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THE LINKAGE BETWEEN MERCURY-CAUSED NEURO- AND GENOTOXICITY VIA THE INHIBITION OF DNA REPAIR MACHINERY: FISH BRAIN MODEL

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Ключевые слова: *тяжелые металлы, токсичность, окислительный стресс, апуриновая/апиримидиновая эндонуклеаза APE1, эксцизионная репарация оснований BER*

Abstract. The linkage between mercury-caused neuro- and genotoxicity via the inhibition of DNA repair machinery: fish brain model. Nedzvetsky V.S., Gasso V.Y., Herrmann B., Novitsky R.O. *Heavy metals in model conditions as well as industrial pollution launch disturbances in neural cells of different animals and human beings. The neurotoxicity of mercury, which is one of the most toxic heavy metals, has been studied for several decades. However, its low doses chronic exposure effects for neural tissue cells are still poorly understood. Therefore, the basic molecular mechanisms of mercury should be clarified. The purpose of our research is to clarify the mechanism of mercury genotoxicity, the role of the DNA repair protein apurinic/apyrimidinic endonuclease 1 (APE1) in neural tissue cells, and the response to inorganic mercury-induced neurotoxicity. In our model, we used juvenile rainbow trout exposed to mercury chloride with a range of doses 9-36 µg/L for 60 days to study the cytotoxicity of chronic exposure. We detected the reactive oxygen species (ROS) production as an index of oxidative stress and APE1 as a marker of cellular DNA damage response in a neural cell. The ROS level was measured by using the fluorometric method based on 2',7'-dichlorofluorescein diacetate reaction. The analyses of markers of the DNA repair (APE1) and apoptosis (B cell lymphoma-2 anti-apoptotic protein – Bcl-2) were carried out with western blotting. The mercury chloride chronic exposure induced statistically significant upregulation of the ROS production in the fish brain. Contrary, the mercury low doses stimulated the downregulation of APE1 expression in the brain tissue. Furthermore, mercury chronic exposure inhibited the expression of Bcl-2 in the animals treated with 18 and 36 µg/L mercury chloride. The harmful effect of mercury could be promoted by oxidative stress generation. The downregulation of APE1 expression could lead to a lack of DNA damage response efficacy and initiate the decline in neural cell functioning. Obtained data on the APE1 expression have shown that the neurotoxic effect of mercury could be mediated, at least partially, by the decline in cellular DNA damage response in the brain. The evaluation of decrease in DNA repair response via detection of the APE1 expression can be a prospective tool to reveal the deleterious effects of toxicants in terms of their neuro- and genotoxicity.*

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Реферат. Зв'язок між індукованими ртуттю нейро- та генотоксичністю через гальмування механізмів репарації ДНК: мозок риб як модель. Недзвецький В.С., Гассо В.Я., Геррманн Б., Новіцький Р.О. *Важкі метали в модельних умовах та при промисловому забрудненні викликають порушення в нейронних клітинах різних видів тварин і людини. Нейротоксичність ртуті, яка є однією з найбільш токсичних важких металів, вивчалася протягом декількох десятиліть. Однак хронічні наслідки впливу її низьких доз для клітин нервової тканини все ще недостатньо зрозумілі, тому основні молекулярні механізми впливу ртуті необхідно прояснити. Метою нашого дослідження є уточнення механізму генотоксичного впливу ртуті, роль ДНК-репараційного білка апуринової/апіримідинової ендонуклеази 1 (APE1) у клітинах нервової тканини та реакція на нейротоксичність, викликану неорганічною ртуттю. У нашій моделі ми використовували ювенільну форель, яку піддавали впливу хлориду ртуті в діапазоні доз 9-36 мкг/л протягом 60 днів для вивчення цитотоксичності при хронічному впливі. Ми визначали утворення реактивних форм кисню (ROS) як індекс окисного стресу та APE1 як маркера реакції пошкодження клітинної ДНК у нервових клітинах. Рівень ROS вимірювали за допомогою флуорометричного методу, заснованого на реакції 2',7'-дихлоро-флуоресцеїн-діацетату. Аналіз маркерів репарації ДНК (APE1) та апоптозу (антиапоптотичний білок BCL-2) проводили за допомогою western blotting. Хронічний вплив хлориду ртуті індукує статистично значуще продукування ROS у мозку риб. Навпаки, низькі дози ртуті стимулювали зниження експресії APE1 у тканинах мозку. Крім того, хронічний вплив хлориду ртуті в концентраціях 18 та 36 мкг/л інгібує експресію BCL-2 у тварин. Шкідливий вплив ртуті може стимулюватися окиснювальним стресом. Зниження експресії APE1 може призводити до недостатньої ефективності репарацій ДНК та викликати погіршення функціонування нервових клітин. Отримані дані щодо експресії APE1 показали, що нейротоксичний ефект ртуті може бути опосередкований, принаймні частково, погіршенням клітинної реакції на пошкодження ДНК у клітинах мозку. Оцінка порушень репарації ДНК за допомогою визначення експресії APE1 може бути перспективним інструментом для виявлення шкідливого впливу токсикантів з точки зору їх нейро- та генотоксичності.*

