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CISPLATIN AND STEROIDS SEPARATE AND JOINT ACTION ON ANTIOXIDANT ENZYMES ACTIVITY IN BRAIN TISSUE OF FEMALE RATS

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The aim of this research was to explore the antioxidant enzyme catalase and peroxidase activity in brain tissue of female rats after the cisplatin, estradiol and progesterone separate and joint *in vivo* action. The results demonstrated the opposite effects of cisplatin and steroids on catalase activity and on peroxidase activity in case of separate application of drugs: the cisplatin treatment decreased while the two steroids separate action increased the activity of the mentioned enzymes by different degrees in the studied tissue. The joint injection of cisplatin and estradiol leaded to recovery of baseline values of both antioxidant enzyme activities. In case of joint action of cisplatin and progesterone, some increase in activity of both enzymes was recorded. In case of combined injection of steroids, their effects on antioxidant enzymes catalase and peroxidase activity were summed up.

The obtained results may be helpful for explaining of antioxidant action mechanism of these steroid hormones as well as for the attenuating effects of estradiol and progesterone in case of its joint use with cisplatin.

Oxidative stress – cisplatin – estradiol – progesterone – antioxidant enzymes – catalase – peroxidase.

Տվյալ ուսումնասիրության նպատակն է հետազոտել հակաօքսիդիչ ֆերմենտներ կատալազի և պերօքսիդազի ակտիվությունը էգ առնետների գլխուղեղի հյուսվածքում ցիսպլատինի, էստրադիոլի և պրոգեստերոնի առանձին և համատեղ *in vivo* ազդեցություններից հետո: Տվյալները վկայում են, որ առանձին կիրառելիս ցիսպլատինը և ստերոիդները հակամետ ազդեցություն են գործում ինչպես կատալազի, այնպես էլ պերօքսիդազի ակտիվության վրա. ընդ որում ցիս-պլատինը ճնշում է, մինչդեռ ստերոիդները տարբեր չափով խթանում են հակաօքսիդիչ ֆերմենտների ակտիվությունը հետազոտվող հյուսվածքում:

Ցիսպլատինի և էստրադիոլի համատեղ ազդեցության արդյունքում վերականգնվում են երկու հակաօքսիդիչ ֆերմենտների ակտիվության ստուգիչ տարբերակի արժեքները, մինչդեռ ցիսպլատինի և պրոգեստերոնի համատեղ ազդեցության դեպքում գրանցվում է նշված ֆերմենտների ակտիվության որոշակի աճ: Ստերոիդային հորմոնների համատեղ ներարկման դեպքում դիտվում է խթանիչ ազդեցության ադիտիվություն:

Ստացված տվյալներն օգտակար կարող են լինել ինչպես ստերոիդային հորմոնների հակաօքսիդիչ ազդեցությունների մեխանիզմները, այնպես էլ համատեղ կիրառման դեպքում ցիսպլատինի առաջացրած անցանկալի ազդեցությունները էստրադիոլի և պրոգեստերոնի կողմից մեղմելու ներգործությունները պարզաբանելու հարցում:

Օքսիդատիվ սթրես – ցիսպլատին – էստրադիոլ – պրոգեստերոն – հակաօքսիդիչ ֆերմենտներ – կատալազ – պերօքսիդազ

Целью данного исследования было изучение активности антиоксидантных ферментов каталазы и пероксидазы в ткани головного мозга самок крыс при отдельном и совместном *in vivo* воздействии цисплатина, эстрадиола и прогестерона. Результаты свидетельствуют о противоположном эффекте цисплатина и стероидов как на активность каталазы, так и на активность пероксидазы в случае их раздельного применения. При этом цисплатин подавляет, а эстрадиол и прогестерон повышают активность каталазы и пероксидазы в разной степени в исследуемой ткани. Совместное введение цисплатина и эстрадиола восстанавливает контрольный уровень активности антиоксидантных ферментов, а при совместном действии цисплатина и прогестерона регистрируется некоторое повышение их активности. Вместе с тем, при совместном введении стероидов наблюдается адитивность их эффектов.

Полученные результаты могут быть полезны как для разъяснения механизмов антиоксидантных эффектов этих стероидных гормонов, так и смягчающего эффекта эстрадиола и прогестерона при совместном с цисплатином применении.

