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Research Article

Sublethal Doses of Inorganic Mercury Induce Dose-Depended Upregulation of RPA1 Content and Inhibit p53 Expression in the Brain of Rainbow Trout (*Oncorhynchus mykiss*)[¥]

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Abstract

Mercury (Hg) is one of most toxic and widespread element of aquatic environment. Almost every kind of the fish can accumulate Hg. Hg-induced peculiarities of cellular malfunction could be used as adequate biomarker to estimate the contamination risk in polluted aquatic ecosystems. The brain cells are high susceptible to the Hg compounds cytotoxicity. Various Hg species have different harmful effects on both structure and function of the brain cells. Neurotoxicity of inorganic Hg remains discussable and studied restrictedly. In this study, we have studied the role of RPA1 and p53 proteins in brain cell response to sublehtal (25% LD_{50} and 50% $LD_{50)}$ doses of inorganic Hg in rainbow trout (*Oncorhynchus mykiss*). LD_{50} value of Hg chloride in presented study was determined as 551 µg/L relate to 96 hours exposure. Two sublethal doses were used in the exposure rainbow trout at 2 and 7 days. The treatment with Hg chloride induced in fish brain dose-dependent increase in ROS level as well as time-dependent growth. Moreover, the exposure to both 25% and 50% LD_{50} Hg doses have caused significant upregulation of RPA1 expression. In the brain tissue of fish exposed to Hg for 2 days, it stimulated slightly expression of p53. Contrary, 7 days exposure induced significant decrease in p53 expression. The results of presented study evidence that sublethal doses of inorganic Hg are extremely neurotoxic and can induce in the fish brain signaling pathways disturbance through decline of stress sensor protein p53. Besides, the increase in RPA1 expression let to assume that brain cells of the fish can repair ROS-induced DNA breaks and prevent genotoxic effect of inorganic Hg. Overall, current data pointed out that inorganic mercury is high toxic to fish brain cells and this question requires future research.

Key words: Rainbow trout, fish brain, inorganic mercury, p53, RPA1, neurotoxicity.

İnorganik Civanın Subletal Dozlarının Gökkuşağı Alabalığı (*Oncorhynchus mykiss)* Beyin Dokusunda Doza Bağlı RPA1 İçeriğini Yükseltmesi ve P53 Ekspresyonunu Engellemesi

Özet

Civa (Hg), su ortamının en toksik ve yaygın unsurlarından biridir. Hemen hemen her balık türü Hg' ya maruz kaldığı zaman bunu dokularında biriktirebilir. Civa ile indüklenen hücresel fonksiyon bozukluğu, sucul ortamdaki kirlenme riskini tahmin etmek için yeterli biyobelirteç olarak kullanılabilir. Beyin hücreleri, civa bileşiklerinin sitoksisitesine karşı oldukça hassastır. Çeşitli civa türleri, beyin hücrelerinin hem yapısı hem de işlevi üzerinde farklı zararlı etkilere sahiptir. Son zamanlarda yapılan çalışmalarda inorganik civanın nörotoksisitesi tartışılmaya devam etse de tamamıyla incelenmemiştir. Bu çalışmada organik civanın subletal dozlarına maruz bırakılan gökkuşağı alabalığının beyin dokusunda rpa1 ve p53 proteinlerinin rolünü araştırdık. Sunulan çalışmada civa klorürün LD_{50} değeri, 96 saat maruz kalma ile 551 µg / L olarak belirlenmiştir. Gökkuşağı alabalıkları 2 ve 7 günlük zaman aralıklarında iki subletal doza maruz bırakıldı. Civa klorüre maruz bırakılan balıkların beyin dokusunda doz

ve zaman bağımlı olarak ROS seviyesinde bir artış belirlenmiştir. Ayrıca, hem % 25 hem de % 50 LD_{50} civa dozlarına maruz kalma süresince RPA1 ekspresyonunun önemli ölçüde upregülasyonuna neden olmuştur. 2 gün süre ile Hg maruz bırakılan balıkların beyin dokusunda, p53 ekspresyonunu zayıf bir şekilde uyarmıştır. Bunun tam tersine olarak, 7 günlük maruz kalma, p53 ekspresyonunda önemli bir azalmaya neden oldu. Sunulan çalışmanın sonuçları inorganik civanın alt dozlarının son derece nörotoksik olduğunu ve stres sensörü proteini p53 ün azalması ile balık beyni sinyal yollarında bozukluğa neden olabileceğini kanıtlamaktadır. Ayrıca, RPA1 ekspresyonundaki artış, balıkların beyin hücrelerinin ROS kaynaklı DNA kırılmalarını tamir edebileceğini ve inorganik Hg'nin genotoksik etkisini önleyebileceğini göstermektedir. Genel olarak, mevcut veriler inorganik civaların balık beyin hücrelerine toksik olduğunu ve bu sorunun gelecekte de araştırılmasının gerekliliğine işaret etmektedir.

Anahtar kelimeler: Gökkuşağı alabalığı, balık beyni, inorganik civa, p53, RPA1, nörotoksisite.

Introduction

Various heavy metal species are recognized as potential health hazard for every type of living organism, particularly in aquatic ecosystems (Has-Schön et al., 2015). The presence of these metals in natural waters and ponds caused by continuous extensive growth of agriculture, chemical and industrial enterprises is worldwide problem (Meena et al., 2017). Mercury (Hg) is one of most toxic and widespread element which present in the environment. Natural sources of this heavy metal include volcanic activity, forest fires, and fossil fuel incineration. However, anthropogenic sources remain as most intensive in local areas as well as extremely dangerous for human and animals health (UNEP, 2013). For instance, environmental contamination by Hg is due primarily to anthropogenic activities and sources such as dumping of urban waste, agricultural products, mining operations, fossil fuel combustion and industrial discharges (Horowitz et al., 2014; Obrist et al., 2018).

