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CISPLATIN AND ESTRADIOL SEPARATE AND JOINT ACTION ON CATALASE ACTIVITY IN LIVER AND KIDNEY TISSUES OF RATS

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АННОТАЦИЯ

Целью данного исследования было изучение активности каталазы в некоторых препаратах печени и почек самок крыс при отдельном и совместном *in vivo* воздействии цисплатина и эстрадиола. Результаты свидетельствуют о противоположном эффекте цисплатина и эстрадиола на активность каталазы в случае их раздельного применения: цисплатин подавляет, в то время, как эстрадиол увеличивает активность каталазы в исследуемых тканях. Совместное введенение цисплатина и эстрадиола восстанавливает контрольный уровень активности каталазы. Эстрадиол способствует снягчению токсичности цисплатина, регулируя количество активных форм кислорода путем активации антиоксидантного фермента каталазы. Полученные результаты могут быть полезны для разьяснения смягчающего эффекта эстрадиола при совместном с цисплатином применении.

ABSTRACT

The aim of this research was to explore the catalase activity in some preparations of liver and kidney tissues of female rats after the cisplatin and estradiol separate and joint in vivo action. The results demonstrated the

opposite effects of cisplatin and estradiol on catalase activity in case of separate application of drugs: the cisplatin treatment decreases while the estradiol increases the enzyme activity in both studied tissues. The joint action of cisplatin and estradiol leads to partial recovery of baseline value of catalase activity. Estradiol can soften the toxicity of cisplatin by regulating the amount of ROS by activating antioxidant enzyme catalase. The obtained results may be helpful for explaining the estradiol attenuating effects in case of its joint use with cisplatin.

Ключевые слова: окситадивный стресс, цисплатина, эстрадиол, антиоксидантные ферменты, каталаз
Kay word: oxidative stress, cisplatin, estradiol, antioxidant enzymes, catalase

Cisplatin is an effective important antitumor drug, which is nowadays widely used in chemotherapeutic practices. Cisplatin has a high cytostatic and cytotoxic effect towards tumor cells. Cisplatin kills cancer cells by damaging DNA, inhibiting replication, transcription, activation of multiple pathways of apoptosis and by inducing oxidative stress [2, 3, 6]. The effectiveness of cisplatin therapy depends on its concentration, but high doses of this drug lead to developing of toxicities. Chemotherapy is associated with different toxicities, such as nephro-, neuro-, hepato-, oto- and other toxicities. That is why the therapeutic use of cisplatin is limited by its undesirable side effects [2, 6]. The exact mechanism of cisplatin-induced toxicity is not fully understood, but it is generally accepted that cisplatin causes the oxidative stress, which has been recognized as an important factor that contributes to cisplatin nephrotoxicity [3]. The oxidative stress is due to the excessive generation and accumulation of reactive oxygen species (ROS). The induction of reactive oxygen species generation and ROS-induced cytotoxicity is an important mechanism by which cisplatin kills tumor cells [3, 6]. ROS can interact with DNA, lipids and proteins, causing DNA and proteins damages and lipid peroxidation, which in turn can trigger cell death [3, 6, 10].

The reactive oxygen species are generated continuously in cells during the oxidative metabolism. In normal physiological conditions the balance is established between the generation and destruction of oxyradicals. Cellular levels of ROS are strongly regulated by detoxifying antioxidant complex system for the protection against damage effects of ROS [3, 10]. It is well known that the main components of detoxifying antioxidant complexes are the antioxidant enzymes, such as superoxide dismutase, catalase, glutathione peroxidase and others, which metabolize intracellular ROS and maintain homeostasis of cells. Different low-molecular weight antioxidants, including flavonoids, ascorbic acids, vitamin E, and glutathione (GSH) are also actively engaged in removing of ROS [3, 9, 10]. There are significant correlations between ROS generation and metabolism, as well as with cellular pathophysiology [9, 10]. However, when ROS generation exceeds the antioxidant capacity of cells, oxidative stress develops, which can damage cells [10]. Intracellular accumulation of ROS has been well accepted as a fundamental mechanism contributing to antitumor mechanism of cisplatin [2, 3, 10]. Nevertheless ROS and oxidative stress recognized as an important factor, that contributes, to cisplatin toxicities [3, 10]. At the same time, it has been argued, that cisplatin induction of ROS generation is consequence of inhibition of antioxidant enzymes activities by this drug [3, 9, 10].

