Acta Poloniae Pharmaceutica – Drug Research, Vol. 69 No. 6 pp. 1372–1379, 2012 ISSN 0001-6837
Polish Pharmaceutical Society

ROLE OF ESTRADIOL IN CHROMIUM-INDUCED OXIDATIVE STRESS

KRZYSZTOF P. REMBACZ[1]*, EWA SAWICKA[1] and ANNA DŁUGOSZ[1]

[1]Chair and Department of Toxicology, Wrocław Medical University,
Traugutta 57/59, 50-417 Wrocław, Poland

Abstract: This study investigated the role of 17β-estradiol in chromium-generated oxidative stress in order to determine whether it has a detoxifying activity or increases the toxic effects of chromium compounds. Reduced glutathione (GSH) levels, membrane lipid peroxidation (levels of malondialdehyde – MDA), glutathione peroxidase (GPx), and superoxide dismutase (SOD) activities were measured in blood. Isolated mitochondria were used to investigate the MDA levels and hydroxyl radical ($OH^{\cdot}$) generation. The results showed a varying influence of estradiol on the chromium-induced oxidative stress. This paper demonstrated, that 17β-estradiol showed a positive effect when erythrocytes were exposed to moderate concentrations of CrVI and increased the levels of erythrocytal GSH. Estradiol did not show any interactions with chromium on the antioxidative enzymes (SOD in erythrocytes and GPx in whole blood) activity measurements. Additionally, estradiol played a generally positive role in the chromium-induced lipid peroxidation in erythrocytes. Unexpectedly, the interaction of estradiol with chromium was found in human mitochondria, where estradiol increased the MDA levels induced by both forms of chromium. Estradiol also increased the $OH^{\cdot}$ generation triggered with CrVI. It appeared that estradiol acted protectively on lipid peroxidation caused by chromium in erythrocytes but gave an interaction with Cr in mitochondria, which partially correlated with hydroxyl radical formation in this organelle.

Keywords: estradiol, chromium, interaction, reduced glutathione, lipid peroxidation, hydroxyl radical

Free radicals occur naturally in the body and are scavenged by antioxidants in physiological processes. Any disturbance of this equilibrium may lead to severe tissue abnormalities, even to cancer. Chromium is widely present in the environment; the toxicity of this metal is directly dependent on its valency. Chromium III (CrIII) is an essential factor of glucose metabolism as a constituent of the glucose tolerance factor GTF; it is occasionally supplemented to human diet. Chromium VI (CrVI) is a proved carcinogen responsible for a number of disorders of variable background in humans. Chromium is one of metalloestrogens that has the ability to replace zinc in zinc fingers area of estrogen receptor (1, 2). It generates reactive oxygen species; CrVI, easier than CrIII, translocates through biological membranes and is subsequently reduced intracellularly to CrV, CrIV and CrIII with a simultaneous reactive oxygen species (ROS) generation (3, 4). Chromium III was in general considered not toxic, it was found, however, able to generate hydroxyl radicals (5), its toxicity has been also demonstrated recently by decreasing the ferric reducing ability of plasma (6) or increasing the lipid peroxidation (7). Additionally CrIII has a well-documented ability of binding to DNA (8).

Estradiol, next to the hormonal activity, is involved in the anti- and pro-oxidative transformations as the inducer or scavenger of ROS. Estrogens have a well-documented ability to scavenge free radicals (9–11), they may, however, induce oxidative stress as well (12, 13). It has also been shown, that peroxidation of low-density lipoprotein is inhibited in postmenopausal women after administration of estradiol (10, 14). Apart from the free radical scavenging activities, estradiol binds to the estrogen receptor (ER) activating the transcription of a variety of estrogen-dependent genes.

Interactions between xenobiotics and estradiol are limitedly described. It has been shown, that the exposure to xenobiotics present in cigarette smoke can abolish the positive effects of the hormone replacement therapy (HRT) in women (15). Estradiol has been shown to minimize the toxic effects of fluoride exposure (16) and to be involved in the ethanol toxicity (17). It was also known, that

* Corresponding author: e-mail: krzysztof.rembacz@am.wroc.pl

alcohol consumption increases the levels of blood estrogens (18). The influence of estradiol on chromium-induced free radical formation has not been characterized to date. Since the chromium supply increases in the form of diet supplements, it seems crucial to investigate this problem further to determine whether chromium exposure may abolish the antioxidative or other biological properties of estradiol. This paper demonstrated that estradiol can play a protective role in the chromium-induced oxidative stress, it may, however, in some cases interact with chromium and aggravate the toxicity of this metal.

