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CHANGES IN MALONDIALDEHYDE LEVEL IN FEMALE RATS BRAIN, KIDNEY AND LIVER CELLS AFTER THE SEPARATE AND JOINT ACTION OF CISPLATIN AND STEROIDS

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The aim of this research was to explore the alterations in malondialdehyde (MDA) quantity in the rat brain, kidney and liver tissues after the cisplatin, estradiol and progesterone separate and joint action. The results confirm that MDA concentration significantly increased in all tissues of female rats after the cisplatin separate.action.

Steroids affect MDA quantity in different ways: either they, similar to estradiol, reduce it in the kidneys and liver, and progesterone in the brain, or they do not affect on the content of this aldehyde.

Cisplatin and steroids joint action manifested the tendency of restoring the baseline quantity of MDA in the tissues of female rats. Steroidal hormones exhibit themselves as antioxidants and essentially neutralize the stimulating action of cisplatin.

In case of joint action the estradiol and progesterone steroid hormones are in competition. It seems to be the result of "crosstalk" between two steroid hormones at the molecular level resolved in favor of either estradiol or progesterone.

The obtained results may be helpful for explaining the estradiol and progesterone attenuating effects in case of their joint use with cisplatin.

Cisplatin-estradiol-progesterone-oxidative stress-malondialdehyde (MDA)

Տվյալ ուսումնասիրության նպատակն է հետազոտել էգ առնետների գլխուղեղի, երիկամների և լյարդի հյուսվածքներում մալոնային երկալդեհիդի (ՄԵԱ) քանակական փոփոխությունները ցիսպլատինի, էստրադիոլի և պրոգեստերոնի առանձին և համատեղ ազդեցությունների ներքո: Տվյալները վկայում են, որ ցիսպլատինի ազդեցության արդյունքում ՄԵԱ-ի քանակն աճում է էգ առնետների բոլոր հետազոտվող հյուսվածքներում: Ստերոիդ հորմոնները տարբերակված ազդեցություն են թողնում. կամ կրճատում են ՄԵԱ-ի քանակը, ինչպես էստրադիոլը երիկամներում ու լյարդում և պրոգեստերոնը գլխուղեղում, կամ էլ չեն փոխում այդ ալդեհիդի քանակը:

Ցիսպլատինի և ստերոիդների համատեղ կիրառման դեպքում հետազոտվող բոլոր հյուսվածքներում ՄԵԱ-ի քանակի ստուգիչ տարբերակի արժեքների վերականգնման միտում է գրանցվում: Ստերոիդներն իրենց դրսևորում են որպես հակաօքսիդիչներ և հիմնականում չեզոքացնում են ցիսպլատինի խթանիչ ազդեցությունը:

Համատեղ կիրառելիս երկու ստերոիդ հորմոնները մրցակցում են: Հավանաբար դա ստերոիդ հորմոնների՝ մոլեկուլային մակարդակում տարվող «բանակցություն-

ների» արդյունք է, որն ավարտվում է կամ էստրադիոլի, կամ պրոգեստերոնի օգտին:

Ստացված տվյալներն օգտակար կարող են լինել համատեղ կիրառման դեպքում ցիսպլատինի առաջացրած անցանկալի ազդեցությունները ստերոիդների կողմից մեղմելու ներգործությունները պարզաբանելու հարցում:

Ցիսպլատին – էստրադիոլ – պրոգեստերոն – օքսիդատիվ սթրես – մալոնային երկալդեհիդ (ՄԵԱ)

Целью данного исследования было изучение изменения количества малонового диальдегида (МДА) в тканях головного мозга, почек и печени самок крыс при отдельном и совместном воздействии цисплатина, эстрадиола и прогестерона. Результаты показывают, что при отдельном применении цисплатин значительно повышает концентрацию МДА во всех тканях крыс самок. Стероиды при отдельном применении по-разному влияют на количество МДА: либо они сокращают его количество как эстрадиол в почках, печени и прогестерон в мозге, либо они не влияют на содержание данного альдегида.