Mercury is confirmed as one of the most toxic environmental contaminants for all living organisms including humans. Global industrialization causes mercury pollution [10] and generates dangerous risk factor for both human and animal health [11] due to high mercury potential bioaccumulation in living organisms [3, 14]. Heavy metals entail disturbances in neural cells of different animals [4, 7]. The neurotoxicity of mercury is known [1, 15] and the fish brain is confirmed as a convenient model to study neurotoxicity mechanisms. There was demonstrated a direct interaction of inorganic mercury with the cells involved in the blood-brain barrier (BBB) functions that induces the disturbance in the BBB integrity [17]. The delayed effect of inorganic mercury in the fish model of low doses mercury exposure was recently approved [14].

The intracellular effect of mercury is multifactorial due to many molecular targets. One of the unspecific consequences of mercury cytotoxicity is a generation of redox imbalance, which can induce lesions of all macromolecules including DNA. The genotoxic effect of mercury is known [3]. However, the effect of mercury exposure on DNA repairing machinery remains uncovered.

One of the most often occurred DNA lesions is single-strand breaks (SSB). Exposure to environmental toxicants can entail a meaningful increase in SSB of DNA [11]. The enzymatic system of the SSB restoring called the base excision repair (BER) is evolutionary conservative and stable in eukaryotic cells. The key enzyme of BER is mammalian apurinic/apyrimidinic endonuclease 1 (APE1). Both upregulation and downregulation of this enzyme are associated with abnormalities in the cell functioning and viability. Taking into account that DNA repair machinery is involved in cellular response initiated by cytotoxic injury, the modulation of mechanisms of DNA lesions restore could reflect the vital adaptive potential to maintain genomic stability and cell viability. The fish brain is generally applicable to study detrimental effects of environmental contaminants including mercury. Similar models can clarify some molecular mechanisms of tissue-specific mercury cytotoxicity in mammals including humans [9].

The purpose of our research is to clarify the mechanism of mercury genotoxicity, the role of APE1 in neural tissue, and the response to inorganic mercury-induced neurotoxicity.

