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INDICATION OF THE BIOCHEMICAL TARGETS OF RARE EARTH ELEMENTS IN BIVALVE MOLLUSCS UNDER MULTIPLE EXPOSURE

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Rare earth elements (REE) are considered the pollutants of priority, and biological effects are studied poorly.

Aim. Our study was devoted to the elucidation of suspected biochemical effects of two representative REEs, gadolinium (Gd) and yttrium (Y), individually and in a mixture with Ca-channel blocker nifedipine (Nfd), using the freshwater bivalve *Unio tumidus* as a model organism.

Materials and Methods. The specimens of swollen river mussel *Unio tumidus* were treated with GdCl3, 30 nM, YCl3, 30 nM, or their mixture with Nfd (10 µM) for 14 days. The set of metal-binding, reductive state, biotransformation activities, and toxicity in the digestive gland of mussels was selected.

Results. All exposures caused the elevation of the ratio of reduced and oxidized forms of nicotinamide adenine dinucleotide ($NADH/NAD^+$ ratio) and increase of the levels of reduced and oxidized glutathione (GSH and GSSG), the total level of cysteine-rich protein metallothionein (MTSH) in the Gd- and Y- groups and non-metalated MTSH in all exposures. The effects on the transformation of Phase I and Phase II enzymes were exposure-dependent. Dynamin-related GTP-ase activity increased in all exposures. The lysosomal membrane integrity and apoptotic activity decreased only in the Gd-group.

Conclusion. REEs in low concentrations cause adaptive metabolic responses in the mussels. The changes in the Zn distribution within the cells and Zn-dependent functions were indicated, confirming the impact of REEs on the functionality of the essential metal. In the combined exposure of REEs with a Ca-channel blocker, a cumulative effect was detected.

Keywords: Gadolinium, Yttrium, multiple exposure, mussels, stress.

Rare earth elements (REE) are considered “strategic elements” [6]. Consequently, the rising of their entry into the surface waters from anthropogenic sources can be expected. Knowledge about the biological role of REEs is currently fragmentary [1]. The REEs can disrupt ion regulation [4]. Therefore, the combined exposure to REEs and Ca-channel blocker nifedipine (Nfd) can elucidate this regularity.

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Aim. The assessment of the impact of two representative REEs, gadolinium (Gd) and yttrium (Y), individually and in a mixture with Nfd, on the metal-related functions and stress response in the bivalve *Unio tumidus*.

Methods. The specimens of swollen river mussel *U. tumidus* from the pristine area were distributed randomly to four groups: untreated mussels (C) and treated with GdCl3, 30 nM, YCl3, 30 nM, or their mixture with Nfd (10 μM) during 14 days. The digestive gland was utilized. The applied methods are described thoroughly in our previous work [7]. Briefly, reduced and oxidized forms of nicotinamide coenzymes (NADH and NAD+) were determined according to the enzymatic cycling assay based on the oxidation of ethanol to acetaldehyde with the reduction of NAD+. Reduced and oxidized glutathione (GSH and GSSG) were quantified by the glutathione reductase recycling assay using 5,5-dithiobis-2-nitrobenzoate (DTNB) for thiols quantification. Metallothionein protein (MTSH) concentration was determined after the ethanol/chloroform extraction utilizing DTNB. For the determination of Zn bound to metallothioneins (ZnMT), these heat-denaturated proteins were isolated by chromatography on a Sephadex G-50. The concentration of Zn in the tissue (Zn t) and ZnMT were measured utilizing the reaction of the complexation of Zn(II) with 5-Br-PAPS. For the evaluation of biotransformation activities, we analyzed the activities of cytochrome P450 (CYP450) related ethoxy resorufin *O-demethylase* (EROD) by indicating the formation of resorufin in the presence of reduced form nicotinamide adenine dinucleotide phosphate (NADPH), glutathione *S*-transferase (GST), using GSH and 1-chloro-2,4-dinitrobenzene as the substrate, and GTPase dynamin utilizing guanosine triphosphate (GTP) as the substrate and malachite green for the determining of the released inorganic phosphate. The caspase-3-related activity assay was based on the cleavage of peptide acetyl-Asp-Glu-Val-Asp p-nitroanilide (Ac-DEVD-pNA). The loss of lysosomal membrane integrity was analyzed utilizing the Neutral Red Retention (NRR) assay. The IBM SPSS Statistics version 26 software for Windows was used for calculations.

