THE EVALUATION OF THE EFFECT OF MICROPLASTIC ON BIVALVE MOLLUSK IN THE SINGLE AND COMBINE WITH IBUPROFEN EXPOSURES

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Microplastic (MP) is now internationally recognized as a ubiquitous pollutant with potentially serious consequences in the environment. It can sorb other substances and change their biological activities of other substances like nonsteroidal anti-inflammatory drugs (NSAIDs). Ibuprofen (IBU) is a common NSAID and one of the most widely distributed pollutants in the aquatic environment. However, the combine effect of MP and NSAIDs in the aquatic animals is studied scarcely. Filter feeders, such as bivalve mollusks, are particularly vulnerable to MP ingestion as they are able to feed directly on MPs. The goal of this study was to elucidate the biochemical responses of freshwater bivalve mollusk to the combine effect of MP and IBU in the environmentally relevant concentrations, utilizing the multi-marker approach. To consider the possible effect of other confounding factors, specimens of mollusk *Unio tumidus* were collected from two populations, located in the pristine and polluted sites. We treated mollusks with microplastic PET particles (MP, 1 mg L^{-1} with a size < 0.5 mm), IBU (0.8 μg L^{-1}), or their combination (IBU-MP) for 14 days. Untreated mollusks from both sites were also examined. The oxidative stress response was assessed from the total antioxidant activity (ABTS test), Mn- and Cu,Zn-superoxide dismutases (SOD) and catalase (CAT) activities, the creation of the lipid peroxidation (TBARS) and protein carbonyls (PC), metallothionein-related thiols (MT-SH), cellular redox index ($NADH/NAD^+$). The activities of biotransformation, namely Cyp450-related (EROD, Phase I) and glutathione *S*-transferase (GST, phase II) were also analyzed. The activity of the main apoptotic executive enzyme caspase-3 and lysosomal protease cathepsin D (total and its efflux from lysosomes, CTDt and CtDe, respectively), cholinesterase (ChE, marker of neurotoxicity) and citrate synthase (CS, marker of metabolic activity) and lysosomal membrane stability (viability) were evaluated to indicate the toxicity of exposure.

The residents of two populations were distinguished by substantially higher level of antioxidant defence, biotransformation, metabolic activity and low apoptotic activity in the specimens from the pristine site. The exposures did not reveal the oppression of viability or neurotoxicity in all experimental groups. The exposure to MP and IBU-MP activated Mn- and Cu,Zn-SOD in both populations. The most common manifestations were the up-regulation of CtDt and CtDe (by 2-3 times) attesting the lysosome participation in the MP detoxification, and the decrease in the redox state (ratio $NADH/NAD^+$). The activation of antioxidant defence (ABTS test, TBARS and PCI) and metabolic activity was confirmed in the exposures to IBU and IBU-MP. In these exposures, EROD activity was depleted. ChE was activated only in the IBU and IBU-MP-groups. The exceptions from this regularity were the opposite responses of SOD, GST, MT-SH, caspase-3, and vitality in the specimens from two populations. The co-exposure to IBU and MP had sinergestic effects on the ABTS, SOD, PC, EROD, GST, ChE and CS responses. The specimens from the polluted site were more vulnerable to the exposures. These comprehensive results confirm the valuability of the utilized model to understand the earlier effects of pharmaceuticals and stress the importanse of the evaluation of the initial resistance of the organism depending on its history of population.

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