Оксидативный стресс – цисплатин– эстрадиол– прогестерон – антиоксидантные ферменты – каталаза – пероксидаза.

Cisplatin (cis-diaminedichloroplatinum II) (CDDP) is one of the most potent antineoplastic agents used for the treatment of a wide variety of human malignancies [2, 4, 15]. Cisplatin treatment is plagued by severe side effects such as neurotoxicity, nephrotoxicity, ototoxicity hepatotoxicity [3,4,12,15,16,18]. About 30% of patients treated with cisplatin have neurotoxicity because it can cross blood brain barrier andbe accumulatedthrough repeated dosage [12,14,15,16,18].

The exact mechanism of cisplatin toxicity is not fully understood but it is generally accepted that cisplatin causes oxidative stress which is due to the generation of reactive oxygen species which interact with DNA, lipids, proteins leading to lipids peroxidation and DNA damage [2, 8]. Since DNA is the primary target of cisplatin, its damage by reactive oxygen species (ROS) could lead to irreversible changes in its molecule, which in turn preventing the cell division or DNA synthesis and its repair mechanism that can trigger cell death and resulting in induction of apoptosis [3,4,8,15]. Moreover, the induction of oxidative stress and ROS formation is considered another mechanism of action of cisplatin [2,10,14]. Cisplatin increases the production of free oxygen radicals and decreases the antioxidants, thus resulting in the disturbance of the oxidant/ antioxidant balance [10,20].

It is well known that reactive oxygen species (ROS) are natural bioproducts of cellular oxidative metabolism and play important roles in the modulation of cell survival, cell death, differentiation, cell signaling, and inflammation-related factor production [5,19]. Disturbed redox homeostasis leads to oxidative damage to biomolecules, such as proteins, lipids, and nucleic acids that resulting harmful effects on cells mediated by interference with cell-signaling mechanisms. By contrast, the modulation of ROS generation enhances activation of key signaling molecules that regulate cell death, survival, differentiation, and proliferation [5,19].

Under various physiological states, ROS are produced as intermediates, and their cellular levels are strongly regulated by various detoxifying enzymes, such as superoxide dismutase, glutathione peroxidase and catalase or by different antioxidants, including flavonoids, ascorbic acids, vitamin E, and glutathione[5,12,19].

The cells have defense systems to cope with routinely generated ROS. Besides the antioxidant enzymes and small molecules with antioxidant capacity described above, cells have some natural antioxidants. Among the latter the steroid hormones occupy a special place [5, 9, 12, 19].

When ROS are not eliminated sufficiently by antioxidant enzymes or small molecules, the cells are damaged by oxidative insults, leading to cell death. Natural antioxidants have been reported to protect against cisplatin toxicity [10, 12]. Several studies have been reported that supplementation with antioxidants prevent neurotoxicity associated with platinum-based chemotherapy which work together to enhance the anticancer activity and reduce adverse effects [9,12,20].

It has been established, those natural antioxidants, such are steroid hormones are capable of protecting against the toxicities of cisplatin [9,12]. Likewise, the brain possesses an inherent endocrine system and synthesizes steroid hormones known as neurosteroids [9,12]. The biological functions of the neurosteroids are exerted either through a conventional genomic process via their receptors (SRs), or through interaction with membrane receptors.Neurosteroids may directly activate G protein coupled transmembrane receptors [9,12].

Regulation of gene expression by steroids is closely related to its antioxidant properties. Up regulation of antioxidant enzymes is the simplest of the antioxidant mechanisms of neurosteroids that are mediated via the genomic steroid receptor (SR) pathway [9, 12]. The female sex steroid 17β-estradiol and progesterone binds to the receptors in the nucleus, activating gene transcription. The genes of antioxidant enzymes, such as superoxide dismutase, catalase and glutathione reductase, are targets of progesterone involved in oxidative stress tolerance [9, 12].

At the same time, it is well known that estradiol and progesterone show a beneficial effect in elimination of cisplatin-induced nephrotoxicity [6, 7,17]. It was shown, that activities of antioxidant enzymes are regulated by steroid hormones in sex dependent manner [6, 7, 17]. Moreover, the antioxidant enzyme activity in liver tissue of female and male rats shows certain dependence on concentration of progesterone and estrogen in the organism [7, 17].

