ROS only inducing in the nuclei and chromatin[27-29]. Taking into account the regulatory role of nuclear lipids[30], the regulation of chromatin activity by their oxidation products cannot be excluded. Since unsaturated fatty acids are the starting material of lipid peroxidation processes, it was important to first determine their amount in the investigated preparations.

Our studies revealed a small quantitative increase in unsaturated fatty acids only when cisplatin and dexamethasone were used separately compared to the control. Similar to other anticancer drugs, cisplatin is known to suppress metabolic processes (including lipids) that promote cell growth[2,31]. Cisplatin has been found not only to be able to suppress the activity of the enzyme fatty acid synthase (FASN), but also to suppress lipid metabolism by activating enzymes that break down lipids and release fatty acids[31,32]. In that regard, the obtained increase of unsaturated fatty acids quantity do not contradict the data proving the ability of cisplatin to suppress lipid metabolism[31]. As for dexamethasone, then like other glucocorticoid, it can stimulate lipase activity as, well, as de novo synthesis of fatty acids and the hepatic de novo lipogenesis pathway[33,34]. Besides the low level of quantitative changes of total unsaturated fatty acids may be associated with the activation of lipid peroxidation processes and the formation of conjugated dienes by cisplatin and dexamethasone. And indeed, the obtained data prove that in the nuclei from rat liver cisplatin increased the amount of conjugated dienes more than 2.5 times (by 184%) compared to the control (Table 1). At the same time, compared to the control modest changes in the quantity of total conjugated dienes were obtained both in case of dexamethasone alone treatment and in case of co-injection cisplatin and dexamethasone.

The results of the intergroup comparison indicate the strength of the effect caused by cisplatin compared to both dexamethasone alone and the combined use of these drugs (Table 1). Both with control and intergroup comparisons also revealed significant quantitative changes in conjugated trienes (Table 1). The obtained data, as well as the values of oxidation indices for conjugated dienes and trienes, show that the applied pro-oxidants stimulate lipid peroxidation processes to a different extent when used separately or together (Tables 1 & 2).

These results clearly indicate the stimulation of lipid peroxidation processes and oxidative stress by cisplatin and dexamethasone.

It is well known that lipid peroxidation of polyunsaturated fatty acids resulting in the production of aldehydes, such as malondialdehyde, 4-hydroxy-2-nonenal (4-HNE), acrolein and other toxic substances. Among these aldehydes 4-hydroxynonenal is the most reactive and specific lipid peroxidation product. It is of great scientific interest due to its toxic effect and signaling functions. However, unlike MDA, the analysis of 4-HNE is more complicated[35]. Considering that MDA is still an accepted marker of oxidative stress and lipid peroxidation levels[12], we preferred to determine the amount of MDA.

An increase in the amount of MDA was recorded as a result of separate and co-injection of cisplatin and dexamethasone (Table 3). Changes in the amount of MDA after exposure to the mentioned pro-oxidants were also recorded by other investigators[33,36].

Thus, it should be noted that both exposure to cisplatin and dexamethasone, separately and to its combined use, causes profound changes in the content of lipid peroxidation products (conjugated dienes, conjugated trienes and MDA) of rat liver tissue across all investigated experimental groups.

It is well known that there is a powerful antioxidant system in the cells, the main components of which are antioxidant enzymes[9,10]. Among these enzymes belongs catalase, the activity of which was recorded in the nuclear fraction obtained from the livers of rats of different experimental groups. According to the results of the research dedicated to the separate and combined influence of cisplatin and dexamethasone

on the activity of the antioxidant enzyme catalase, applied pro-oxidants reduce the activity of this enzyme both, when used alone and when co-injected. The decrease in catalase activity indicates the way cisplatin and dexamethasone affect lipid peroxidation processes[1,2,10].

Cisplatin and dexamethasone are known to induce hepatotoxicity by stimulating oxidative stress, ROS accumulation, and lipid peroxidation processes. In order to visualize the hepatotoxic effect of applied pro-oxidants, histological studies were carried out. The obtained results are shown that although cisplatin and dexamethasone are both pro-oxidants, they cause different histological changes of liver tissue. The histological studies revealed pathological changes in the liver tissue of rats after cisplatin exposure in compare to baseline (Fig. 1-I, b and B). These changes manifested as various destructive alterations after cisplatin exposure in compare to baseline (Fig. 1-I, b and B). The accumulations of lymphocytes in the peripheral areas of the liver lobes and the foci of necrosis and steatosis were revealed after cisplatin exposure. The latter indicates the development of inflammatory processes (Fig. 1-I, b and B). In the livers of rats, from experimental group of animals with cisplatin treatment, partial destruction of collagen fibers were also observed (Fig. 1-II, b and B). The similar discernible changes in the connective tissue support were revealed also in case of combined use of cisplatin and dexamethasone (Fig. 1-II, d and D).