As a rule low levels of Hg is naturally present in aquatic environment. However, the concentrations in the aquatic compartments are dependent on the distance from contamination sources. Particularly, both organic and inorganic Hg species are widely distributed in aquatic ecosystems. Hg have been detected in the sediment and surface water of diverse aquatic systems around the world almost in concentration above the maximum limits established (Eagles-Smith et al., 2016a: Eagles-Smith et al., 2016b). Once it enters into water ecosystem, Hg can be absorbed by living organisms that initiate various toxic consequences. The most meaningful bioaccumulation of Hg is therefore considered in aquatic organisms in compare to other heavy metals (Orihel et al., 2007; Eagles-Smith et al., 2016a). Hg can induce multifaceted cellular damages in the fish tissues as well as mammals (Carocci et al., 2014; Chang et al., 2017). The bioaccumulation of Hg species was determined in several fish types, especially *Cyprinus carpio, Salmo salar*, *Pomatoschistus microps*, *Liza aurata* in the gills, kidneys, liver, muscle and brain (Simon and Boudou, 2001: Amlund et al., 2007; Vieira et al., 2009; Brandão et al., 2015; Gómez-Oliván et al., 2017).

The fish brain was recognized to be one of target for Hg species toxicity. In aqueous environment the fish are generally exposed to both organic and inorganic Hg species either through diet or water. Totally, Hg is mainly presented in its inorganic form (Laurier et al., 2004). Neurotoxic effect of Hg confirmed to its different forms. In the most cases there was established that the organic Hg develops more neurotoxicity caused by its possibility to cross the blood-brain barrier (BBB) and accumulate in the brain tissue (Wang and Wong, 2003; Pletz et al., 2016; Cariccio et al., 2019). Numbered results have showed most high accumulation of organic Hg compared with inorganic form in the fish brain independently on the exposure source. This dependence was observed in the brain of the fish from Hg contaminated areas of the sea (Pereira et al., 2014). However, several studies have demonstrated the ability of inorganic Hg to accumulate in fish brain with different concentrations and deposition patterns (Korbas et al., 2012; Rooney, 2014; Pereira et al., 2015). Recently, Lohren and coauthors presented the results of in vitro study which demonstrate that organic and inorganic Hg can develop different capability to cross the BBB (Lohren et al., 2016). Inorganic Hg compounds possess a low capability to cross BBB as a rule. However, it can interact directly with the cells which construct this barrier and consequently to disturb the integrity of BBB (Zheng et al., 2003). The results of several study showed the presence inorganic Hg content in the fish brain (Mieiro et al., 2011; Pereira et al., 2014; Harayashiki et al., 2019). Recently, Pereira and coauthors demonstrated study that inorganic Hg can attain to brain cells several days later of the exposure to environmentally realistic doses (Pereira et al., 2015).

The mechanism of Hg transfer across BBB is undiscovered. Recently, Takahashi and colleagues

demonstrated that methyl Hg can induces BBB damage via upregulation of VEGF expression in rat chronic intoxication model (Takahashi et al., 2017). The expression of this growth factor was detected in BBB associated astrocytes which capable to accumulate inorganic Hg species (Lohren et al., 2015). Unfortunately, molecular mechanisms of inorganic Hg neurotoxicity in fish remain undiscovered.

The brain cells are high sensitive to the Hg compounds toxicity. Moreover, Hg ions consumed with the water and the food can initiate severe damage in the brain as well as other tissues. Several Hg compounds have different harmful effects on both structure and function of the brain cells. The absorption of any Hg forms from polluted environment induces the increase of this heavy metal into the blood as first step of Hg toxicity. The absorbance of Hg by individual cell types depends on the content of Hg-binding chemicals, especially thiol-containing compounds. Metalothioneines and low molecular weight thiols are important factors to mediate the transport and distribution of Hg. The mechanisms of transport and accumulation Hg species in brain cells remain discussable (Aschner and Aschner 1990; Pereira et al., 2019). Number data support the opinion that inorganic Hg compounds cannot cross BBB. However, there are several study which demonstrate undoubted neurotoxic effects of inorganic Hg. Recently Lohren and coauthors have shown have shown that organic Hg compounds exerted much higher cytotoxic effect on the barrier building cells (Lohren et al., 2016). Moreover, there was presented data that inorganic Hg appeared more toxic than methyl Hg to neurons and glial cells in immature aggregate cell cultures (Monnet-Tschudi et al., 1996).

Despite of the number of researcher data on Hg toxicity in various terrestrial and aquatic organisms, mechanism of neurotoxicity initiated by this heavy metal remains unknown. Limited number of studies presented direct evidences of molecular base of Hg-induced neurotoxicity. The absorption of Hg by glial cells can initiate neuronal degeneration (Ohgoh et al., 2000). Molecular mechanisms of neuronal surviving after Hg exposure as well as the range of cellular response to Hg cytotoxicity undisclosed too. Most known consequence which accompanied with Hg toxicity is overproduction of reactive oxygen species (ROS) and as result the generation of oxidative stress (Simmons et al., 2011). Other feature of Hg toxicity mediated through its affinity to hemoglobin that can drive the brain to hypoxic injury (Giblin and Massaro, 1975).

Various cellular damages induced by Hg are confirmed as a consequence of ROS overproduction. Fish, similarly to all other aerobic organisms, are susceptible to ROS increment with consequently developed antioxidant defense system. The authors propose to use the monitoring of antioxidant systems response against Hg exposure in the fish tissues as valid biomarker of environmental toxicity (Van der Oost et al., 2003; Santovito et al., 2012; Tolomeo, 2016). However, antioxidant enzyme activity modulated with various factors has common features which are independent on nature of contaminant kind. The number of differ pollutants can induce the same antioxidant system response. Anyway, the measuring of both oxidative stress indexes and antioxidant system activity remain important addition to study heavy metal toxicity (Antunes Dos Santos et al., 2018).

