EFFECTS OF SUBCHRONIC TONALIDE EXPOSURE ON ZEBRAFISH, *DANIO RERIO*

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EFEKTI SUBHRONIČNOG IZLAGANJA ZEBRICE, *DANIO RERIO* SINTETIČKOM MOŠUSU

Apstrakt

Policiklična jedinjenja sintetičkog mošusa su veoma rasprostranjena i koriste se u velikim količinama kao miris u proizvodima široke potrošnje. Zbog njihove česte upotrebe i otpuštanja mirisa, ova jedinjenja su postala prisutna svuda u životnoj sredini. Toksikološka istraživanja su potvrdila da jedinjenje mošusa predstavlja opasnost za vodene ekosisteme. Cilj ovog istraživanja bio je da se ocene efekti subhroničnog izlaganja sintetičkom mošusu tonalidu na rast riba i odgovore na oksidativni stress kod zebrice (*Danio rerio*). Test rasta kod mlađi zebrice izvršen je prema OECD metodu broj 215. U toku 28 dana, riba stara 30 dana je bila izložena koncentraciji tonalida (50; 500; 5.000 and 50.000 ng/l). Na kraju eksperimenta, sve ribe su žrtvovane, izmerene, određena je njihova specifična prosečna stopa rasta po tanku, a selektivni oksidativni stress markeri su analizirani u homogenatu celog tela (glutation S-transferaze, glutation reduktaze, glutation peroksidaze, katalaze i lipidne peroksidaze). U našem ekperimentu, nismo pronašli značajne razlike između kontrolne i eksperimentalne grupe u specifičnom prirastu, telesnoj težini i dužini. Međutim, primetili smo značajne promene kod većine oksidativnih stres markera naročito kod eksperimentalne grupe koja je bila izložena najvećoj koncentraciji mošusa (tonalida).

U poređenju sa kontrolnom grupom, značajna povećanje je konstatovano u aktivnostima glutation S-transferaze (za koncentracije – 5.000 i 50.000 ng/l) i kataze (za koncentracije – 500, 5.000 i 50.000 ng/l). Sa druge strane, primećeno je značajno smanjenje aktivnosti glutation peroksidaze (za koncentraciju – 500 ng/l) u poređenju sa kontrolnom grupom. Nisu konstatovane promene u aktivnosti glutation reduktaze i nivou lipidne peroksidaze u poređenju sa kontrolnom grupom. Naši rezultati pokazuju da izlaganje zebrice tonalidinu

ima značajan uticaj na oksidativne stres markere i enzime za detoksifikaciju. Promene u aktivnostima antioksidantnih enzima se mogu tumačiti kao adaptivni odgovor koji bi zaštitio organizam ribe od toksičnosti prouzrokovane tonalidinom.

Ključne reči: riba; test rasta toksičnosti; policiklična jedinjenja mošusa; oksidativni stres; zagađenje vodene sredine
Keywords: fish; growth toxicity test; polycyclic musk compounds; oxidative stress; contamination of aquatic environment

INTRODUCTION

Polycyclic musk compounds are widely used as fragrances in consumer products in very large quantities. Due to their high use and release, they have become ubiquitous in environment. Toxicological studies confirmed that all musk compounds pose risk for aquatic ecosystems and can be considered toxic for aquatic invertebrates. Tonalide and galaxolide are the two most important compounds in group of polycyclic musk compounds. They probably share over 95% of the market for polycyclic musk compounds (Balk and Ford, 1999ab; Ramirez et al. 2009). The aim of this study was to investigate the effects of subchronic exposure to tonalide on fish growth and oxidative stress responses in zebrafish, *Danio rerio*.

MATERIALS AND METHODS

Juvenile growth tests were performed on *D. rerio* according to OECD method No. 215. For 28 days, fish at an initial age of 30 days were exposed to the environmental tonalide concentrations (50; 500; 5.000 and 50.000 ng/l). The fish were randomly distributed into 20 l glass aquaria, 50 specimens per each. Each test group was performed in duplicate. The experiment was conducted in a flow-through system and the volume of each test solution was replaced twice a day. The fish were fed with dried *Artemia salina* without nutshells to the amount of 8% of their body weight per day. Experimental procedures were in compliance with national legislation (Act No. 246/1992 Coll., on the Protection of Animals Against Cruelty, as amended, and Decree No. 207/2004 Coll., on the Protection, Breeding and Use of Experimental Animals as amended).