Thus, the antioxidant effects of these neurosteroids are similar. Estradiol and progesterone protect neuronal cells from oxidative stress by up regulating antioxidant enzymes via genomic pathway [9].

Taking into consideration the above information it seemed important to estimate the activity of antioxidant enzyme catalase and peroxidase in brain tissue of female rats under the cisplatin, estradiol and progesterone separate and joint action.

Materials and methods. The investigation was performed on adult female albino rats (120-150 g weight). The animals were divided into 7 groups. The group 1 was a control group of animals without treatment. Animals of groups 2, 4 and 6 received a single dose of cisplatin (8 mg/kg). Cisplatin was injected peritoneal. Exposition time for cisplatin was 24 hours. The group 3 was treated with estradiol (200 mcg/kg, injected peritoneal), the group 5 received a single dose of progesterone (30 mcg/kg, injected peritoneal). Exposition time for steroids was 4 hours. Animals from the groups 4 and 6 were received the same single dose of estradiol and progesterone respectively within 20 hours after the cisplatin injection (4 hours before decapitation). The animals of group 7 were simultaneously treated with estradiol and progesterone (respectively 200 mcg/kg and 30 mcg/kg, injected peritoneal). Exposition time for steroids was 4 hours.

All animals were decapitated through corresponding time after the inhalation anesthesia with chloroform. Then, animals were sacrificed, and extracted brain tissues from each group of animals. The extracted organs were homogenized in ten volumes of ice-cold TM buffer (50mM Tris-HCL pH 7.4, 3mM $MgCl_2$). The homogenates were centrifuged at 1000×g for 10 min at 4°C. The supernatants were collected and stored at-20˚C for biochemical determination of protein and enzymes activity. Quantitative determination of protein in investigated preparations was carried by spectrophotometric method [11].

The activity of catalase (EC 1.11.1.6) was determined by measuring the decrease in the hydrogen peroxide (H_2O_2) concentration at 410 nm. The method of defining of catalase activity is

based on developing of stable blue colored complex in result of ammonium molybdate reaction with H_2O_2 and subsequent photometric measurement of the recovered complex [13].

The assay medium consisted of 1 ml Tris-HCl buffer solution (50 mM, pH 7.8), 0,1 ml of homogenate sample and 2 ml of 0.03% H_2O_2. The reaction mixture was left for 10 min at room temperature, in darkness. The reaction stopped after 10 min by adding 1 ml of 4% ammonium molybdate solution. The absorption measurement was conducted at λ=410 nm, and the activity of catalase was expressed in micromoles μM of the transformed H_2O_2 /min per mg of protein [13].

The activity of peroxidase (EC 1.11.1.7) was determined by [20]. It is well known, that peroxidase has two substrates, to one of which to H_2O_2 the enzyme exhibits strict specificity.A second substrate is needed to oxidize by hydrogen peroxide. As second substrate can be glutathione, guaiacol, benzidine and other substances. We used benzidine or 4.4 diaminodiphenyl as a second substrate for peroxidase [20].

The reaction results in a color complex called benzidine blue with a maximum absorption at 520nm. The reaction mixture contains 2 ml 2.5mM benzidine, 0.5ml 3% H_2O_2 and 0.1 ml of investigated homogenate. The optical density is measured at 520 nm after the 1 minute. The activity of peroxidase is expressed in μM/ min, mg protein [20].

All results were expressed as M±m from 4 independent experiments. Statistical differences in the results between groups were evaluated by the Student's t-test.

Results and Discussion. Results of the studies of cisplatin and steroids separate and joint action on catalase activity in supernatant of first centrifugation of female rats' brain homogenate are shown in Table 1 (tab.1).

These results showed that cisplatin 24 h *in vivo* action caused a significant decrease in the catalase activity in supernatant fraction of brain tissue homogenate by 14.4% (tab.1 and f an increase of this antioxidant enzyme activity correspondingly by 40% and 30% in brain tissue (tab.1 and fig.1). The joint action of cisplatin and estradiol restored the initial level of catalase activity (tab.1 and fig.1). Animal combined injection of cisplatin and progesterone not only neutralizes the overwhelming effect of antitumor drug, but even increases the activity of catalase activity by 18% (tab.1 and fig.1). In case of joint action of both steroid hormones, their stimulating effects are summed up. However, the total stimulus effect is less than the mathematical sum: enzyme activity increase instead of 70% is equal to 52% as compared with baseline (tab.1 and fig.1). Apparently, steroid hormones act in a competitive manner, preventing the full integration of their stimulating effects.