Совместное действие цисплатина и стероидов продемонстрировало тенденцию восстановления контрольного уровня количества МДА в тканях крыс самок. Стероидные гормоны проявляют себя как антиоксиданты и по сути нейтрализуют стимулирующее действие цисплатина.

При совместном действии стероидные гормоны эстрадиол и прогестерон конкурируют. По видимому это результат “переговоров” на молекулярном уровне между двумя стероидными гормонами, которые завершаются в пользу эстрадиола или прогестерона.

Полученные результаты могут быть полезны для разъяснения смягчающего эффекта эстрадиола и прогестерона при совместном с цисплатином применении.

Цисплатин – эстрадиол – прогестерон – оксидативный стресс – малоновый диальдегид (МДА)

Cisplatin is one of the most commonly used cytotoxic agents in the treatment of a variety of tumors [1, 5, 13]. Although treatment with this drug is often effective, serious side effects such as nausea, nephrotoxicity, neurotoxicity, hepatotoxicity and ototoxicity often occur [1, 5, 13, 16].

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 [1, 5, 13, 16]. Several mechanisms are believed to mediate cisplatin-induced apoptosis [1, 5, 16]. The “traditional” mechanism involves covalent binding of cisplatin to guanine bases on DNA, formation of inter- and intra-strand chain cross-linking, induction of p53, cell cycle arrest and apoptosis [1, 13, 16]. Moreover, it is suggested that there is also another mechanism of cisplatin cytotoxicity, associated with the oxidative stress and reactive oxygen species (ROS) formation [1, 13]. Antiproliferative and cytotoxic effects of cisplatin are linked to ROS-induced damage and its ability to increase oxidative stress. [1, 16]. It has been shown that ROS generated by cisplatin could increase lipid peroxidation, which in turn alters DNA molecule, enzymes and structural proteins and directs the cell to an apoptotic pathway [1, 16].

Oxidative stress is caused by an imbalance between the production of reactive oxygen species and biological system’s ability to readily detoxify the reactive intermediates or easily repair the resulting damage [9, 23]. Reactive oxygen species express a variety of molecules and free radicals physiologically generated from the metabolism of molecular oxygen, including superoxide anion, which is the precursor of most ROS [9, 23].

Disturbances in normal redox state can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids and DNA. Oxidative stress plays a key role in the pathogenesis of many diseases [9, 18, 23].

The main targets of ROS and free radicals are polyunsaturated fatty acids that involve one or more double bindings. ROS and free radicals attack both free and incorporated in various lipids fatty acids. These interactions result in formation of lipid oxidation products [2, 6]. The latter undergo fragmentation to produce a broad range of reactive carbonyl intermediates such as unsaturated aldehydes. Malondialdehyde (MDA) is one of these intermediates [2, 4, 6]. Malondialdehyde that is formed from decomposition of lipid peroxidation products is the most frequently used biomarker of oxidative stress [4]. The intensity of oxidative stress is determined by the quantity of malondialdehyde [4, 15].

Oxidative stress and resulting lipid peroxidation are involved in numerous pathological states [6, 21]. Oxidative stress and the formation of free radicals are accompanying effects of various drugs action, including cisplatin [3, 23]. Moreover, oxidative stress is the main cause of the intoxication in cisplatin therapy of tumors [10, 23].

Nowadays 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 the best adjuvants to cisplatin which are able to prevent intoxication [7, 8, 10, 17]. The ability of steroids to mitigate unwanted side effects of cisplatin due to their antioxidant properties is well known [7, 8, 12, 17]. Steroids can reduce oxidative stress at two levels, by preventing ROS generation and by scavenging free radicals [12, 17, 19]. As already mentioned the intensity of oxidative stress is determined by the quantity of malondialdehyde [4, 15].

