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THE BIOCHEMICAL RESPONSES OF BIVALVE MOLLUSCS TO NEUROLEPTIC CHLORPROMAZINE ARE COMPARABLE WITH THE RESPONSES OF HIGHER VERTEBRATES

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Pharmaceuticals belong to the novel emerging aquatic contaminants. They can seriously threaten the health of non-target organisms [1]. Neuroleptic chlorpromazine (Cpz), known as dopamine D_2 receptor antagonist [2] has been recently nominated among the most promising coronaviruses inhibitors in human cells" [3]. Its antitumoregenic effects were also discovered [4]. However, the information concerning its effects on the non-targeted aquatic organisms are scant and controversies [5]. Bivalve molluscs are well recognized bioindicators of environmental pollution. Their ability to serve as a model of human biochemical responses is discussed.

Aim. The aim of the study was to compare the specificity and sensitivity of responses of stress and toxicity to Cpz in marine and freshwater species of bivalve molluscs.

Methods. The bivalve molluscs *Mytilus galloprovincialis* and *Unio tumidus* were collected in Messina, Italy and Ternopil region, Ukraine correspondingly. Two low Cpz concentrations (Cpz I: 12 ng L^{-1}; Cpz II: 12 (*M. galloprovincialis*) or 18 (*U. tumidus*) μg L^{-1}) were administered to molluscs for 14 days. The set of 16 studied parameters included the cytotoxicity indexes (lysosomal membrane stability, apoptotic enzymes caspase-3 and cathepsin D (CtD)), o xidative/reductive stress responses (superoxide dismutase (SOD) and catalase (CAT) activities, the levels of protein carbonyls (PC) and products of lipid peroxidation (TBARS), glutathione (GSH&GSSG), NAD^+&NADH), metallothionein (total and Zn-bound) concentration, the biotransformation enzymes CYP450-dependent EROD, glutathione *S*-transferase (GST), and GTP-ase dynamin in the digestive gland with some inter-species differences in this spectrum. The applied spectrophotometric and chromatographic methods are described thoroughly in our previous work [6].

Results and Discussion. In the hepatocytes of rat, Cpz causes the biotransformation up-regulation [7]. In our study, the activity of EROD increased both in *M. galloprovincialis* and *U. tumidus,* depending on the concentration and, particularly, in the Cpz II group of *U. tumidus* (by three times). This conversion of Cpz can result in the reactive metabolite formation [7]. According to this data, the inhibition or absence of the changes in the EROD activity in the Cpz I group of *U. tumidus* and Cpz II group of *M. galloprovincialis* can delay Cpz toxicity. On the other hand, the toxic product of biotransformation, quinoneimine is known to be eliminated by the GSH-dependent GST which activity was increased up to 63% in the Cpz I and II groups of marine mussels. Since in the freshwater mussels GST activity decreased in both exposures, the higher toxicity of Cpz was

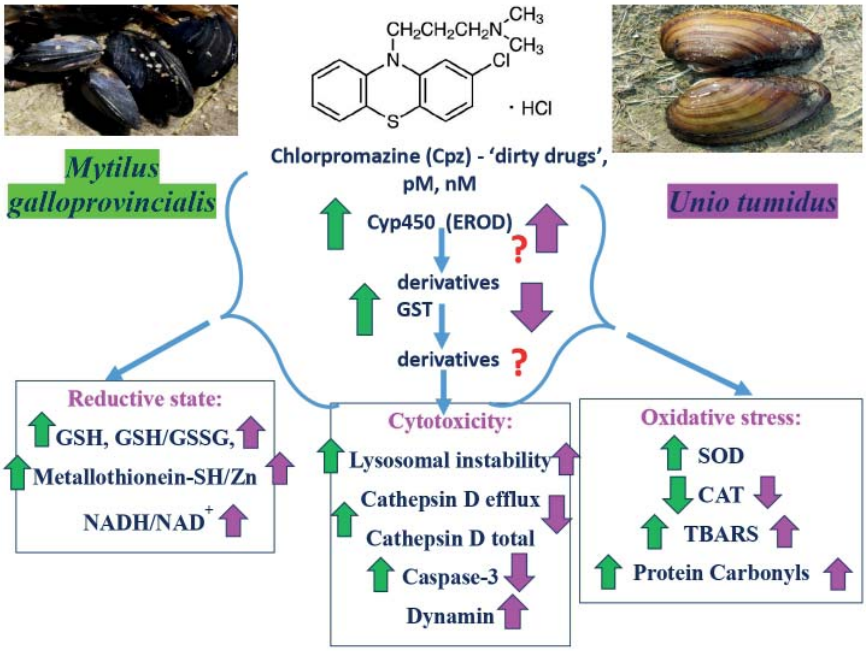

Fig. **Graphical presentation of results**

expected. Moreover, the GTP-ase dynamin activation in the Cpz I group of *U. tumidus* confirms the impact of this pharmaceutical on the clathrin-dependent endocytosis [8].

The oxidative stress response indicated the misbalance between the SOD (activated or not changed) and CAT (decreased) accompanied by the oxidative injury to lipids (TBARS) and protein carbonyls in both species. These signs confirmed the toxic consequences of the Cpz biotransformation, probably to peroxides, particularly in the *M. galloprovincialis*. In both species the similar responses of cellular low weight thiols was detected. The concentration of GSH increased in all exposures with the elevation of the (GSH/GSSG ratio almost in all groups. It was indicated the increase of the concentration of metallothioneins as well (both from their SH-groups and Zn-contained form). Importantly, the ratio of NADH/NAD^+, evaluated in the *U. tumidus,* increased substantially (Figure). This strengthening of the reductive state is typical for obligate anaerobes in the stressful conditions [6].

Second known effect of Cpz in the higher vertebrate, namely in the mammalian cell lines, is the selective increase of the lysosomal membrane permeability with the efflux of CtD [9]. In the present study, the loss of the lysosomal membrane stability was confirmed in all exposed groups. However, the specific response of the CtD efflux was detected only in the marine mussel. Similar difference was shown for the cytosolic executive apoptotic enzyme capase-3 (Figure).

Conclusions. Summarizing, adverse outcome pathways related to the effect of Cpz on vertebrates in the μM concentration, were indicated in the mussels under the pM — nM concentrations with distinct inter-species concentration-related dependence, and the responses of stress were similar in both species. Particularly, marine molluscs can be a perspective model for evaluating Cpz adverse effects intrinsic for the higher vertebrates.

Key words: pharmaceuticals, bivalve mollusc, ecotoxicity, oxidative stress, biotransformation, apoptosis.

Authors' contribution. KY: sampling, biochemical and data analysis, presentation, FI: sampling, cytology, VM: biochemical analysis, sampling, data analysis, CRM: sampling, cytology; MZ: sampling, chromatography; VK: data analysis, presentation, TM: biochemical and data analysis.

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