The aim of this study was to investigate the role of 17β-estradiol in chromium-generated oxidative stress in order to determine whether it has a detoxifying activity or increases the toxic effects of chromium compounds. Analyses described in this study were performed in an *in vitro* model of whole human blood or purified erythrocytes and mitochondria isolated from placenta obtained from physiological deliveries. The results show varying influence of estradiol on the chromium-induced oxidative stress.

EXPERIMENTAL

Human blood, erythrocytes, or placental mitochondria were used in experiments. Mitochondria were isolated from human placenta from natural deliveries essentially as described (19). Fresh human blood taken on EDTA (for GSH, SOD, and MDA determination) or heparin (for GPx measurements) was obtained from healthy donors of the Academic Hospital and used directly (GPx) or after fractionation into erythrocytes. Erythrocytes were isolated by the whole blood centrifugation for 10 min at room temperature (3000 rpm). Subsequently plasma and white blood cells were rejected and the resulting pellet of erythrocytes was washed three times in a phosphate buffered saline (PBS). Erythrocytes were suspended in PBS at 50% density for GSH determination or 10% density for SOD and MDA measurements.

Mitochondrial protein content was measured with a method of Lowry (20). Mitochondrial lipid peroxidation [malondialdehyde (MDA) levels] was determined with thiobarbituric active reagent species (TBARS) method (21) and expressed in nmol per milligram of protein (nmol/mg). Hydroxyl radical ($OH^{\cdot}$) formation in mitochondria was measured with deoxyribose degradation method of Rice-Evans (22) and expressed in nmol/mg protein.

Reduced glutathione (GSH) content of erythrocytes was determined with Ellman's modified method (23) and expressed in mmol per gram of hemoglobin (mmol/gHb). SOD activity was measured with a RANSOD kit from Randox Laboratories according to manufacturer's instructions and expressed in U/gHb (24). GPx acivity was determined with a RANSEL kit from Randox Laboratories and expressed in U per liter (25).

$CrCl_3$ (chromium chloride hexahydrate, Riedel de Haën) and $K_2Cr_2O_4$ (P.P.H. POCh S.A.) were used as CrIII and CrVI sources, respectively. Chromium concentrations of 0.05 to 10.0 μg/mL were used. The exact Cr concentrations for every parameter tested are specified in the text. Estradiol (E_2; 17β-estradiol; >98%, Sigma-Aldrich) was dissolved in 96% ethanol and used in concentrations 0.75 nM; 1–10 μM (exact concentrations for each parameter are specified in the text).

All statistical analyses were performed with a Student's *t*-test, differences between the groups were considered significant at $p < 0.05$. Pearson correlations of the linearity of estradiol concentrations used to the effects invoked were determined as negative or positive (r_{xy}"+" or r_{xy}"–") and were considered significant at $p < 0.05$.

RESULTS

Estradiol did not show harmful interactions with chromium on SOD and GPx activities measured in human erythrocytes. The data obtained demonstrate, that 17β-estradiol does not influence the activities of antioxidant enzymes upon chromium exposure.

Previous studies have shown that estradiol (E_2) plays a protective role on the thiol (-SH) groups of mitochondria exposed to NaF (16) and that CrVI reduces the levels of glutathione (GSH) in erythrocytes exposed to 5 and 10 μg/mL CrVI (6). In this work, the protective ability of 17β-estradiol in concentrations of 1.0, 5.0 and 10.0 μM towards GSH was tested on erythrocytes exposed to 5 and 10 μg/mL of chromium VI and III. No interactions between CrIII and E_2 influencing the GSH content in erythrocytes were noted as the erythrocytes exposed to both CrIII and E_2 did not show any significant differences when compared to control treated with CrIII alone.