In light of the foregoing it seems interesting to explore the level of oxidative stress by the quantity of malondialdehyde in rat kidney and brain cells after the separate and joint action of cisplatin, estradiol and progesterone.

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 peritoneally. Exposition time for cisplatin was 24 hours. The group 3 was treated with estradiol (200 mcg/kg, injected peritoneally), the group 5 received a single dose of progesterone (30 mcg/kg, injected peritoneally). 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 peritoneally). Exposition time for steroids was 4 hours (tab.1).

All animals were killed by decapitation through appropriate time, after the inhalation anesthesia with chloroform. Then, animals were sacrificed, brain, kidneys and liver from each group of animals were used for 10% homogenate preparation. For homogenization 25 mM Tris-HCl buffer (pH 7, 4), 0,175 M KCl was used. Half of each homogenate was kept in the refrigerator and the other half was used for centrifugation 15 min at 1000 g. We use the homogenate and supernatant for estimation of MDA amount. Quantitative determination of protein in samples of investigated preparations was carried by spectrophotometric method [14].

Malondialdehyde (MDA) amount was estimated by method [22]. The assay is based on a condensation reaction of two molecules of thiobarbituric acid with one molecule of MDA, in which the reaction rate depends on temperature, pH and concentration of thiobarbituric acid. The reaction is carried out in acidic solution and temperature of 100^0C within one hour time course and most of MDA is produced during reaction process from decomposition of products of lipid peroxidation.

The MDA and other aldehydes have been reacting with TBA to give a pink coloured species that absorbs at 532 nm. The method involved heating of biological samples with thiobarbituric acid reagent for 20 min in a boiling water bath. The reaction mixture contains 30% trichloroacetic acid, 5 N HCl, 0, 8% solution of thiobarbituric acid and biological sample. After cooling the solution was centrifuged at 3000 rpm/min for 10 min and the precipitate obtained was removed [22]. The absorbance of the supernatant was determined at 532 nm against a blank that contained all the reagents without the biological sample. The MDA concentration was calculated by formula

$$C = \frac{D}{\varepsilon lm} xd$$

where C is MDA concentration, (nM/ mg protein);
D is optical density of the studied sample;
ε is extinction coefficient: 1,56x105 M^{-1} cm^{-1} at 532 nm wavelength;
m is amount of protein in biological samples;
l is light optic pathway length l sm
d is measure of dilution

The obtained results were treated by statistics and 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. The concentration of MDA in the brain, kidney and liver tissues of female rats are shown in Table 1 (tab.1). It should be noted that in brain and kidney of control group of rats approximately the same quantities of MDA were obtained. On the contrary, in the rat liver the concentration of this aldehyde is substantially higher. These differences in MDA content in different rat tissues is likely linked to specificity of metabolism. On the other hand the MDA is considered main biomarker of oxidative stress [4]. Therefore it can be assumed that the brain and kidney tissues of female rats have the nearly same basic level of oxidative stress. On the contrary, in the liver tissue the basic level of oxidative stress is much higher.

Table 1.

MDA concentration in the rat brain, kidney and liver tissues of control and experimental groups (in nM/ mg protein) *-p<0.05

Experimental groups	Brain	Kidney	Liver
Baseline	1.50±0.03	1.26±0.071	2.65±0.17
Cisplatin	2.03±0.05	*1.74±0.07	*3.40±0.25
Estradiol	1.44±0.03	*1.10±0.031	*1.74±0.21
Cisplatin + Estradiol	1.58±0.02	*1.02±0.036	*2.11±0.10
Progesterone	1.10±0.02	1.28±0.035	2.40±0.02
Cisplatin + Progesterone	1.46±0.02	1.20±0.036	2.40±0.15
Estradiol + Progesterone	1.48±0.02	1.17±0.060	*2.34±0.05

The results confirm that MDA concentration significantly increased in the brain and kidney as well as in the liver of female rats after the cisplatin separate administration compared to the baseline (tab.1). Changes in MDA quantities of rat brain, kidney and liver tissues caused by cisplatin expressed as a percentage are presented in the Figure 1 (fig.1).