Results and Discussion. The responses of reductive shift belong to almost unified responses to stress in facultative anaerobes like bivalve mollusks [7]. The present study confirms these previous findings. Indeed, all exposures caused the elevation of the NADH/NAD+ ratio from 0.56 to 1.03-1.15, particularly in the Mix-group. The level of GSH and GSSG increased simultaneously in all exposures, particularly in the Gd-group (GSH about five times), resulting in the relatively stable rate of GSH/GSSG ratio (Fig. 1). The level of cysteine-rich protein MTSH increased in the Gd- and Y-groups but was corresponding to control value in the Mix-group. The part of ZnMT changed not so prominently, and, consequently, in all treated groups, the part of non-metallated protein increased, promoting the redox state of SH-groups [7]. The Zn t increased in all exposures by 15-32%, leading to a rise of Zn partitioning among other than ZnMT cellular targets. The biotransformation manifestations were more particular depending on the exposure (Fig. 1).

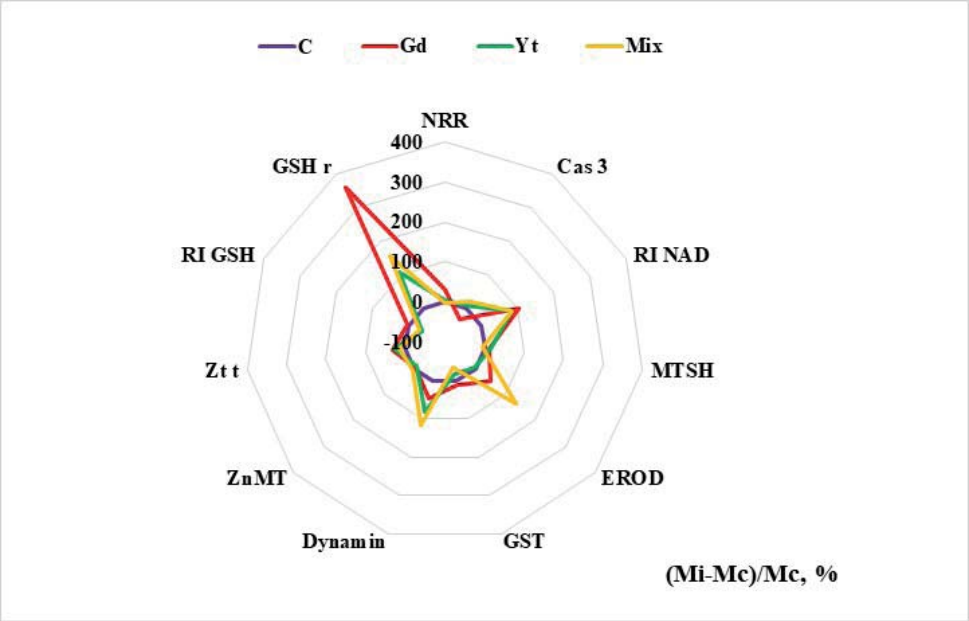

Fig. 1. **Graphical presentation of relative to control (C) responses of biomarkers in the exposures of *Unio tumidus* to the Gadolinium (Gd), Yttrium (Y), and their mixture with nifedipine (Mix)**

EROD activity increased in the Gd- and, particularly, in the Mix-groups, whereas GST was activated by Gd and depressed by Y and Mix. Significantly, dynamin-related GTPase activity increased in all exposures (up to two times in the Mix-group). The lysosomal membrane integrity decreased only in the Gd-group (by 32%). The caspase-3 activity was oppressed in the Gd-group, probably due to the highest level of unbound to MT Zn, its inhibitor [5], but increased by Mix.

The comparison of the reactions indicated, in most cases, the higher limits of responses in the Gd-group. Lifting of biotransformation and apoptotic activities demonstrated the cumulative effect in the presence of Nfd [2, 3].

Conclusion. Low nM concentrations of REEs cause adaptive metabolic responses in the mussels. The dependence on the Zn distribution within the cells was indicated, confirming the interaction between REEs and essential metals activities and its distorting in the multiple exposures with Ca-channel blocker.

Authors' contribution

VK: data analysis, presentation, analysis, interpretation, drafted manuscript; KY: sampling, biochemical and data analysis, presentation; VM: biochemical analysis, sampling, data analysis; MZ: biochemical and data analysis; VH: biochemical analysis, sampling.

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