There is extremally limited data on the mechanisms of inorganic Hg toxicity in brain tissue. Recently, Wang and coauthors have shown that inorganic Hg exposure can modulate the expression of several proteins which mediate abnormality cytoskeleton assembly and metabolic disorders in a brain (Wang et al., 2015). However, the other types of mercury-induced molecular damage remain undiscovered. In several reports Hg has recognized as a cytogenotoxic agent. Genotoxic effect of Hg associated with its prooxidant capacity. Hg exposure can modify many proteins, including cytoskeleton components and specific proteins which are involved in DNA repair. Several studies have been carried out on mammals (Schmid et al., 2007; Crespo-López et al., 2009). Some studies have been carried out with using comet assay and micronucleus test of Hg genotoxicity in fish. Gómez-Oliván and coathors have shown genotoxic effect of Hg in blood cells of common carp (Gómez-Oliván et al. 2017). Genotoxic effect of inorganic Hg in the brain has no presented in literature.

Neural tissue cells potent to respond against cytotoxic injury. The proliferative activity of cells is regulated by various factors and depends on the expression DNA replication associated proteins. Replication protein A (RPA), the major eukaryotic single-stranded DNA binding protein. RPA family consists of three RPA1, RPA2 and RPA3 members where RPA1 plays crucial role in DNA breaks repaire. RPA1 is required for replication, repair, and recombination of DNA. Especially, RPAs are required for both initial and elongation phases of DNA replication. In course of the initiation, RPA balances single-stranded DNA region consisting of initial relaxation. The DNA-binding activity is mediated by the domains RPA1 70-kDa subunit of the RPAs complex. RPA1 interposes the proteins into the newly formed replication fork (Zou and Elledge, 2003). Defects in RPA-associated cellular activities initiates genomic imbalance. Genomic

instability is the main initiating factor in the pathogenesis of cancer and other diseases. RPA1 also serves as the coordinator of DNA metabolism, cell cycle and the response to DNA damage provoked by abnormal cellular functioning (Choi et al., 2010).

Universal and multifunctional regulatory protein that plays vital role in most cell types is p53. p53 provides cell viability through many signaling pathways. The expression of p53 is sensitive to growth of ROS content and oxidative damages that direct p53 to modulate several signalling pathways. The expression level of p53 is limited by endogenic factors in physiology normal state. Any type of cellular stress can switch cell events to critic changes in the cell cycle and/or genotoxic damages. p53 protein participates as one of key regulator of programmed cell death and anti-tumor protection in stressed cells. Especially in Zebra fish brain, activated p53 protein allows the destruction of damaged cells by stopping the cell cycle and initiating apoptosis (Hu et al., 2015). Besides, p53 protein is a transcription factor which control vital processes in a course of cell rectivity, including cell viability, DNA repair and apoptosis (Bensaad and Vousden, 2007).

Taking into account that p53 and RPA1 involved in cellular response initiated by cytotoxic injury, the expression both of them can reflect the capability of brain cells to switch compensatory mechanisms to maintain cell viability.

Rainbow trout is wide used as adequate object to study harmful effect of various environmental contaminants, including Hg species (Ciardullo et al., 2008; Kenšová et al., 2012; Liu et al., 2013).

The purpose of present study was to clarify the role of RPA1 and p53 proteins in brain cell response against inorganic Hg-induced neurotoxicity.

Materials and Methods

Fish model and treatment

The experimental model of inorganic Hg neurotoxicity was carried out in Agriculture Aquaculture Laboratory and Molecular Biology Laboratory of Bingöl University.

Rainbow trout (*Oncorhynchus mykiss*) (59.43 ± 3.73 g and 17.24 ± 1.64 cm) was purchased from fish farm in Keban district of Elazig province and removed into transport aerated tank to the laboratory. The fish brought to the laboratory were placed in 600 liters tanks to acclimatize during 15 days. The fish were fed with a commercial product two times per day in a volume accordingly 2% of their weight. The water temperature was 14 ± 3 °C, the dissolved oxygen level was 8.24 ± 0.5 mg/L and the pH was 7.3 ± 0.2.

In accord the rules to determine LD50 value, $HgCl_2$ was exposure to seven experimental groups of rainbow trout in 10, 100, 200, 500, 750, 1000 and 1500 µg/L dose per group. The fish in every group were monitored during 96 hours 6 times per day to remove the died individuals from the aquarium. LD_{50} value was calculated as 551 µg/L relate to 96 hours Hg chloride exposure. After calculating the LD_{50} value, 2 subletal doses (25% LD_{50} = 138 µg/L and 50% LD_{50} = 276 µg/L) were determined. Four groups of fish were exposure to reated with sublethal (25% and 50% LD_{50}) doses at 2 and 7 days. Every of treated fish group as well as untreated control group was formed with 15 fish individuals.

Tissue sampling

The fish were applied to sacrificed procedures accordingly the rules of local ethic committee rules. The brain was removed and washed with 0.9% NaCl to remove blood from the tissues. Obtained brain tissues were frozen at -80 °C and kept until protein samples extraction.

Tissue homogenization

Frozen brain tissue samples individually were homogenized in a 1:10 (w/v) ratio (10 mM Tris-buffer (pH = 7.4), 0.1 mM NaCl, 1% TritonX-100, 0.2% SDS, 2.5 mM ethylenediaminetetraacetic acid, 6.5 µM aprotinin, 1.5 µM pepstatin A, 23 µM leupeptin, 1 mM phenylmethylsulfonyl fluoride, 1 µM sodium orthovanadate 5 µM soybean trypsin inhibitor glass-glass homogenizer. During the homogenization processes, all the procedures were carried out on the ice to prevent protein degradation caused by protease activity. After 1 hour protein extraction in lysis buffer at +4 °C the homogenates were centrifuged at 60.000 g for 60 minutes at + 4 °C in a cooled centrifuge. Obtained supernatants which contain total protein extract were removed in microcentrifuge tubes and stored at –80 °C before Western blotting analysis.

Determination of reactive oxygen species

The level of Reactive Oxygen Species (ROS) was determined individually in every brain tissue sample obtained by centrifugation of according homogenates. The ROS content was measured by using fluorometric method based on DCFH-DA. Every procedure was performed with according ROS assay kit producer. Each sample was tested in trice parallels.