Those results show that the quantity of MDA in brain increased by 35 %, in kidney increased by 38 % and in liver by 28 % compared to baseline (fig.1). It is

interesting to note that in tissues with a relatively low basic oxidative stress level, the change in MDA quantity is greater, than in the liver, where the initial level of oxidative stress is rather high (fig.1).

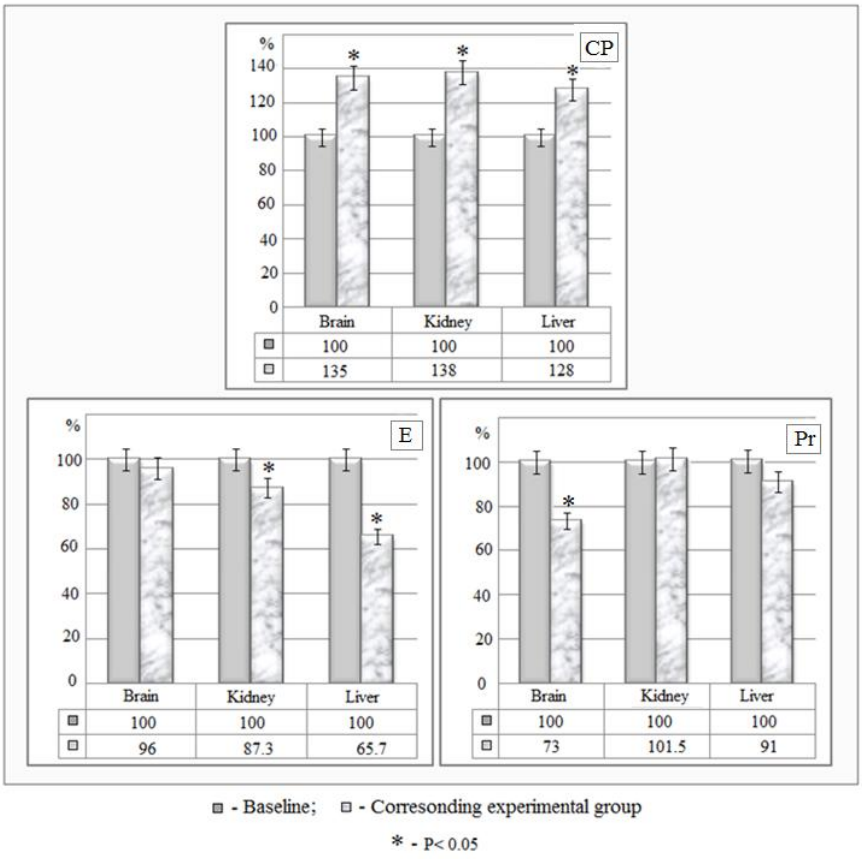

Fig. 1. The alteration (in percent) of MDA concentration in the rat brain, kidney and liver of control and experimental groups
CP – after the cisplatin separate action, E – after the estradiol separate action, Pr – after the progesterone separate action

It is well known that cisplatin is able to induce oxidative stress by stimulating the formation of reactive oxygen species [5, 11, 16]. Cisplatin is capable of stimulating the oxidative stress by suppressing the activity of the enzymes of the antioxidant system of the cell and thus contributing to the accumulation of ROS [11, 24]. These reactive oxygen species in turn trigger cell death by damaging DNA and other important cell molecules [11, 16, 20]. Furthermore there is suggestion that the induction of ROS is one of the major mechanisms of cisplatin cytotoxic action [9, 23].

However it should be noted, that oxidative stress is the main cause of undesirable side effects accompanying cisplatin therapy [23]. Furthermore there is suggestion that the induction of ROS is one of the major mechanisms of cisplatin cytotoxic action [9, 23]. In this respect the increase of MDA quantity after the cisplatin action is quite understandable.