Table 1. Cisplatin, estradiol and progesterone separate and joint action on catalase activity (μM/min, mg protein) in supernatants of first centrifugation of rat brain tissue homogenate

#	Variants	Activity of catalase μM/ min, mg protein	Activity of peroxidase μM/ min, mg protein
1	Baseline	382.20±10.61	437.00±8.34
2	Cisplatin	*327.10±7.60	*383.74±11.50
3	Estradiol	*534.74±6.70	*534.74±17.00
4	Cisplatin+ Estradiol	385.00±11.00	441.20±11.55
5	Progesterone	*497.00±14.54	*654.15±17.34
6	Cisplatin +Progesterone	*452.30 ± 8.44	*490.00 ±14.60
7	Estradiol + Progesterone	*582.00±42.00	*587.32±42.00

Table 1 also presents the results of activity of another antioxidant enzyme of peroxidase. That is, after the cisplatin separate action peroxidase activity was decreased by 12% in supernatant fraction of brain tissue homogenate as compared with baseline (tab.1 and fig.3). The separate injection of steroid hormones estradiol and progesterone caused an increase of peroxidase activity correspondingly by 22.4% and 50% in brain homogenate (tab.1 and fig.2).

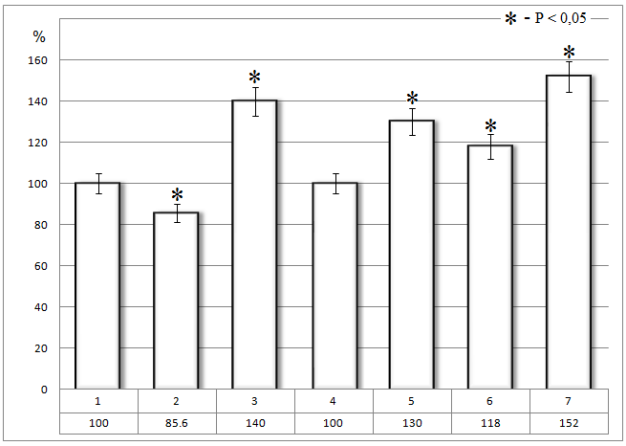

Fig.1. Catalase activity alterations in supernatant of female rats' brain cells under the cisplatin and steroids separate and joint action (1 –baseline, 2 –after the cisplatin action, 3 – after the estradiol action, 4 – after the cisplatin and estradiol joint action, 5 – after the progesterone action, 6 – after the cisplatin and progesterone joint action, 7 – after the estradiol and progesterone joint action).

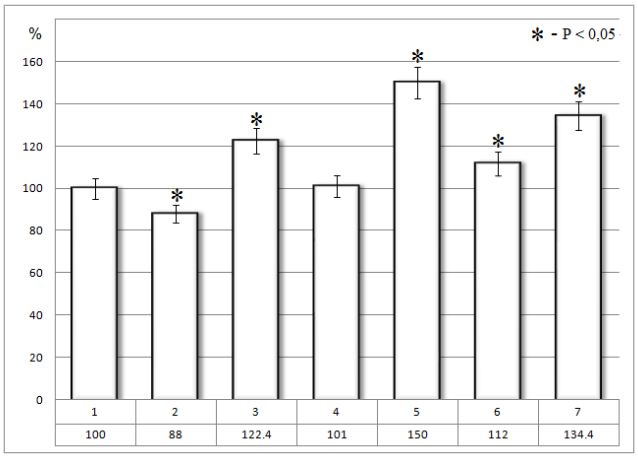

Fig.2 Peroxidase activity alterations in supernatant of female rats' brain cells under the cisplatin and steroids separate and joint action (1 –baseline, 2 –after the cisplatin action, 3 – after the estradiol action, 4 - after the cisplatin and estradioljoint action, 5 - after the progesterone action, 6 - after the cisplatin and progesteronejoint action,7 – after the estradiol and progesterone joint action)