Recently many efforts have been made to employ drugs and other medicines as candidate adjuvants to cisplatin to minimize its undesirable influence. Steroid hormones such as estradiol and progesterone are presently considered as the best adjuvants to cisplatin which are able to prevent intoxication [4, 5, 9]. Estradiol and progesterone showed a beneficial effect in elimination of cisplatin-induced nephrotoxicity [4. 5, 9]. It was shown, that activities of antioxidant enzymes are regulated by steroid hormones in sex dependent manner [4, 5, 9]. Moreover, the enzyme activity of the antioxidant system in liver tissue of female and male rats show certain dependence on concentration of progesterone and estrogen in the organism [5, 9].

Taking into consideration the above information it seemed important to estimate the activity of antioxidant enzyme catalase in some tissues of female rats under the cisplatin and estradiol separate and joint action.

Materials and methods. The investigations were performed on 24 adult female albino rats (120-150g body weight). The animals were kept in 20-22ºC and 12 hours dark/light conditions with free access to water and food (animals were fed with commercial rat feed) in the animal house of faculty of biology of Yerevan state univercity.

Experiences were fulfilled according to the "International Recommendations on Carrying out of Biomedical Researches with use of Animals" (CIOMS, 1985), to the "Human Rights and Biomedicine the Oviedo Convention" (CE, 1997), to the European Convention for the Protection of Vertebral Animals Used for Experimental and Other Scientific Purposes (CE, 2005) and approved by the National Center of Bioethics (Armenia).

The animals were divided into 4 groups. Group 1 is a control group of animals without treatment. Animals of groups 2 and 4 received single dose of cisplatin (8 mg/kg, Sigma-Aldrich). Cisplatin was injected peritoneal. Exposition time for cisplatin was 24 hours. Group 3 was treated with estradiol (200 mcg/kg, Sigma-Aldrich, injected peritoneal). Exposition time for estradiol was 4 hours. Animals from group 4 within 20 hours after the cisplatin injection (4 hours before decapitation) received the same single dose of estradiol.

All animals were decapitated through corresponding time after the inhalation anesthesia with chloroform. Then, animals were sacrificed, and extracted liver and kidney tissues from each group of animals and used for isolation of nuclei by the method of Blobel and Potter [1]. 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 catalase activity. Quantitative determination of protein in investigated preparations was carried by spectrophotometric method [7].

For the estimation of catalase (EC 1.11.1.6) activity we used the supernatants of first centrifugation of female rats liver and kidney homogenates and the nuclear fractions of observed tissues. The activity of catalase was determined by measuring a decrease in the hydrogen peroxide (H_2O_2) concentration at 420 nm. The method of defining of cayalase activity is based on developing of stabily blue colored complex in result of ammonium molybdate reaction with H_2O_2 and subsequent photometric measurement of the recovered complex [8].

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 minuts at room temperature, in darkness. The reaction stopped after 10 min by adding 1 ml of 4% ammonium molybdate solution. The absorbtion measurement was conducted at λ=420 nm, and the activity of catalase was expressed in micromoles μM of the transformed H_2O_2 /min per mg of protein [8].

All results were expressed as M±m from 6 independent experiments. Statistical differences in the results between groups were evaluated by the Student's t-test.

Results and discussion. Results of our studies of cisplatin and estradiol separate and joint action on catalase activity in supernatant of first centrifugation and nuclear fractions of investigated preparations are shown in Table 1 and 2. Results suggest that the catalase activity is unevenly distributed among the supernatant and nuclear fraction of liver tissue from four studied groups of female rats. The 65-67% of catalase total activity is registered in supernatant of female rats liver tissue from all variants, whereas only 34-40% of enzyme activity is defined in nuclear fraction (Tab.1).

Table 1

Cisplatin and estradiol separate and joint action on catalase activity (μM/min, mg protein) in supernatants of first centrifugation of rat liver tissue homogenate and in nuclear fractions of rat liver tissue.