Similar results were obtained when evaluating the interactions between estradiol and chromium VI used at the highest concentration of 10 μg/mL, where the levels of GSH remained reduced and did not differ significantly from the control erythrocytes exposed to CrVI. However, a positive effect of

estradiol on the GSH levels was evident in the chromium VI induced oxidative stress used at lower (5 μg/mL) concentrations. Erythrocytes exposed to 5 μg/mL of CrVI alone showed reduced level of GSH (2.70 mmol/gHb) when compared to control erythrocytes not treated with chromium (3.01 mmol/gHb). After addition of estradiol (0.75 nM; 1 and 10 μM) the levels of GSH were increasing and reached the concentration of 2.92 μmol/gHb with 10 μM E_2. While this value of GSH concentration was somewhat lower than of control erythrocytes not treated with CrVI, it was shown statistically significantly higher ($p = 0.00$) than of erythrocytes exposed to chromium VI at 5 μg/mL (Fig. 1). The relation of estradiol concentrations used to the gradual increase of GSH in 5.0 μg/mL chromium VI treated erythrocytes was confirmed with a Pearson linear correlation test, where $r_{xy} = 0.805$ demonstrating a very strong, linear and positive correlation with the significance of $p = 0.00$ (Fig. 1).

Estradiol in erythrocytes showed generally positive effect on lipid peroxidation caused by both

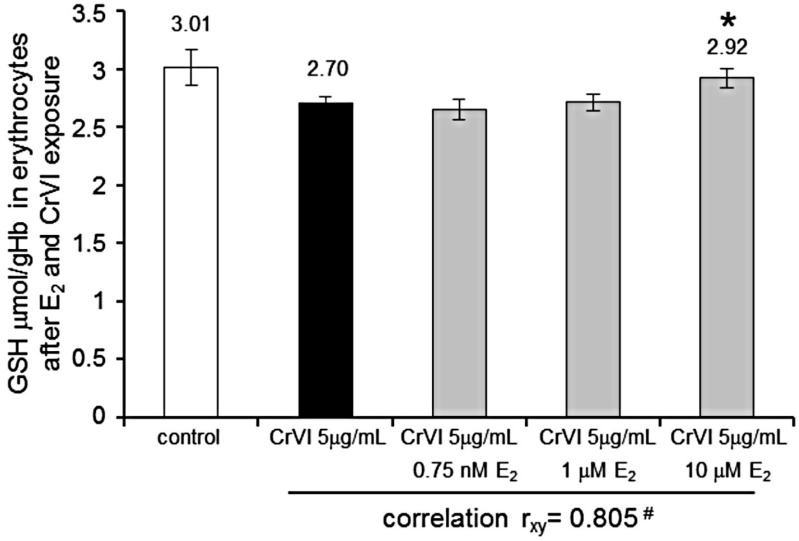

Figure 1. Influence of estradiol on GSH levels in erythrocytes exposed to chromium VI.
* Significant differences *vs*. control containing chromium without estradiol (black bar) # Significant correlation of estradiol influence are given

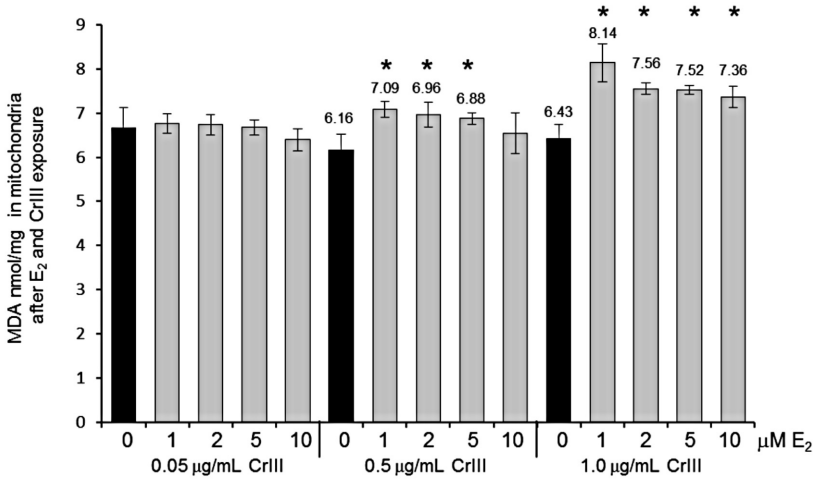

Figure 2. Influence of estradiol on MDA levels in mitochondria exposed to chromium III.
* Significant differences *vs*. control without estradiol.

