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MOLECULAR TARGETS OF ZINC OXIDE NANOPARTICLES IN THE DIGESTIVE GLAND OF BIVALVE MOLLUSKS AND THEIR RESPONSES UNDER THE COMPLEX EXPOSURES

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Nanooxide ZnO particles (n-ZnO) became one of the most common types of metal-based nanoparticles used in electronics and personal care products. Last studies detected high effectivity of its composites with the coal fly ash in the sorption of endocrine disruptor organotin compounds in the water [Ayanda, 2015]. However, nZnO concentration in the aquatic environment in Europe has reached μg/L. This could be of great concern for the aquatic animals, particularly sessile filter-feeder organisms, bivalve mollusks and in the cooling reservoirs of coal-fired power plants which have constantly elevated temperature and polluted by the coal combustion residuals. Whereas vertebrate animals like fish or frog are able to release Zn from the nanoparticles [Falfushynska, 2014; 2017], the bioavailability of Zn from nanoparticles in mollusks is questionable. The aim of this study was to elucidate the ability of mollusks to utilize Zn from n-ZnO in the digestive gland. For that, we detected the binding of Zn with Zn-buffering proteins metallothioneins (MT). The effect of nanoparticles *per se* was evaluated from the integrity of lysosomal membranes and the activity of the ATP-dependent system of the efflux of xenobiotics from the cells (*p*-glycoproteins). Male specimens of *Unio tumidus* from two cooling reservoirs (DPP and BPP) were exposed for 14 days to nZnO (3.1 μM), Zn^{2+} (3.1 μM) at 18 °C, elevated temperature (T, 25 °C), or nZnO at 25 °C (nZnO+T). Control groups were held at 18 °C.

All Zn-containing exposures resulted in the elevated concentrations of total and Zn-bound metallothionein (MT and Zn-MT) in the digestive gland demonstrating the bioavailability of Zn from nZnO. This cellular response was distinct from the response of the mussels from the reference pond studied previously [Falfushynska, 2015]. The exposures to nZnO caused the decrease of *p*-glycoproteins activity in the mussels from both cooling ponds. The lysosomal membrane stability in hemocytes was initially low in both DPP- and BPP-groups and was decreased in all Zn-related exposures but elevated or stable under the exposure to heating reflecting the adaptation of mussels to elevated temperature in both ponds. The exposures to Zn and nZnO led to increase of ATP level and lipid stores (nZnO) in the digestive gland in DPP- and BPP-mussels. However, the heating caused the decline of the lipids (by 62.5 % in BPP-group) and ATP levels and diminished most responses to nZnO. Most common responses were the decrease of pyruvate concentration and increase in the Lactate/Pyruvate ratio in digestive gland. The up-regulation of cathepsin D total and/or free activity demonstrates the activation of the processes of autophagy in the tissue.BPP-groups were distinguished by increased level of cadmium in the tissue reflecting the highest level of pollution.

Hence, the mussels from the ponds of coal power plants can release Zn from the nanoparticles unlike the specimens from the pristine pond studied previously. However, their stress responses to additional heating could exceed the limits of adaptation.

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(recently has been attracting researchers' attention for use in aquatic animals) for fish health using markers of aerobic and anaerobic capacity in the gills, muscle, hepatic, and cardiac tissues of rainbow trout. The alanine (ALT) and aspartate aminotransferases (AST), lactate dehydrogenase (LDH) activity, lactate and pyruvate levels were assessed in the gills, muscle, hepatic, and cardiac tissues of rainbow trout exposed to chlorine dioxide and compared to untreated control.

In the present study, ALT activity after chlorine dioxide exposition was significantly increased by 26.5 % (p=0.033) in the cardiac tissue compared to control value. Hepatic and gill tissues showed the decrease of ALT activity by 21.4 % (p=0.008) and 18 % (p=0.000), respectively. In case of muscle tissue, ALT activity was non-significantly decreased compared to control. AST activity was increased significantly in the cardiac tissue by 24 % (p=0.011) but decreased in the cardiac tissue by 16.7 % (p=0.002) as compared to control value during chlorine dioxide disinfection. AST activity in the muscle and hepatic tissues after chlorine dioxide exposition showed usual trend of decreased as compared to control value (by 30.7 %, p=0.033 and by 12.7 %, p=0.002, respectively). LDH activity, likewise ALT and AST in the cardiac tissue, was significantly increased after chlorine dioxide exposure. In muscle and hepatic tissues, the statistically significant decreased LDH activity was recorded by 51.6 % (p=0.000) and by 110 % (p=0.002), respectively. The recovery pattern in case of LDH activity after disinfection by chlorine dioxide was in the following order: muscle tissue > gills > cardiac tissue > hepatic tissue. Like transaminases, muscle LDH activity was also reduced during disinfection as a compensatory response to overcome the stress. Reduced activity of LDH during disinfective measurement may be due to lower synthesis rate of lactate and pyruvate and less energy demand as fishes are in recovery period after disinfection. Lactate level after exposition of chlorine dioxide was significantly decreased by 28 % (p=0.017) in the muscle tissue of the trout compared to control value. Gill tissue also showed similar results as in the muscle tissue such as decrease of lactate level by 23 % (p=0.045). In case of cardiac tissue, lactate level was significantly increased by 79 % (p=0.001) compared to control. In case of lactate level, pattern was in the following order: cardiac tissue > hepatic tissue > gills > muscle tissue. Pyruvate level was decreased significantly in the gill tissue by 35 % (p=0.033) but non-significantly decreased in the muscle and hepatic tissue as compared to control value.

The increased activities of ALT, AST, and LDH in the cardiac tissue of trout may represent a metabolic compensatory mechanism employed by cardiac tissue in an attempt to mitigate the effects of the chlorine dioxide through changes in it metabolic functions [Malarvizhi et al., 2012]. The chlorine dioxide may induce an increased metabolism of nutritional carbohydrate and protein, and the upregulation of LDH, AST and ALT may be a response to resolve such an energy crisis [De Smet, 2001; Malarvizhi et al., 2012]. Therefore, the present findings, combined with previous research, demonstrate that the increased levels of LDH, AST and ALT in the cardiac tissue of trout following disinfection to chlorine dioxide may be a response to an increase in metabolism, which may suggest structural damage and dysfunction in fish hearts [Loteste et al., 2013; Zhang de et al., 2016]. Therefore, these biochemical indices can be considered as indicators for assessment of disinfective effects, although further studies are required for investigating the mechanism involved in this pattern.

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BIOINDICATION OF THE TOXICITY OF INDUSTRIAL EFFLUENTS BY THE APPLYING OF THE BIOCHEMICAL RESPONSES OF AQUATIC ANIMALS

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The validity of molecular biomarkers of stress and exposure to reflect the level and different types of pollution in aquatic animals is proved in a broad number of experimental exposures.

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