Analysis of proteins by western blotting technique

Western blotting was performed with using SDS-PAGE electrophoresis in 10% acrylamide gel.

Separated in polyacrylamide gel proteins were transfered onto polyvinylidenefluoride (PVDF) membrane, washed with PBS and incubated in blocking buffer (3% BSA) at 60 min. After blocking, the membranes were incubated overnight at +4 °C with the respective primary antibody, anti-p53 (Santa Cruz, sc-126,1:1000 dillution), anti-RPA1 (Santa Cruz, sc-48425, 1:1000 dillution) and anti-β-actin (Abcam, ab8226, 1:3000 dillution). After the incubation with primary antibodies the membrane were trice washed with tris buffer saline contained 0.05% Tween-20 (TBS-T). Washed membranes were incubated with anti-mouse HRP-labeled secondary antibody 60 min. at room temperature. After the membranes were washed trice with the same TBS-T. The imaging of western blot results was developed with ECL kit based on chemiluminescence method with using the automatic X-ray machine (Carestream Heath Inc., USA). BIO-RAD imaging system was used to scan the films with western blot results. Band densities were evaluated as densitometry using Image-j program.

Statistical analysis

Statistical analysis of the results obtained from the experiment was performed using SPSS 20 statistical program. The statistical analysis of the changes in the examined parameters of control and experimental group fish was determined by using variance and Duncan multiple comparison test. Quantitative data of laboratory parameters are shown as mean values ± SD. For all tests, $P < 0.05$ was considered statistically significant.

Results and Discussion

Obtained in presented study results about RPA1 content in the brain of control and treated with Hg salt rainbow trout groups presented on a Figure 1. The western blot results of RPA1 content measuring have showed the same increase of RPA1 protein in the brain samples of all fish groups which were treated with Hg chloride. Unexpected data were determined in comparative analyses of the results obtained in groups treated with 25% LC_{50} and 50% LC_{50} doses. The increase of RPA1 content in the brain of fish exposure to low (25% LC_{50}) was observed as more high then increment of same index in a group treated with 50% LC_{50} dose the same while. However, Hg chloride initiated more meaningful increment of RPA1 content in all fish groups exposure 2 days to compare the groups treated during 7 days. The treatment with both 25% LC_{50} and 50% LC_{50} doses of Hg chloride has induced RPA1 overexpression in the brain of rainbow trout. Hg chloride exposure to these doses develops reciprocal time-depended effect where 25% LC_{50} inorganic Hg dose initiate more high increment of RPA1 content in compare with 50% LC_{50} dose.

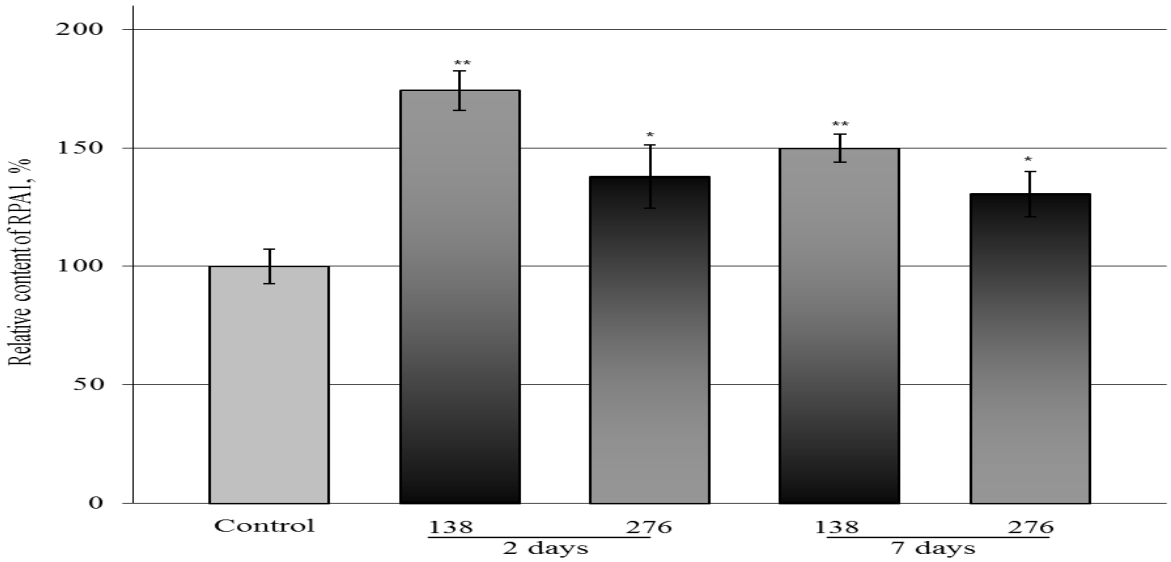

Figure 1. The effects of Hg chloride on RPA1 content in rainbow trout brain.
*The fish were treated with sublethal doses 25% LC_{50} (138 µg/L) and 50% LD_{50} (276 µg/L) during of 2 and 7 days exposure. The values correspond to the means ± SE of 5 independed experiments. * - $P < 0.05$, ** - $P < 0.01$ compaired with the control group.

The measuring of p53 content shown both up- and downregulation in depends on exposure time. The opposite directed changes of p53 expression observed in extracted from fish brain of treated with Hg chloride 2 days and fish groups treated during 7 days. The changes of p53

expression were determined in all Hg-treated groups relate to control. The induced insignificant increment of p53 content was observed in a group treated with low 25% LC_{50} dose of inorganic Hg salt in both 2 and 7 days while of exposure. However, the treatment with the same while of exposure to 50% LC_{50} dose induced oppositely directed changes of p53 expression compared to 25% LC_{50}. Surprisingly that obtained data on downregulation of p53 content have a high statistically significant. The results obtained in control and all treated groups about the effect of Hg ions on p53 expression in rainbow trout brain presented on Figure 2.