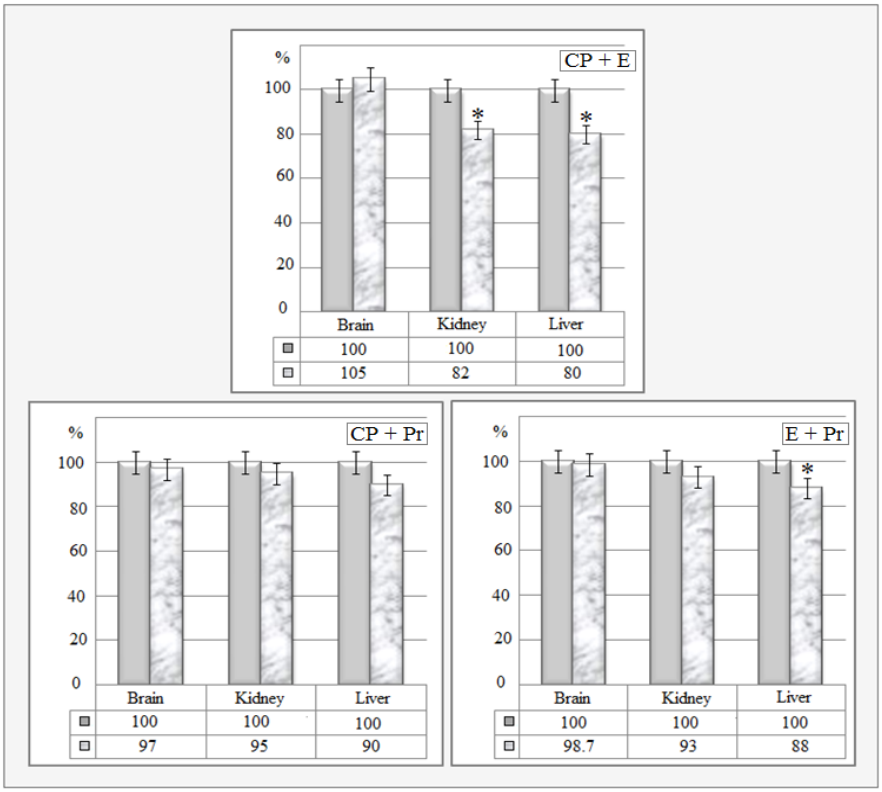

Fig. 2. The alteration (in percentage) of MDA concentration in the rat brain, kidney and liver of control and experimental groups
CP+E – after joint action of cisplatin and estradiol, CP +Pr – after joint action of cisplatin and progesterone,
E + Pr – after the joint action of estradiol and progesterone

Steroids have been shown to be antioxidants and capable of suppressing oxidative stress at two levels, by preventing ROS generation and by scavenging free radicals [7, 8, 17, 19]. The antioxidant properties of steroids are also evidenced by the results of our research [24]. The data on the separate action of estradiol show that the quantity of MDA decreased in the kidney and liver, whereas alterations that were obtained in the brain were unreliable (tab.1). These alterations expressed as a percentage are accounted for 12,7 % and 34,4 % in rat kidney and liver respectively (fig.1). On the contrary, the other steroid hormone progesterone injection decreased the MDA quantity only in the brain (by about 27 %), while in the other two tissues the amount of MDA remains unchanged or are not altered (tab.1 and fig.1).

The results we've obtained point to differences in antioxidant properties of these steroids as well as to its selective effects in cells of different rat tissues (tab.1 and fig.1). Taking into account that steroid hormones works as antioxidants and are able to prevent cisplatin toxicity we decided to investigate their combined effect on the malondialdehyde quantity.