MATERIALS AND METHODS OF RESEARCH

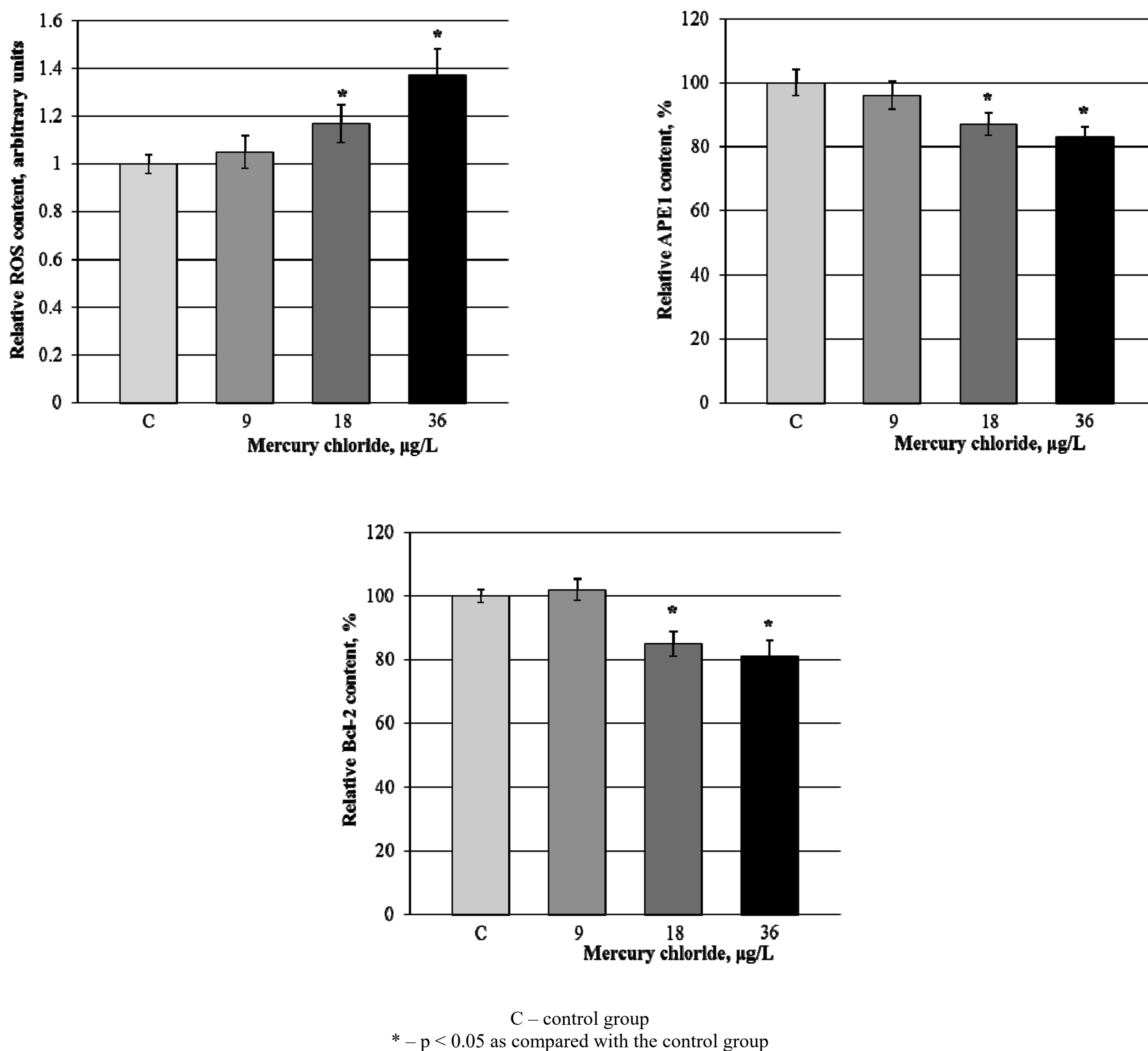
Rainbow trouts (*Oncorhynchus mykiss* (Walbaum, 1792)) were divided into 4 groups (7 fish each) and exposed to low doses of 9, 18, and 36 μg/L $HgCl_2$ for 60 days. After 60 days treatment, the fish were sacrificed according to the procedures of the Oles Honchar Dnipro National University Bioethics Committee rules and to the principles outlined in the Helsinki declaration. The reactive oxygen species (ROS) level was measured by using the fluorometric method based on 2',7'-dichlorofluorescein diacetate (DCFHDA) reaction [5]. The analysis of markers of the DNA repair (APE1) and apoptosis (B cell lymphoma-2 anti-apoptotic protein – Bcl-2) was carried out with western blotting [13]. We use ANOVA and Duncan multiple comparison test and employ the IBM SPSS Statistics 28.0.1.0,

License Subscription 15-Dec-2021 [8]. Results are shown as a mean ± standard error (SE) of seven independent experiments. Comparisons were considered statistically significant in the case of $p<0.05$.

RESULTS AND DISCUSSION

We measured the relative ROS content in the brain samples of fish from the control and exposed groups to mercury chloride. A concentration-dependent increase in ROS content was observed in all exposed to mercury fish groups in comparison with the untreated control (Fig. 1).

In order to investigate the possible genotoxic effect of inorganic mercury on the fish brain, relative APE1 content was measured with western blot in the brain extracts. The decline in APE1 expression was detected in groups exposed to 18 and 36 µg/L mercury chloride doses. The detection of anti-apoptotic protein Bcl-2 as a marker of programmed cell death has shown that exposure to inorganic mercury is potent to inhibit this apoptosis suppressor. The most representable data on APE1 and Bcl-2 expression in control and exposed to mercury chloride fish groups measured with western blot analysis presented in figure 2.



C – control group
* – $p < 0.05$ as compared with the control group

Fig. 1. The effects of mercury chloride on the ROS level, APE1 and Bcl-2 expressions in the brain of rainbow trout

Taking together the results obtained in our study, the cytotoxicity of inorganic mercury is accompanied the dose-dependent increase of ROS and the decline in the content of APE1 and Bcl-2 in brain tissue.

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In spite of number of reports, molecular mechanisms of mercury neurotoxicity remain undiscovered. Mercury is involved in the initiation of BBB disruption and neural tissue cell abnormalities [2]. Since inorganic mercury neurotoxicity remains poorly understood, we have tested the chronic effect of its low doses in the brain cells. Oxidative stress is confirmed to be a widespread cause of DNA damage, irreversible injury, and programmed cell death [12]. Mercury-induced mitochondrial dysfunction accompanied by the DNA oxidizing can switch cell fate to death through genome instability [2].