Table 1. Influence of estradiol on MDA levels in erythrocytes exposed to chromium.

CrIII	0.05 µg/mL					0.5 µg/mL					1.0 µg/mL				
E_2 µM	0	1	2	5	10	0	1	2	5	10	0	1	2	5	10
MDA nmol/gHb	0.432	0.421	0.444	0.473	0.350	0.422	0.372	0.387	0.397	0.394	0.509	0.434	0.439	0.516	0.486
SD	0.01	0.05	0.01	0.02	0.01	0.20	0.01	0.08	0.02	0.03	0.06	0.01	0.04	0.06	0.06
p value *vs.* CrIII		0.66	0.17	0.00*	0.00*		0.01*	0.94	0.20	0.20		0.06	0.11	0.88	0.58
CrVI	0.05 µg/mL					0.5 µg/mL					1.0 µg/mL				
E_2 µM	0	1	2	5	10	0	1	2	5	10	0	1	2	5	10
MDA nmol/gHb	0.432	0.479	0.436	0.418	0.413	0.432	0.540	0.451	0.459	0.436	0.456	0.455	0.415	0.405	0.408
SD	0.01	0.02	0.01	0.01	0.01	0.02	0.03	0.02	0.01	0.01	0.01	0.01	0.02	0.05	0.02
p value *vs.* CrVI		0.00*	0.50	0.10	0.02*		0.00*	0.10	0.01*	0.61		0.88	0.00*	0.00*	0.00*

* Difference significant *vs*. control without estradiol.

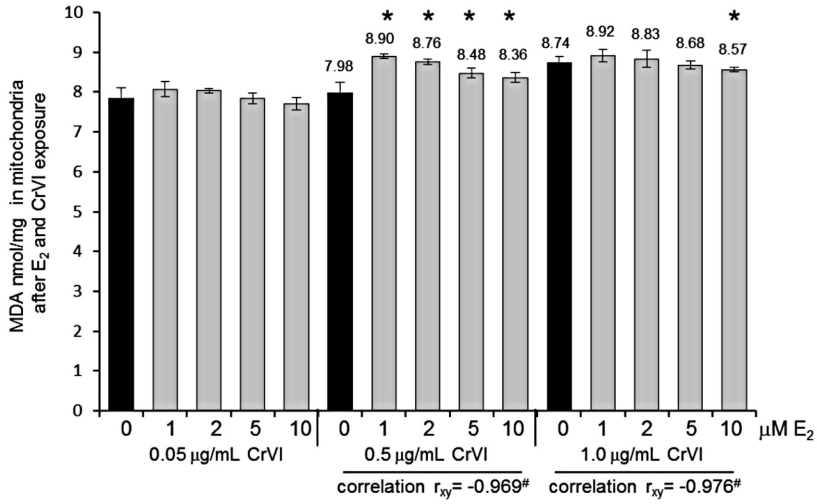

Figure 3. Influence of estradiol on MDA levels in mitochondria exposed to chromium VI. * Significant differences *vs*. control without estradiol. # Only significant correlations of estradiol influence are given

CrIII and CrVI measured as MDA levels. In chromium treated erythrocytes, estradiol caused a decrease of MDA when compared to control erythrocytes exposed to chromium. Estradiol prevented the lipid peroxidation in erythrocytes exposed to moderate concentrations of CrIII (0.5 µg/mL). Estradiol at concentrations of 1, 2 and 5 µM decreased the MDA levels in erythrocytes to 0.373; 0.387 and 0.397 nmol/gHb, respectively, compared to control exposed to 0.5 µg/mL chromium (0.422 nmol/gHb) with a statisctical significance of p = 0.00 (Table 1). A positive influence of estradiol on erythrocytal membrane lipid peroxidation caused by a high CrVI concentration (1 µg/mL) could also be noted. Estradiol at concentrations 2, 5 and 10 µM decreased the levels of MDA to 0.415; 0.405 and 0.408 nmol/gHb, respectively, when compared to control erythrocytes exposed to CrVI (0.432 nmol/gHb MDA) with p = 0.00 (Table 1). Minor interactions of estradiol with both chromium forms

could be noted as well, they are, however, sporadic and not linear (MDA level measured in these limited cases were not corresponding to estradiol concentration range used) and thus should be regarded as inconclusive (Table 1).