Cisplatin and estradiol joint action manifested the tendency of restoring the baseline quantity of MDA in the brain of rat. In case of rat kidney and liver the MDA content was even decreased by 18 % and 20 % respectively (tab.1 and fig.2). In fact, estradiol does not only neutralize the stimulating effect of cisplatin in the kidneys and liver, but also suppresses the formation of malondialdehyde in these tissues (tab.1 and fig.2). Joint action of cisplatin and progesterone leads to the restoration of the baseline level of malondialdehyde quantity in all investigated tissues (tab.1 and fig.2). Thus, these steroids, due to their antioxidant properties, neutralize the stimulating action of cisplatin.

Significantly high antioxidant properties of estradiol and progesterone are detected when comparing the results of the co-use of cisplatin and hormones with the data of cisplatin separate injection (taking these for 100 %) (tab.1 and fig.3). In case of cisplatin and estradiol joint action the quantity of MDA decreased in the brain by 22 %, in the kidney by 41 %, and in the liver by 38 %. (tab.1 and fig.3).

Cisplatin and progesterone joint action leads to decrease in the amount of MDA by 27 %, 33 % and 32 % in brain, kidney and liver respectively by comparison to cisplatin separate action (tab.1 and fig.3). These results demonstrate the strong antioxidant properties of steroid hormones, which are more evident in case of joint action with cisplatin.

We also explored the joint action of two steroid hormones estradiol and progesterone on content of MDA in the brain, kidney and liver tissues of rat. In this case there is a perceptible change (by 12 %) in MDA quantity of rat liver tissue, whereas in the brain and kidney of rat obtained changes were negligible and unreliable (tab.1 and fig.2). Quantitative changes in MDA reveal some competition in the behavior of these hormones. Results suggest that the effect of estradiol is predominating in the brain tissue of rat in case of joint action of two steroids. Estradiol neutralizes the suppressive effect of progesterone and restores the basic level of MDA. In the rat kidney cells, the progesterone advantage neutralizes the suppressive effect of estradiol, while in the liver cells of female rat's estradiol effect is again dominant. The foregoing seems to be the result of "crosstalk" between two steroid hormones at the molecular level resolved in favor of estradiol or progesterone.

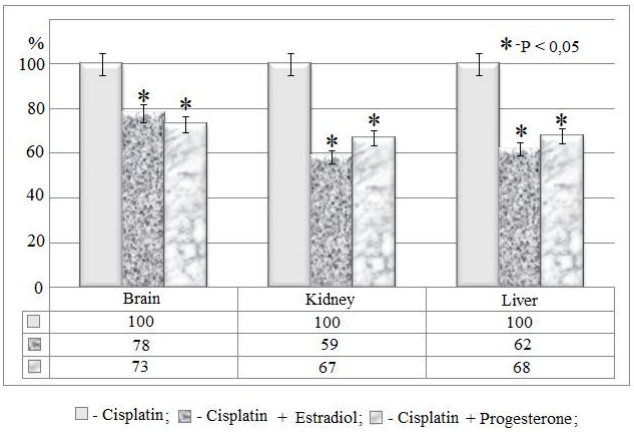

Fig.3. The alteration (in percentage) of MDA concentration in the rat brain, kidney and liver tissue. Data of cisplatin separate action was taking for 100 %
Cisplatin – after the cisplatin separate action, Cisplatin +Estradiol – after joint action of cisplatin and estradiol,
Cisplatin +Progesterone – after joint action of cisplatin and progesterone.

Thus, when used separately, cisplatin stimulates the formation of MDA, and hence oxidative stress in all investigated tissues. Estradiol separate injection does not affect the quantity of MDA in the brain, but reduces its amount in the kidney and in the liver tissues. Progesterone, when used separately, reduces the amount of MDA in the brain, and does not affect its quantity in the kidney and liver cells. In case of joint action cisplatin and estradiol as well as progesterone neutralize the stimulating effect of cisplatin. The estradiol and progesterone steroid hormones are in competition in case of joint action.

The obtained results may be helpful for explaining the estradiol and progesterone attenuating effects in case of their joint use with cisplatin.

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