At the end of the experiment, the fish were killed, weighed and their tank-average specific growth rates were deterimined. Then, fish were immediately frozen, and stored at –85 °C until spectrophotometric analyses of oxidative stress biomarkers such as activities of glutathione S-transferase (GST), glutathione reductase (GR), glutathione peroxidase (GPx), catalase (CAT) and level of lipid peroxidation using thiobarbituric acid (TBARS). The activity of GST was determined by measuring the conjugation of 1-chloro-2,4-dinitrobenzene with reduced glutathione at 340 nm (Habig et al. 1974) and was expressed as the nmol of the formed product per min per mg of protein. The activity of GR was determined spectrophotometrically by measuring NADPH oxidation at 340 nm (Carlberg et al. 1975). The activity of GPx was calculated from the amount of NADPH oxidation by the reaction with GR at 340 nm (Flohe & Gunzler 1984). The activities of GR and GPx were expressed as the nmol of NADPH consumption per min per mg of protein. Protein concentration was determined by a Bicinchoninic Acid Protein Assay Kit (Sigma-Aldrich, St. Louis, MO, USA) using

bovine serum albumin as a standard (Smith et al. 1985). To check lipid peroxidation, malondialdehyde was measured by the TBARS method as described by Lushchak et al. (2005) at 535 nm. The concentration was expressed as nmol per gram wet weight of tissue.

Statistical analysis was performed using Unistat 5.6 software. Indices were tested for normal distribution and after testing of homogeneity of variance across groups, an analysis of variance (one-way ANOVA) was used. Differences between control and each experimental group were assessed with the Dunett test and $p<0.05$ was chosen as the level of significance.

RESULTS AND DISCUSSION

In test groups exposed to sublethal concentrations of tonalid we did not notice any changes in fish behaviour or food intake. We also did not find out any significant differences in specific growth rate, body weight and length among control and experimental groups.

On the other hand, we observed significant changes in most of oxidative stress markers especially in experimental groups exposed to the highest concentrations (Table 1).

Activity of GST significantly ($p<0.001$) increased in experimental groups exposed to tonalide at 5.000 ng/l and 50.000 ng/l compared to the control group. Significant increases were also observed in activity of CAT in experimental groups exposed to tonalide at 500 ng/l ($p<0.05$), 5.000 ng/l ($p<0.001$) and 50.000 ng/l ($p<0.001$) compared to the control group. In case of GPx acitivity, we found out significant decrease ($p<0.05$) but only in experimental group exposed to 500 ng/l. No changes were found in GR activity and level of lipid peroxidation.

Table 1. Results of oxidative stress markes in *D. rerio* after tonalide exposure; data are expressed as mean ± standard error of mean; **p<0.05 ***

Group	**GPx** (nmol/min/mg protein)	**GR** (nmol/min/mg protein)	**GST** (nmol/min/mg protein)	**CAT** µmol/min/mg protein)	**TBARS** (nmol/g)
control	99.96 ± 21.20	15.60 ± 0.65	180.28 ± 5.17	69.42 ± 2.89	12.99 ±0.93
50 ng/l	88.51 ± 19.11	13.69 ± 0.45	197.77 ± 4.48	86.80 ±4.77	15.58 ±1.28
500 ng/l	**44.15 ± 9.72***	14.55 ± 0.53	184.86 ± 4.29	**90.66 ±5.69***	13.62 ± 1.04
5000 ng/l	71.02 ± 16.55	15.53 ± 0.59	**215.62 ± 5.65***	**115.42 ±5.13***	14.47 ±2.74
50000 ng/l	108.64 ± 12.59	14.80 ± 0.71	**238.94 ± 9.39***	**104.55 ±7.85***	17.54 ± 0.72

CONCLUSION

Our results showed that tonalide exposure had profound influence on the oxidative stress markers and detoxifying enzyme of the exposed zebrafish. The changes in antioxidant enzyme activities could be an adaptive response to protect the fish from the tonalide induced toxicity.

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