In mitochondria, estradiol showed an advert interaction with both CrIII and CrVI leading to an increase of mitochondrial membrane lipid peroxidation. At the highest CrIII concentrations of 1.0 μg/mL for example, the MDA levels in mitochondria statistically significantly ($p = 0.00$) increased upon 1, 2; 5 and 10 μM estradiol addition to 8.14, 7.56, 7.52 and 7.36 nmol MDA/mg protein, respectively, compared to control mitochondria exposed do CrIII (6.43 nmol/mg) (Fig. 2). Pearson correlations of the linear dependence of estradiol concentrations used to the effect of MDA level reduction were in the range of $-0.94 < r_{xy} < -0.92$ in the background of CrIII 0.5 and 1.0 μg/mL, which depicts a strong, negative dependence, they were, however,

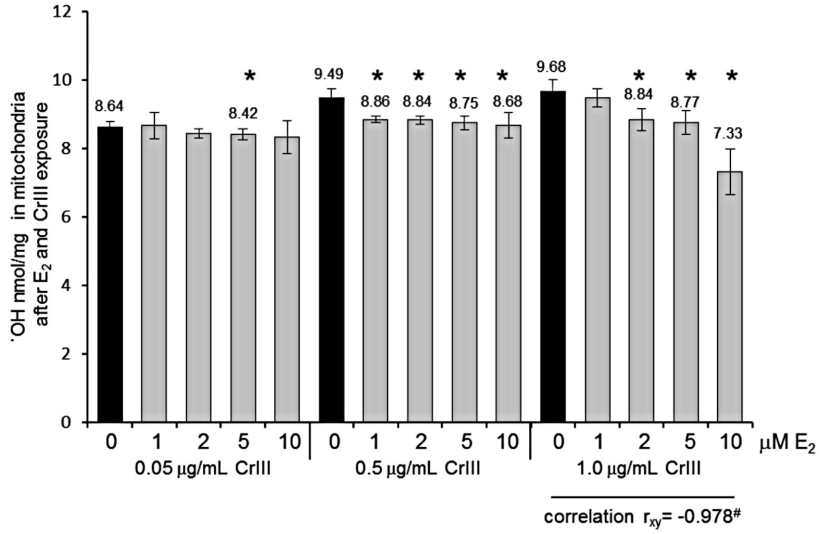

Figure 4. Influence of estradiol on hydroxyl radical levels in mitochondria exposed to chromium III. * Significant differences *vs.* control without estradiol. # Only significant correlations of estradiol influence are given

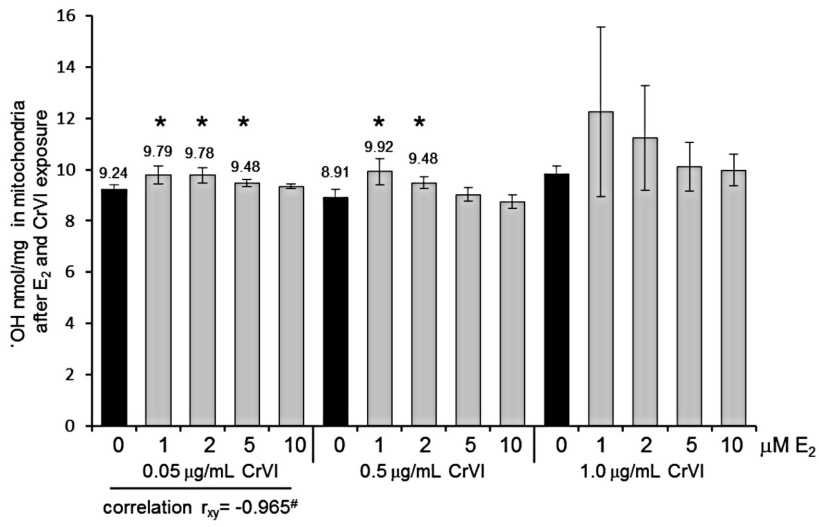

Figure 5. Influence of estradiol on hydroxyl radical levels in mitochondria exposed to chromium VI. * Significant differences *vs.* control without estradiol. # Only significant correlations of estradiol influence are given