In this case, the steroids also have a stimulating effect on peroxidase activity. The joint action of cisplatin and estradiol restored the initial level of enzyme activity, whereas cisplatin and progesterone combinedinjection leaded to increase in peroxidase activity by 12% (fig.2). In case of joint treatment of animals with both steroid hormones, their stimulating effects are unusually summed up and is equal to 34.4% as compared with baseline, instead of 72.4% (fig.2). It is assumed that in case ofjoint action of estradiol and progesterone on activity of antioxidant enzymes there is competition between two steroid hormones, preventing them from summing up their stimulating effects.

The stimulating effect of steroid hormones is more evident when comparing the results of joint action of these drugs with the results of separate action of cisplatin on catalase and peroxidase activity. In this case cisplatin and estradiol joint action caused change of both antioxidant enzymes catalase and peroxidase activity correspondinglyup to 18% and 15% (fig.3).

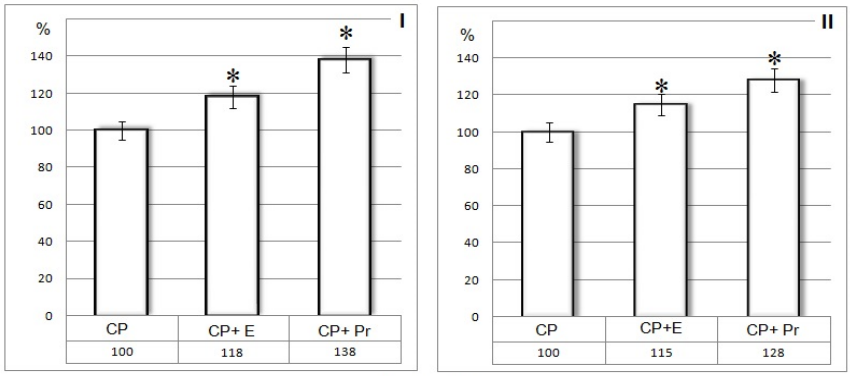

Fig.2. Catalase (I) and peroxidase (II) activity alterations in brain tissue of female rats after the cisplatin alone and in combination with steroids action.Results of catalase and peroxidase activity, obtained in case ofcisplatin separateinjection were accepted for 100%. (CP –after the cisplatin alone action, CP + E - after the cisplatin and estradiol joint action, CP + Pr - after the cisplatin and progesterone joint action).

The combined treatment with cisplatin and progesterone leads to increased activity of catalase and peroxidase respectively by 38% and 28% (fig.3).In both cases, steroid hormones not only neutralize the overwhelming effect of cisplatin, but even increase the activity of the studied enzymes (fig.3).

It is well known, that cisplatin induced neurotoxicity characterized by a significant reduction in antioxidant enzymes activity, among them the catalase and peroxidase activity in rats brain tissues [8]. Additionally found that the activity of antioxidant enzymes such as catalase was significantly decreased in the cisplatin injected rats in comparison with the normal control rats [8, 20].

As already noted antioxidant enzymes are the part of the antioxidant defense system of cell and catalyzed hydrogen peroxide (H_2O_2) and other peroxides degradation into oxygen and water. It has been demonstrated, that the level of catalase in the brain tissues markedly decreased after the cisplatin treatment compared to the control [8, 20].

It is well known, that cisplatin induced neurotoxicity characterized by a significant reduction in antioxidant enzymes activity, among them the catalase and glutathione peroxidase activity in rat's brain tissues. Additionally found that the activity of antioxidant enzymes such as catalase was significantly decreased in the cisplatin injected rats in comparison with the normal control rats [8, 20]. Our results also show the regulation of antioxidant enzymes activity by sex steroid hormones, which are considered natural antioxidants. In this way the combined use of cisplatin and steroid hormones results in the neutralization of the inhibiting effect of cisplatin on the activity of antioxidant enzymes. This in turn can help to reduce oxidative stress, thereby reducing neurotoxicity and unwanted side effects of cisplatin.

The obtained results may be helpful for explaining of antioxidant action mechanism of these steroid hormones as well as for the attenuating effects of estradiol and progesterone in case of its joint use with cisplatin.

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