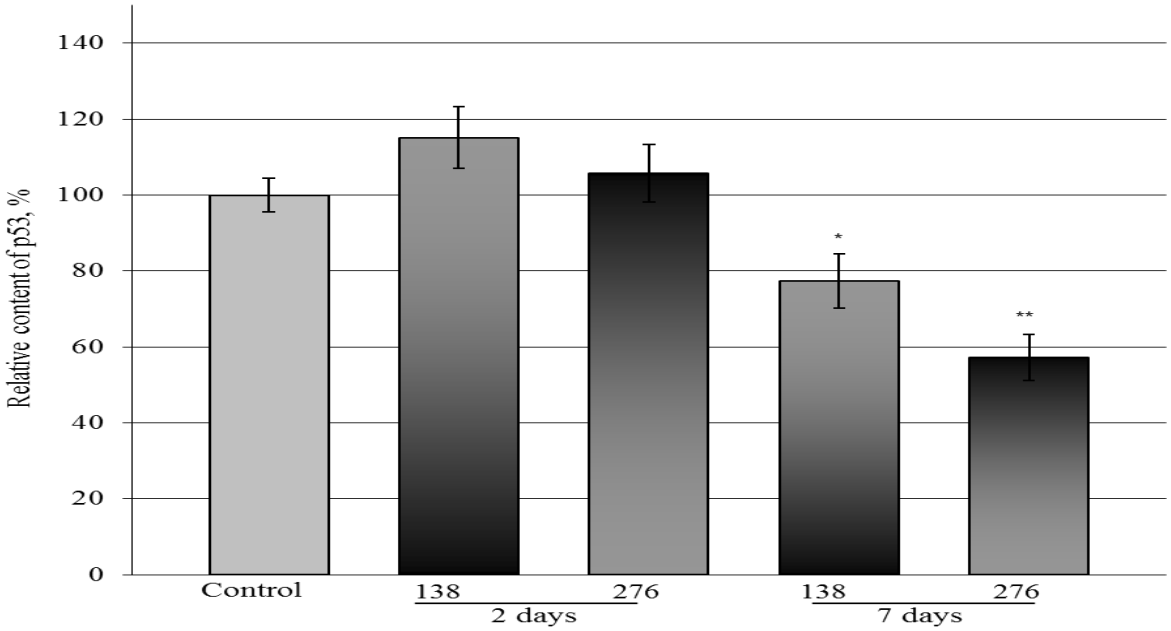

Figure 2. The effects of mercury chloride on p53 content in rainbow trout brain.
*The fish were treated with sublethal doses 25% LC_{50} (138 µg/L) and 50% LD_{50} (276 µg/L) in the course of 2 and 7 days exposure. The values correspond to the means ± SE of 5 independed experiments. * - P < 0.05, ** - P < 0.01 compaired with the control group.

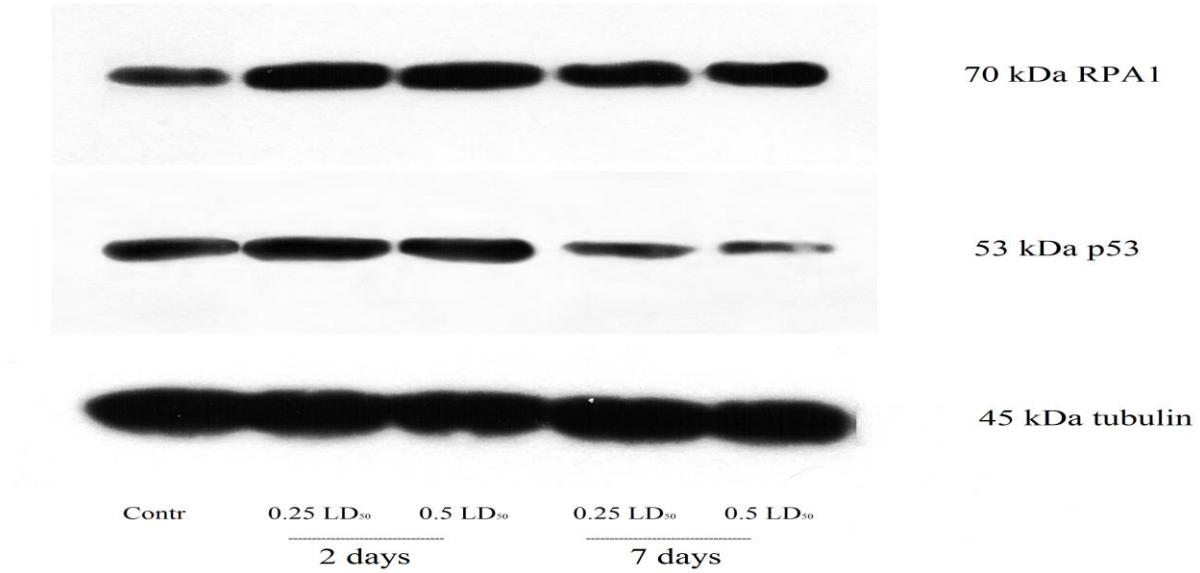

Figure 3. Representative western blot results of p53, RPA1 and tubulin content.
*The data p53 and RPA1 content presented on Fig. 1 and Fig. 2 were calculated with regard to tubulin content.

The intensity of ROS generation and oxidative stress rising are recognized as common consequence of the exposure to various types of heavy meal ions. Taking into account this heavy

metal feature, the ROS level was determined in the brain of all studied fish groups. The data of the ROS level measuring in the brain of fish groups exposure to inorganic Hg presented as relative content compared to untreated control on a Figure 4.