not significant. Similar results were obtained for mitochondria exposed to 0.5 μg/mL CrVI, where estradiol (used in 1, 2, 5 and 10 μM solutions) statistically significantly ($p = 0.00$) increased the mitochondrial membrane lipid peroxidation expressed as MDA levels to 8.90; 8.76; 8.48; 8.36 nmol/mg, respectively, compared to the control (7.98 nmol/mg) (Fig. 3). Pearson correlations of the linear dependence of estradiol concentrations used to the effect of MDA level reduction were in the range of $-0.976 < r_{xy} < -0.969$ in the background of CrVI 0.5 and 1.0 μg/mL, which depicts a strong, negative dependence with a significance $p < 0.03$ and $p < 0.02$. respectively (Fig. 3).

To get further insight into the cause for the mitochondrial lipid peroxidation increase resulting from the interaction between chromium and estradiol, the hydroxyl radical formation was evaluated in mitochondria exposed to CrIII, CrVI and 17β-estradiol. This experiment showed, that estradiol differentially influences the OH formation resulting from mitochondrial exposure to both forms of chromium. In chromium III exposed mitochondria estradiol decreased the $OH^{\cdot}$ generation. As an example, in 1.0 μg/mL CrIII treated mitochondria, where the hydroxyl radical levels of control mitochondria were 9.68 nmol/mg protein, estradiol in concentrations of 1, 2, 5 and 10 μM decreased the $OH^{\cdot}$ levels to 9.49; 8.84; 8.77 and 7.33 nmol/mg, respectively (Fig. 4). Correlation of the linear dependence of estradiol concentrations used to the effect of $OH^{\cdot}$ level reduction was shown with Pearson coefficient equaling –0.978 (strong and negative) with $p = 0.02$ for this concentration (Fig. 4). An opposite effect was noted for CrVI, for example, in 0.05 μg/mL of CrVI, where 1, 2, 5 and 10 μM of estradiol increased the $OH^{\cdot}$ levels to 9.79; 9.78; 9.48 and 9.35 nmol/mg, respectively, when compared to control mitochondria exposed to CrVI alone (9.24 nmol $OH^{\cdot}$/mg of protein) (Fig. 5). Correlation of the linear dependence of estradiol concentrations used to the effect of $OH^{\cdot}$ level increase was shown with negative Pearson coefficient values in the background of CrVI and at the chromium VI concentration of 0.05 ˙g/mL equaled –0.965 (strong and negative) with $p = 0.035$ (Fig. 5).

DISCUSSION

In this paper, it was investigated the role of 17β-estradiol in a variety of chromium-induced free radical reactions. In human erythrocytes exposed to chromium VI estradiol played a positive role and partially ameliorated the negative effects of this metal on the reduced glutathione (GSH) content in these cells. This effect could be seen at moderately high (5 μg/mL) chromium VI concentrations only. The response to estradiol treatment was linear and GSH levels were correlating with the increasing concentrations of estradiol used, demonstrating the specificity and biological relevance of this process *in vitro*. It also conformed to the previously published data on the estradiol-based increase of the general content of -SH groups in human mitochondria (16). At the highest concentration of chromium VI (10 μg/mL), the positive effect of estradiol on the erythrocyte GSH levels was not observed. This chromium concentration appeared too excessive for the cellular (erythrocyte) system to accommodate, and thus the effect of estradiol under these conditions might have been missed. It was known, that high chromium concentration increases the apoptosis and destroys the glutathione antioxidant system in L-41 cell line (26). Most probably, no cellular activities (and thus no estradiol influence) could be detected after exposure of erythrocytes to such high chromium VI concentration.

Most striking were the differences between the influence of estradiol on lipid peroxidation caused by chromium in erythrocytes and mitochondria. In general, E_2 showed a positive effect on lipid peroxidation caused by chromium in erythrocytes, however, an opposite, negative interactions were observed between estradiol and chromium in mitochondria. These differences might be attributed to the biochemical differences between erythrocytes and mitochondria. Human erythrocytes are non-nucleated cells lacking the majority of organelles (27). The energy for metabolic processes in erythrocytes is derived only from glycolysis; in general, these cells of the human body are moderately active metabolically. Consequently, the redox processes in isolated erythrocytes are characterized by a low intensity, thus the free radical formation is minimal. Isolated erythrocytes that lack the mitochondria – an organelle with a high affinity to estradiol, are also apparently less sensitized to estrogen influence. Estradiol alone did not influence the levels of erythrocytal GSH (data not shown).