Thus, as evidenced by the data of our study, cisplatin and dexamethasone exhibit pro-oxidative properties of different strengths. This is evidenced by the quantitative changes in the primary products of lipid peroxidation, conjugated dienes and trienes, the values of the oxidation index, the changes in the amount of MDA and catalase activity when cisplatin and dexamethasone are used separately. The non-additive, slightly mild effect of these drugs when used together is also evident. The results of histomorphological studies indicate the hepatotoxic effects of cisplatin and dexamethasone, although the histopathological changes they cause are different. The results of the combined effect of these drugs are similar to the changes caused by cisplatin, but due to the presence of dexamethasone, they are milder. The different magnitude of the effects observed is probably due to the different molecular mechanisms of action of cisplatin and dexamethasone in terms of oxidative stress stimulation and ROS formation.

It is known that the main target of cisplatin is DNA. However, only 5-10% of the amount of imported cisplatin is covalently bound to DNA. 75-85% of it binds to non-DNA targets. The cisplatin molecule modified in the cell can also interact with other molecules containing nucleophilic groups[37]. Due to the strong reactivity of platinum compounds against S-donor molecules, the most important non-DNA target of cisplatin is probably the tripeptide glutathione (GSH). Glutathione as antioxidant is important in maintaining redox balance in the cell. Moreover, cisplatin may affect the activity of enzymes, and other proteins through binding to sulfur atoms of cysteine and/or methionine residues and to nitrogen atoms of histidine residues. The resulting functional protein damage may also contribute to the biochemical mechanism of cisplatin cytotoxicity[37,38].

The cisplatin also impairs mitochondrial function by binding to mtDNA. Due to this, the impairment of mtDNA and ensuing mitochondrial dysfunction give rise to the generation of unbound reactive oxygen species (ROS) and trigger oxidative stress-mediated reactions[39].

Dexamethasone is a synthetic glucocorticoid (GC) that exerts its effects through receptor-mediated regulation of genetic activity. According to the classical mechanism of GC action, these hormones are binding to cytosolic receptors. Then, after activation, the hormone-receptor complex translocated to the nucleus and, through binding to the glucocorticoid response elements (GRE), the transactivation or transrepression of multiple genes[40,41]. Glucocorticoid receptor (GR) is a ligand activated transcription factor that controls gene expression in a complex with hormone[40,41]. It has been shown, that GCs generate a massive transcriptional response that affects up to 5558 genes and more than 1151 transcripts[40].

It is known that dexamethasone exhibits strong anti-inflammatory and immunomodulatory effects[16,17,40,41]. Dexamethasone can both upregulate and downregulate expressions of the genes that facilitates anti-inflammatory effects and immunosuppression[40,41].

It has been shown that dexamethasone-induced increases in ROS production and this process is also dependent on binding to the GRE of the hormone-receptor complex. Glucocorticoids can contribute to oxidative stress and ROS formation by suppressing the expression of various antioxidant enzymes and mRNA synthesis[40,41]. The ROS formed can in turn induce lipid peroxidation and disrupt

multiple metabolic pathways[40]. In contrast to glucocorticoids, other steroid hormones, such as progesterone and estradiol, do not affect ROS production[40].

Mitochondria have been proposed as emerging GC targets since they possess standard GRE in their genome[41,42]. Some of the rapid effects of GCs have been attributed to the mitochondrial site of action[41]. GRs in mitochondria of different cell types, GRE in mitochondrial DNA sequences, and translocation of GC–GR complexes to mitochondria have been reported[41]. Moreover dexamethasone, like other glucocorticoids, also exhibits non-genomic effects, which are associated with the translocation of GR into mitochondria. The GC dexamethasone induced mitochondrial apoptotic pathway leads to the disruption of the mitochondrial membrane-potential and the release of key apoptosis inducing factors like Cytochrome C[42].

Thus, as the above facts demonstrate, although cisplatin and dexamethasone are pro-oxidants and exhibit hepatotoxic effects, they manifest their own abilities in different ways. Probably, the differences in molecular mechanisms of action of cisplatin and dexamethasone are responsible for the different intensity of the effects of these pro-oxidants. In addition, it is likely that different molecular mechanisms of action also determine the outcome of the "negotiation" between cisplatin and dexamethasone at the molecular level and the "deterrent" role of dexamethasone in the case of their combined use.

Conclusion

The quantitative changes of conjugated dienes, conjugated trienes and MDA recorded as a result of this research along with their oxidation index values and decrease of catalase enzyme activity once again confirms the pro-oxidant nature of cisplatin and dexamethasone.

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 appears to act as a buffering factor for the effect of cisplatin.

At the same time, histological studies revealed that although cisplatin and dexamethasone are both pro-oxidants, they cause different histological changes. When used alone, the pro-oxidant cisplatin and dexamethasone induce different histological changes in liver cells, moreover cisplatin inducing more profound changes. Although the effect of pro-oxidants combined use is similar to the changes recorded with the injection of cisplatin alone, they are milder due to the presence of dexamethasone.

The different magnitude of the effects observed is probably due to the different molecular mechanisms of action of cisplatin and dexamethasone in terms of oxidative stress stimulation and ROS formation.

In addition, the "deterrent" effect of dexamethasone, formed as result of differences in molecular mechanisms of action of these pro-oxidants, in combination with strong anti-inflammatory and immunomodulatory properties of steroid, helps him to mitigate the side effects of cisplatin in case of its combined action.

Conflict of interest

All authors declare no conflicts of interest.

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