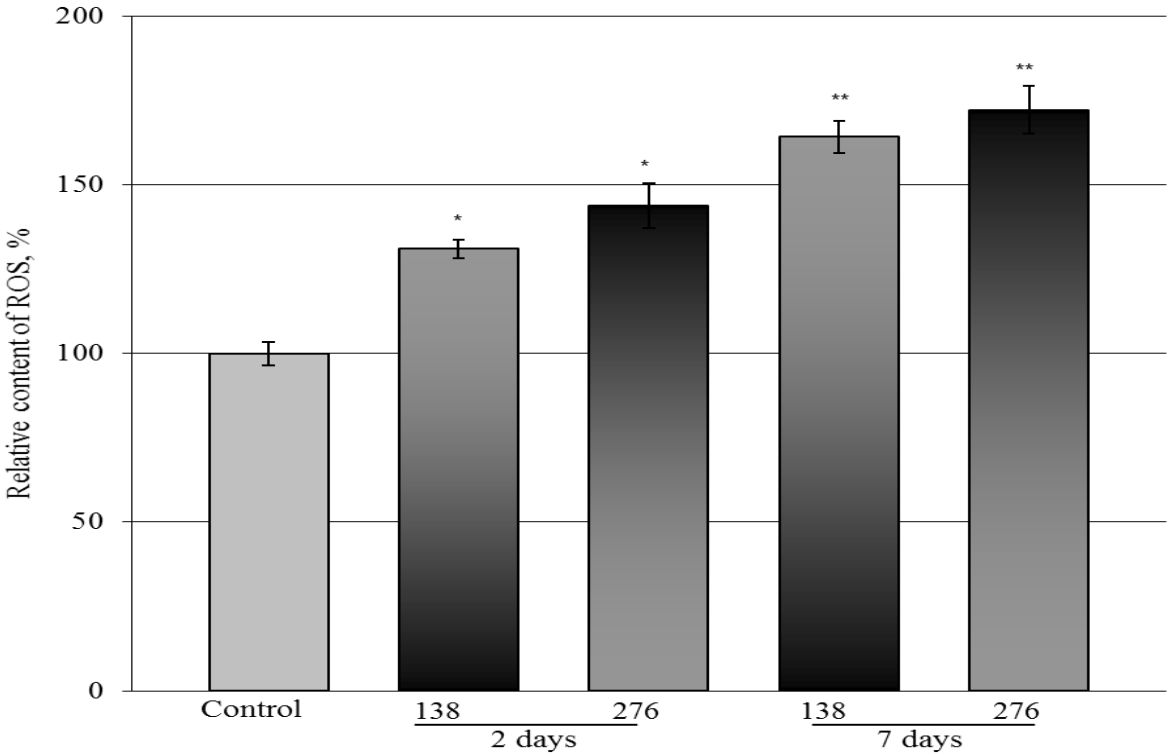

Figure 4. The effects of Hg chloride on ROS production in rainbow trout brain.
*The fish were treated with sublethal doses 25% LC_{50} (138 µg/L) and 50% LD_{50} (276 µg/L) in the course of 2 and 7 days exposure. The values correspond to the means ± SE of 5 independed experiments. * - P < 0.05, ** - P < 0.01 compaired with the control group.

Observed content of ROS in fish brain of all studied groups presented the clear dose-dependent effect of inorganic Hg in brain cells of rainbow trout. Overall, the exposure to Hg chloride in both 25% LC_{50} and 50% LC_{50} doses during 2 days and 7 days developed time-dependent effect.

Taking together presented in our study results, we can assume the presence of dose-dependent increase of ROS level and the modulation of both p53 and RPA70 content in a rainbow trout brain in a course of inorganic Hg exposure in sublethal doses during 2 and 7 days.

Total heavy metal pollution of the environment initiates large number disturbances caused by plural to toxic effects of these ions in all living organisms (Leonard et al., 2004). Last decades, harmful effect of heavy metals as well as the mechanisms of action has been intensively studied. Despite of large number of researcher's data, concrete molecular mechanisms of Hg toxicity, including neural tissue cytotoxicity are still unclear.

Hg is the one of most neurotoxic pollutant which has been recognized as extraordinarily dangerous to human and animal health (Carocci et al. 2014). Two main organic and inorganic forms of Hg species are recognized as critic environmental pollutants. Methyl Hg is main organic form and its harmful effect is known significantly better then the cytotoxicity of inorganic Hg. Last decades study showed that the toxicity of various Hg species seems to be different (Simmons et al., 2011). Especially, neurotoxicity of methyl Hg which observed in several fish types could relate to its capability to initiate the BBB damages (Takahashi et al., 2017). Organic Hg species are widespread in aquatic environment and is recognized most dangerous pollutant among heavy metals compounds. The main sources Hg contamination of freshwater and sea waters are the pollution with industrial products and geologic inorganic Hg. In modern world the emissions of natural Hg not exceed average 40% of global emissions (Pirrone and Mason, 2009). Many sources of environment pollution with Hg are considered as the potential

risk for human poisoning with methyl Hg. This conclusion was based by discovering of microorganisms which capable to transform inorganic Hg to methyl Hg in aquatic medium (Jensen and Jernelöv 1969). This type of Hg transformation provokes organic Hg accumulation in the sea food chain which is finished in human consumption.

Most of fish species can accumulate Hg. Toxic effect of Hg induces specific to this contaminant injury in the cells that could be used as adequate biomarker to estimate the pollution risk in aquatic ecosystems. Recently Łuczyńska and coauthors have shown positive correlation between Hg pollution and weight of fish (Łuczyńska et al., 2018). The application of experimental fish model to study various Hg doses effect can discover molecular mechanisms of tissue-specific Hg cytotoxicity. The mechanisms of Hg toxicity in fish most studied about lipid peroxidation, activities and gene expression of antioxidant enzymes in hepatic, muscle, gill and brain (Sevcikova et al., 2015; Zeng et al. 2016; Naïja et al, 2018). Hg induces mitochondrial dysfunction, which accompanied with a lack of ATP synthesis, increase lipid peroxidation, protein and DNA oxidizing.

The participation of oxidative stress in neurotoxic action of Hg ions remains uncovered in details. One of important feature of Hg to develop harmful effect is the capability to bind thiol groups of different macromolecule types (Szunyogh et al., 2015). Besides, Hg has a high affinity to thiol groups and can bind amino acids which are substrate for the biosynthesis of powerful brain antioxidant GSH (Carocci et al., 2014). Hg species can cause the inactivation of enzimes which have SH-groups into active centre (Miller et al., 1986). Other part of harmful effect of Hg mediated with the inhibition of important antioxidant system which based on glutathione recycling that can provoke oxidative stress (Rensburg et al., 2019). Brain cells are more susceptible to oxidative damages then other cell types because they possess high rate oxigen consumption and deficiency in antioxidant power. Totally, the exposure to Hg ions can induce irreversible disturbances of vital function of the brain.