Activity of catalase μM/ min, mg protein (M ± m);	Supernatants of first centrifugation of female rats liver tissue homogenate			
	1	2	3	4
	685.40±9.85	*533.10±7.35	*822.00±20.77	*600.00±16.73
Activity of catalase μM/ min, mg protein (M ± m);	Nuclear fraction of female rats liver tissue			
	1	2	3	4
	340.90±8.56	*280.30±7.50	*420.00±10.62	318.00±10.62

1 – baseline, 2 – after the cisplatin separate 24 hour action, 3 – after the estradiol separate 4 hour action, 4 – after the cisplatin and estradiol joint action., relative to baseline. *P < 0.05

These results showed that cisplatin 24 h *in vivo* action caused a significant decrease in the catalase activity level in supernatant fraction of liver tissue homogenate as well as in nuclear fraction correspondingly by 22.2% and 11.8% (Tab.1 and Fig.1). In contrast to cisplatin action, estradiol 4 h *in vivo* treatment caused an increase of catalase activity in both liver homogenate and nuclear fractions correspondingly by 20% and 32% (Fig.1). At the same time the joint action of cisplatin and estradiol caused a decrease of catalase activity by 12-13% (Fig.1). On the other hand, comparing with the level of catalase activity, estimated in case of cisplatin separate action, an increase of enzyme activity by 12.5% was revealed (Fig.1).

One can mention that cisplatin and estradiol combined injection restored the initial level of catalase activity in nuclear fraction of rat liver cells because the decrease of the of catalase activity values registered after the cisplatin separate and joint with estradiol action leads to increase of enzyme activity by 13.5% ((Fig.1). Thus, in case of separate action cisplatin and estradiol demonstrated its own abilities suppressing or stimulating the metabolic processes. However, in case of joint action these effects it seems that were summed up. It must be mentioned that «crosstalk» between these two agents with divergent properties in molecular level concluded in favor of estradiol stimulating ability.