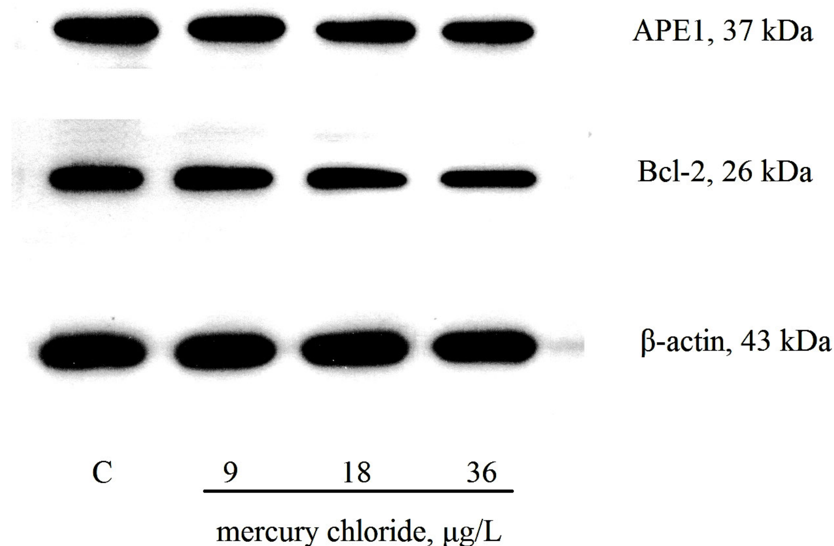

Fig. 2. The western blot results of APE1 and Bcl-2 in the brain of control (C) and exposed to mercury chloride fish groups

Observed in our study dose-dependent ROS production in the brain is agreed with literature data on the effect of sublethal mercury doses [14]. Besides, ROS upregulation is confirmed as the initiator of DNA breaks. In the presented study, we have investigated the APE1 as a marker of DNA repair in the brain to elucidate the possible mechanisms of inorganic mercury genotoxicity. The activity of APE1 mainly is associated with DNA repair pathways. Moreover, the APE1 is a key player in the BER pathway and provides the vital function of genome stability in the various cells including neural tissue.

Detected ROS production in the brain may induce DNA breaking. Observed in our study decline in APE1 content reflects the inhibition of BER. BER is an evolutionary conservative pathway directed to restore the SSB caused by different factors The SSB of DNA is one of the common DNA damages, which can block the transcription processes [6].

The defects in DNA repairing can directly initiate a decline in cell viability and a lack of neuronal functioning. Thus, the detection of DNA repair enzymes in the brain cells exposed to toxic agents is a prospective way to evaluate the both neurotoxic and genotoxic effects of environmental contaminants including mercury. Total coordination of cellular response initiated by DNA lesions launches the DNA damage response and the BER pathway is the largest part of the total DNA damage response machinery.

Taking into account obtained results, APE1 could be considered as one of the important target for mercury genotoxicity. Furthermore, the decline in APE1 expression could be proposed as prospective biomarker to evaluate mercury-induced genotoxicity.

Despite the known genotoxicity of mercury, its link with neurotoxicity is poorly understood [16]. The suppression of APE1 in neural cells can inhibit DNA repair response, increase genomic instability and trigger the cells to apoptosis. Thus, similar irreversible abnormalities disturb brain cell functions as well as their viability. Moreover, mercury-induced oxidative stress can directly initiate programmed cell death via the initiation of mitochondria-dependent regulators of apoptosis. The members of Bcl-2 family are mitochondria-associated pro-apoptotic and anti-apoptotic proteins. All of them are susceptible to intracellular signaling, which regulates cell viability as well as can trigger the damaged cells to the programmed cell death.

As observed in our study, the decrease in anti-apoptotic protein Bcl-2 is evident that mercury exposure initiates apoptotic changes. Therefore, mercury is potent to induce programmed cell death in neural tissue cells via two independent effects: genome instability and mitochondria-associated apoptosis. Actually, the found increase in APE1 expression may be a convenient biomarker of mercury neurotoxicity.

CONCLUSIONS

1. Exposure to low doses of inorganic mercury induces oxidative stress, inhibits DNA repair response, and activates mitochondria-dependent apoptosis in neural cells.
2. The evaluation of decrease in DNA repair response via detection of the apurinic/apyrimidinic endonuclease 1 expression can be a prospective tool to reveal the deleterious effects of environmental contaminants as well as their genotoxicity.

Contributors:

Nedzvetsky V.S. – conceptualization, investigation, writing – original draft, and supervision;

Gasso V.Y. – formal analysis, visualization, writing – review & editing;

Herrmann B. – methodology, resources;

Novitsky R.O. – investigation, validation.

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