Malondialdehyde is formed (synthesized) in erythrocytes as a result of chromium-derived ROS action. Since (isolated) red blood cells are characterized by a low metabolic rate, the actual response of these cells to chromium will most probably be slow. Apparently, estradiol influences this process by scavenging free radicals before they could damage the membrane lipids in cells with a lower metabolism. Estradiol also might chelate chromium ions

more quickly than it takes for the MDA formation, prior to their entry into erythrocytes or already in the cytosol. All of these proposed mechanisms exclude the involvement of estrogen receptor influence. It is known, that human erythrocytes do not possess estrogen receptors at their membrane (28), however, some positive influence of estradiol on erythrocyte shape in rats was documented (28, 29).

Mitochondria, on the other hand, are described as the energy center of the eukaryotic cell. They are responsible for the intense oxidative phosphorylation reactions and house the majority of the redox reactions. They are responsible for the formation of ROS that might cause the lipid peroxidation. It is also known, that mitochondria serve as the intracellular reservoir of estradiol, where extracellularly added estrogens are efficiently transported into this organelle (30). The increase of MDA levels in mitochondria under the influence of estradiol in chromium-induced lipid peroxidation might be caused by a high metabolic activity of these organelles. In this environment, even a minor free radical reaction initiation triggers a cascade response that might result in variety of mitochondrial protein and lipid damage. Additionally, estradiol alone has been shown to promote the free radical formation in mitochondria (31, 32). In the background of free radicals formed by chromium ions in highly metabolically active mitochondria, estradiol not only does not show a positive influence on lipid peroxidation, but also interact with this metal. This most probably is caused by the ability of estradiol alone to induce oxidative stress in this organelle (31, 32), that in turn accelerates the negative effects of chromium and diminishes the ability of this estrogen to block the lipid peroxidation triggered by either CrIII or CrVI in mitochondria.

This proposed mechanism is reflected in Pearson correlation tests of the linear dependence of estradiol concentrations and exhibited effects in this study. Linear correlations showed that MDA formation in mitochondria negatively correlated with estradiol concentration and that at lowest concentrations of estradiol, interaction with chromium was the strongest. This demonstrated that in highly metabolically active mitochondria already small amounts of estradiol interact with chromium and accelerate the lipid peroxidation. The levels of MDA were lowering with increasing estradiol concentration, (which was probably due to partially retained ability of this estrogen to scavenge free radicals); however, the overall balance was always shifted towards acceleration of MDA formation at all the concentrations of estradiol used. Estradiol never exhibited a positive influence on mitochondrial lipid peroxidation in the background of chromium-induced oxidative stress and interacted with chromium accelerating the lipid peroxidation in this organelle.

Additionally, estradiol showed an interaction with CrVI and promoted the hydroxyl radical formation in mitochondria. This corresponded to MDA increase described in this paper and suggested that this radical was responsible for the lipid peroxidation in CrVI exposed mitochondria. An opposite effect was noted for $OH^{\cdot}$ generated by chromium III where estradiol reduced the levels of hydroxyl radicals generated. Since in the mitochondria exposed to CrIII and estradiol, reduced levels of MDA were observed, it suggested that radical different from $OH^{\cdot}$ is responsible for lipid peroxidation in CrIII exposed mitochondria.

CONCLUSIONS

Estradiol showed a differential influence on the ROS generation upon erythrocyte and mitochondria exposure to chromium.

In erythrocytes, estradiol reduced the MDA formation caused by chromium, while in mitochondria it showed an interaction with this element and aggravated the negative effect of exposure to both forms of chromium.

It appeared that estradiol played a protective role in the lipid peroxidation caused by chromium in erythrocytes but showed an interaction with Cr in mitochondria, which partially correlated with the hydroxyl radical formation in this organelle.