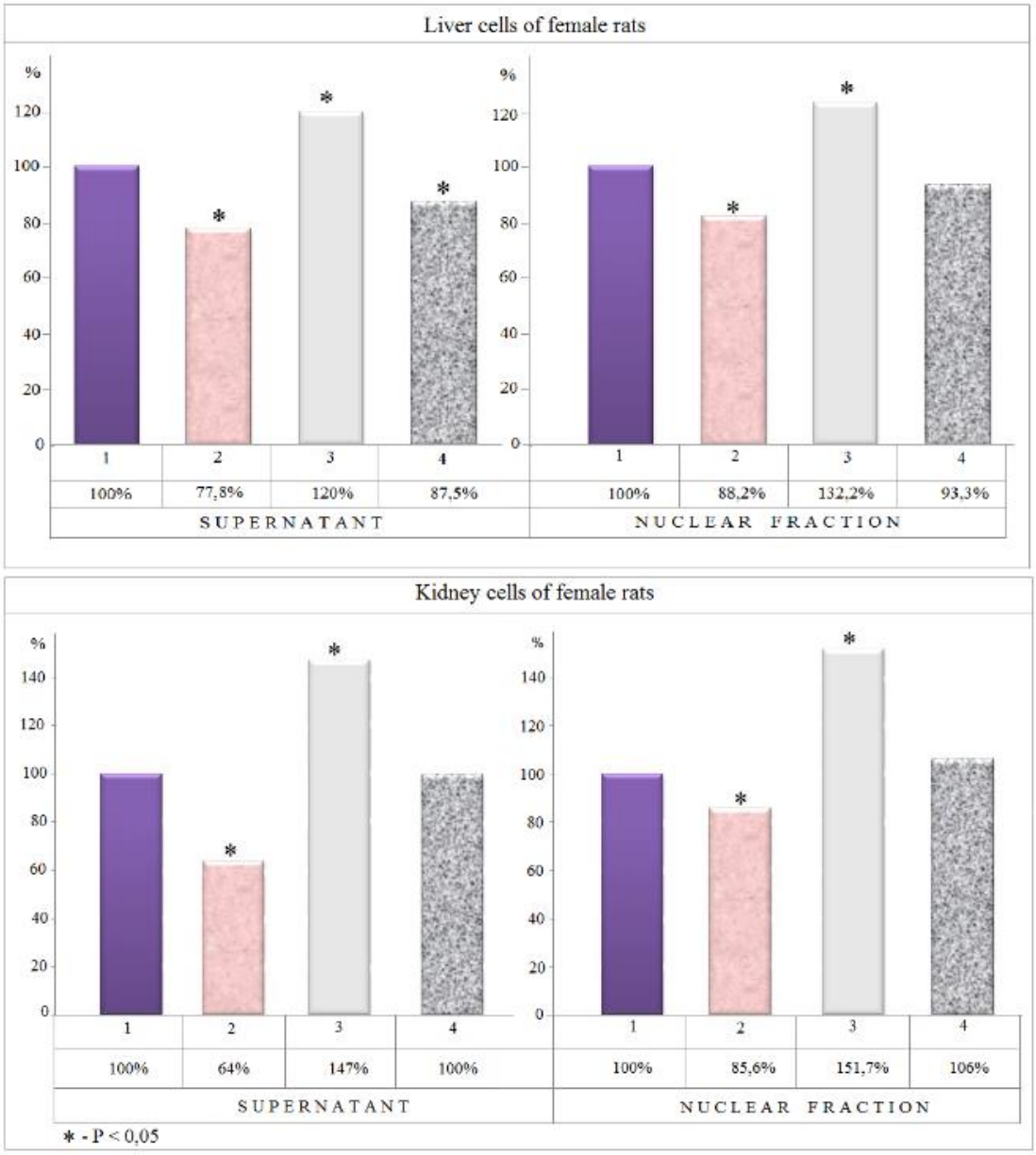

Fig.1 Catalase activity alterations in supernatant and nuclear fractions of female rats liver and kidney cells under the cisplatin and estradiol separate and joint action
1 –baseline, 2 –after the cisplatin action, 3 – after the estradiol action,
4 - after the cisplatin and estradiol joint action
enzyme activity by 6-7% is not reliable (Tab.1 and Fig.1). However, the comparison

The catalase activity we determined also in supernatant and nuclear fraction of four groups of rats kidney tissue. Results of these investigations are provided in Table 2 (Tab.2).

Table 2.

Cisplatin and estradiol separate and joint action on catalase activity (μM/min, mg protein) in supernatants of first centrifugation of homogenates and in nuclear fractions of female rats kidney tissue.

Activity of catalase μM/ min, mg protein (M ± m);	Supernatants of first centrifugation of female rats kidney tissue homogenates			
	1	2	3	4
	553.27±21.60	*354.15±9.66	*814.00±11.60	522.20±12.88
Activity of catalase μM/ min, mg protein (M ± m);	Nuclear fractions of female rats kidney tissue			
	1	2	3	4
	213.00±8.70	*182.34±6.00	*323.14±8.90	226.00±10.24

1 – baseline, 2 – after the cisplatin separate 24 hour action, 3 – after the estradiol separate 4 hour action, 4 – after the cisplatin and estradiol joint action. *P < 0.05, relative to baseline.

These results also showed that as in case of liver tissue, the catalase activity unevenly distributed among the supernatant and nuclear fractions. In baseline more than 72% of catalase total activity was revealed in supernatant, and the remaining 28% was registered in nuclear fraction (Tab.2). In case of cisplatin separate action, the 34% of catalase total activity was registered in nuclear fraction and 66% in supernatant of kidney tissue of rats. In case of estradiol separate action more 28% of enzyme total activity was revealed in nuclear fraction and more than 71% in supernatant of kidney cells (Tab.2). About 30% of catalase total activity registered in nuclear fraction and the remaining 70% in supernatant of kidney tissue of rats (Tab.2).

The separate action of cisplatin decreased the catalase activity in supernatant fraction of kidney tissue by 36% in comparison with baseline level, while in nuclear fraction of the same tissue this diminution was more less (near 14%) (Fig.1). In case of estradiol separate action, the catalase activity was increased both in supernatant and nuclear fractions correspondingly by 47% and 52% (Fig.1). Cisplatin and estradiol combined action restored the initial level of catalase activity in both supernatant and nuclear fractions. However, in comparison with the results demonstrated after the cisplatin alone action the increased level of catalase activity in both supernatant and in nuclear fractions of kidney tissue was revealed. Thus, in supernatant catalase activity was increased by 47.5%, whereas in nuclear fraction of kidney cells the enzyme activity increased only by 24% (Fig.2).

Consequently, the obtained results confirm that cisplatin and estradiol demonstrated opposite effects on catalase activity in supernatant and nuclear fraction of both studied tissues. These drugs in case of their separate action exhibit their own abilities to suppress or to stimulate metabolic processes. However, in case of joint action of these drugs, due to stimulus effect of estradiol, the baseline level of catalase activity is achieved. It seems that the stimulating effect of estradiol on catalase activity neutralizes the diminution action of cisplatin.

Catalase is one of main enzyme components of antioxidant complex for the protection against damage effects of ROS [3, 4, 9]. Due to its stimulus effects estradiol may reduce the level of cisplatin toxicity and eliminate its negative side effects in case of combined use with cisplatin. It may be assumed that estradiol can soften the toxicity of cisplatin by regulating the amount of ROS by activating antioxidant enzyme catalase.

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