Neurons and glial cells differ in the susceptibility to Hg effects. Macro- and microglia are the key guard cell types in a brain to maintain the neuronal functions. Recently, there was demonstrated data on methyl Hg-induced dramatic gliotoxicity. Human astrocyte culture exposure to methyl Hg revealed abnormal astrocyte reactivity and the malfunction in the cytoskeleton and the rearrangement of filamentous network proteins (Malfa et al., 2014). However, there is no clear evidence on direct neurotoxicity of inorganic Hg species. Obtained in our study dramatic decrease of p53 content in a brain of fish exposure to 50% LC_{50} doses of Hg chloride could be estimated as index of critic regulatory pathway disturbance.

A few data have shown the effect of Hg on transcriptional regulation. There was demonstrated that both inorganic and organic Hg species can suppress transcriptional regulation and increase production of superoxide (Jebbett et al., 2013). Additionally, Hg can induce mitochondrial membrane stress through sulfhydryl group binding in astrocytes. Besides, astrocytes accumulate redox active transition metals and as a result generate free radicals. Inorganic Hg exposure initiates plural changes in the structure and the accumulation both Hg and iron in mitochondria (Brawer et al., 1998). Thus the increase of ROS generation closely relate to Hg-induced pathogenic disturbances in brain cells. Observed in presented study results develop dose-dependent ROS production in brain of rainbow trout groups treated with sublethal (25% LC_{50} and 50% LC_{50}) doses of Hg chloride.

Oxidative stress generation is recognized as the most prevalent pathogenic initiator in cytotoxicity of heavy metal ions. ROS overproduction can play an important role in the lack of neural tissue cell functions. Harmful effect of Hg species closely relates to oxidative damages of all macromolecule types. Especially DNA damages may initiate irreversible injury in the brain cells which turn to cell death.

Metal-induced generation of ROS is well known consequence of both redox active and redox inactive metals (Jomova and Valko, 2011). Total cellular injuries which associated with metal-induced ROS production lead to malfunctions in signal transduction, cell cycle, and programmed cell death initiation. The role of Hg in these vital processes remains undiscovered. Overall, oxidative stress can induce lipid peroxidation, receptor and enzyme inactivation, DNA breaks that decrease cell viability. DNA breaks caused by oxidative stress are critic to initiate various types of cell death. Every eukaryotic cells possess effective system to repair DNA breaks initiated by toxicants exposure. Numbered environment factors can cause DNA breaks and as a result genotoxic stress in the cells. On other hand, DNA damages initiate the signals to activate cellular responses directed to repair DNA breaks.

The number of the study of inorganic Hg-induced neurotoxic effect in the fish is limited. There is data on detrimental effect a inorganic Hg in the brain of white seabream. The authors identified the decrement of the number of neuronal and glial cells in fish treated with low dose (2 µg/L) during 7

days. I addition, Hg induced changes in the brain functions, especially swimming behavior (Pereira et al., 2016). Observed a lack of cell viability in the brain may be initiated by the malfunction of regulatory pathways and functional cell decline. Hg exposure can suppress transcriptional activity, cell cycle and limit the repair of DNA breaks. The unrepaired DNAs lead to genomic instability and trigger pathways to programmed cell death.

RPA is one of critic player to repair double stranded DNA breaks. RPA develops repairing function as a complex formed by 70, 34 and 14 kDa subunits named RPA1, RPA2 and RPA3 accordingly. RPA family is highly conserved proteins which have the homologues practically in every eukaryotic cell (Ishibashi et al., 2001; Wold, 1997). The domain structure of RPA is similar in every type o living organisms (Iftode et al., 1999; Wold, 1997). RPAs bind single stranded DNA with high affinity. RPA1 is the key player in repairing DNA initiation because it contents four DNA-binding domains. Moreover, RPAs are main participants the elongation phases in course of DNA replication. There are known two principal pathways of DNA double-strand breaks repair in which RPA is involved as key participant (Binz et al., 2004).

Various brain tissue abnormalities accompanied with genotoxic stress and nucleotide excision repair that is extremely important to maintain neural tissue cells viability (Jensen et al., 2018). Taniguchi and coauthors have shown the role RPAs to provide vital brain functions, especially RPA1 indirectly recovered plural cellular functions, including RNA splicing, cell cycle, and transcriptional regulation (Taniguchi et al., 2016). RPAs together with other DNA replication proteins maintains CAG/CTG trinucleotide repeats stability that safe normal brain function (Mason et al., 2014). Most harmful result of genotoxic stress is uncontrolled mutations. RPAs in a complex with cyclins can participate in cell cycle promotion in astroglia (Kanakis et al., 2011). Thus, the level of RPA expression could be useful prognostic indicator in patients with astrocytomas.

In mammalian cells there was shown that RPA mediates homologous recombination repair pathway. RPA family protects the single-stranded DNA intermediates and load into repairing complex other components of the DNA repair machinery (Ruff et al., 2016). There are no data on the expression of RPA in fish treated with heavy metal ions. There is only one report on the modulation of RPA affinity to bind cisplatin-DNA intrastrand adduct (Patrick and Turchi, 1999).

Zn ion is essential for the conformation of RPA1 tertiary structure. The structure of RPA1 has compact conformation caused by Zn-mediated interaction with polypeptide chain. RPA contains coordinated Zn ion into zinc-binding domain that maintains the conformation of nucleoprotein complexes formed by DNA and RPAs during DNA repairing (Eckerich et al., 2001). Several metal ions especially Cd and Hg develop the competition with Zn to bind RPA1 (Jancsó et al., 2013). Besides, there was presented the results on competing binds the polypeptide specifically designed for binding of both Zn^{2+} and Hg^{2+}. The authors demonstrated that HS accommodates both metal ions and metal ion exchange was observed between individual peptides (Szunyogh et al., 2015). Thus, Hg can develop competitive propriety to force out Zn ion from RPA1. Zink replacement can provokes the changes in RPA conformation and disrupt DNA repair machinery.