REFERENCES

1. Stoica A., Katzenellenbogen B.S., Martin M.B.: Mol. Endocrinol. 14, 545 (2000).
2. Darbre P.D.: J. Appl. Toxicol. 26, 191 (2006).
3. Kalahasthi R.B., Raghavendra Rao R.H., Krishna M.R.B., Kumar M.K.: Chem. Biol. Interact. 164, 192 (2006).
4. Panda S.K., Chaudhury I., Khan M.H.: Biol. Plant. 46, 289 (2003).
5. Shrivastava H.Y., Ravikumar T., Shanmugasundaram N., Babu M., Unni N.B.: Free Radic. Biol. Med. 38, 58 (2005).
6. Długosz A., Rembacz K.P., Pruss A., Durlak M., Lembas-Bogaczyk J.: Pol. J. Environ. Stud. 21, 329 (2012).
7. Sawicka E., Srednicka D., Dlugosz A.: Adv. Clin.Exp. Med. 17, 539 (2008).

8. Sobol Z., Schiestl R.H.: Environ. Mol. Mutagen. 53, 94 (2012).
9. Ruiz-Larrea M.B., Leal A., Martin C., Martinez R., Lacort M.: Steroids 60, 780 (1995).
10. Bednarek-Tupikowska G., Tupikowski K., Bidzinska B., Bohdanowicz-Pawlak A., Antonowicz-Juchniewicz J., Kosowska B. et al.: Gynecol. Endocrinol. 19, 57 (2004).
11. Ruiz-Larrea M.B, Martin C., Martinez R., Navarro R., Lacort M., Miller N.J.: Chem. Phys. Lipids 105, 179 (2000).
12. Gomez-Zubeldia M.A., Corrales S., Arbues J., Nogales A.G., Millan J.C.: Gynecol. Oncol. 86, 250 (2002).
13. Liehr J.G.: J. Lab. Clin. Med. 128, 344 (1996).
14. Sack M.N., Rader D.J., Cannon R.O.: Lancet 343, 269 (1994).
15. Mueck A.O., Seeger H.: Curr. Med. Chem. Cardiovasc. Hematol. Agents 3, 45 (2005).
16. Dlugosz A., Roszkowska A., Zimmer M.: Basic Clin. Pharmacol. Toxicol. 105, 366 (2009).
17. Długosz A., Pruss A., Lembas-Bogaczyk J.: Acta Pol. Pharm. Drug Res. 67, 713 (2010).
18. Ginsburg E.S.: Steroid Biochem. Mol. Biol. 69, 299 (1999).
19. Dlugosz A., Piotrowska D.: Toxicol. In Vitro 16, 649 (2002).
20. Lowry O.H., Rosbrough N.J., Farr A.L., Randall R.J.: J. Biol. Chem. 193, 265 (1951).
21. Dlugosz A., Sawicka E., Marchewka Z.: Toxicol. In Vitro 19, 581 (2005).
22. Rice-Evans C.A., Diplock A.T., Symons M.C.R.: Techniques in Free Radical Research, Elsevier, Amsterdam, London, New York, Tokyo 1991.
23. Ellman G.L.: Arch. Biochem. Biophys. 82, 70, (1959).
24. Mistra H.P., Fridovich I.: Arch. Biochem. Biophys. 181, 308 (1985).
25. Paglia D.E., Valentine W.N.: J. Lab. Clin. Med. 70, 158 (1967).
26. Asatiani N., Sapojnikova N., Abuladze M., Kartvelishvili T., Kulikova N., Kiziria E., Namchevadze N., Holman H.Y.: J. Inorg. Biochem. 98, 490 (2004).
27. Craig JIO, Haynes AP, McClelland DBL, Ludlam CA. Blood disorders. in Davidson's principles and practice of medicine. Haslet C, Chilver ER, Boon NA, Colledge NR EDS., p. 889–956, Churchill Livingstone, New York 2002.
28. Doucet D.R., Bonitz R.P., Feinman R., Colorado I., Ramanathan M., Feketeova E., Condon M., Machiedo G.W., Hauser C.J., Xu D.Z., Deitch E.A.: Principles and practice of medicine. pp. 889–956, Churchill Livingstone, New York 2002.
29. Machiedo G.W., Zaets S., Berezina T., Xu D.Z., Spolarics Z., Deitch E.A.: J. Trauma 56, 837 (2004).
30. Świtalska M., Strządała L.: Postepy Hig. Med. Dosw. 61, 541 (2007).
31. Felty Q., Roy D.: J. Carcinog. 4, 1 (2005).
32. Tatoyan A., Giulivi C.: J. Biol. Chem. 273, 11044 (1998).