Presented in our study results on overexpression RPA1 initiated by Hg sublethal doses exposure in rainbow trout brain evidence high sensitivity of this DNA replication mechanism to neurotoxic effect of inorganic Hg. However, observed dose-dependent increase of RPA1 expression could reflect the adaptive capability of brain cells against oxidative DNA damage. The results our study on dose-dependent growth of ROS level in fish brain correspond to the increment of RPA1 content in the groups treated with inorganic Hg in 25% LC_{50} and 50% LC_{50} doses. Presented results have shown that brain cells possess adaptive system to repair DNA-strand breaks caused by Hg exposure. On other hand, we have observed more high content of RPA1 in fish group treated with 25% LC_{50} dose compared with the fish exposure to 50% LC_{50} dose. This fact could be explained with extremely harmful effect of 50% LC_{50} dose on cell viability. Sublethal 50% LC_{50} Hg chloride dose can initiate irreversible damages and cell death in the fish brain. I addition, relative decrease of RPA1 content in fish group treated with 50% LC_{50} dose in compare to 25% LC_{50} dose group may be a result of total suppressing of protein synthesis as well as cellular response.

Protein p53 is universal regulator of cell viability. One of first discovered function of p53 protein was confirmed as a stress sensor. Protein p53 is involved in the regulation of many vital processes, including cell cycle, the initiation of apoptosis, transcriptional activity and DNA repair (Lieberman et al., 2017). Later, number evidences were lighted that p53 regulates gene expression to maintain efficacy of cell adaptation to environmental context. Moreover, p53 is implicated in translational regulation, controls microRNA processing and can modulate protein activity through direct interaction (Marcel et al., 2018). One of main p53 function is to control cell cycle and

programmed cell death as the response to oxidative damages (Fuschi et al., 2017). Recently, there was demonstrated that p53 mediates DNA repair (Jiang and Rusling, 2019).

The role of p53 expression in the response of brain cells to heavy metal exposure remains unknown. There is extremely limited number of the reports about the p53 participation in cellular response to nickel, copper, lead and chromium exposure. Kim and coauthors have sowed that functional inactivation of p53 is accompanied with excision repair activity in the cells treated with low concentration of nickel (Kim et al., 2018). Copper exposure in extracellular trace concentration can induce a lack of p53 activity that associated oxidative stress, DNA damages and cell death activation (Du et al., 2008). Other authors demonstrated that lead and chromium can induce decline of cell viability mediated with p53-independent apoptosis (Bagchi et al., 2001; Loikkanen et al., 2003). Thus, in spite of oxidative stress as common index of heavy metal toxicity, every heavy metal can initiate individual features of cytotoxicity events.

Taking into account all abovementioned, the study of Hg neurotoxicity in aquatic organisms is actual and is extremely important to understand the hazard of modern environment contamination. There are no any research data on inorganic Hg effect on DNA repairing machinery in fish brain. In presented study we have investigate the sublethal dose exposure to inorganic Hg to clarify the primary effect of this heavy metal ion on the cellular response against the genotoxicity in fish brain. Presented in our study results have shown slight increase of p53 content in the brain of fish treated with 25% LC_{50} dose of Hg chloride. Contrary, intense downregulation of p53 content was determined in fish group exposured to 50% LC_{50} dose. According literature data, the increase of p53 expression occurs as result cellular response to various types of cell damage. However, several experimental results evidence that the suppression of p53 expression is the mechanism to regulate of transcriptional activity too, especially the inhibition with microRNA or hydroxyapatite nanoparticles (Hu et al., 2007; Cho et al., 2016).

The exposure to sublethal doses of inorganic Hg initiates multifaceted cellular stress. As a rule, the cells stressed with toxic factors develop a lack of metabolic activity. Stressed cells can survive if they can generate effective cellular response via stress-specific pathways. Cellular response can provide cell surviving and/or programmed cell death to prevent necrosis. Recently, there was reported that cellular response to DNA breaks accompanied with an increase of p53 level by oscillatory manner (Porter et al., 2017). This mechanism of p53 modulation adapts cell-fate regulation during the DNA damage response. Alike, observed in our study down regulation of p53 can serve as a part of global strategy for cell viability in a course total inhibition of metabolism. In addition, presented results have shown that downregulation of p53 level accompanied with upregulation of RPA1 in the brain of fish treated with sublethal 50% LC_{50} dose. Thus, upregulation of RPA1 expression could be one of critic factor of cell adaptation in stressed with inorganic Hg fish brain. Taking together presented in our study results, we can assume the presence of interaction between Hg-induced ROS generation and the modulation of both p53 and RPA1 content during cellular response to Hg cytotoxicity in a rainbow trout brain.

Presented results in our study are reported the first time that a lack of p53 and upregulation of RPA1 in fish brain after inorganic Hg exposure. The revealed RPA1 upregulation in fish brain may be at least a part of common principle of cellular response to DNA breaks caused by inorganic Hg. Thus, the increase of RPA expression can serve as marker of DNA disturbances which induced with Hg ions exposure. Taken together presented results, oxidative tissue damages and the complex of cellular events including cell stress sensor p53 and key component of DNA-repairing RPA1 are involved in response against inorganic Hg neurotoxicity.

Exactly based on abovementioned, future study of Hg neurotoxicity is extremely critic to clarify the potential risk of environment pollution with Hg containing compounds.

Conclusion

The modulation of stress sensor p53 and DNA breaks repair RPA1 contents is associated with the oxidative stress generation in fish brain caused inorganic Hg exposure in sublethal doses. Dramatic downregulation of p53 in rainbow trout brain induced with Hg chloride 50% LC_{50} dose exposure could be concerned to irreversible malfunction in signaling pathways. Subsequent study of Hg neurotoxicity in various vertebrate types is important to understand the human and animals risk of the environment contamination with Hg-containing compounds.

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