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VITELLOGENESIS AND BIOTRANSFORMATION ACTIVITIES IN FROGS EXPOSED TO COBALT, ZINC AND THEIR ORGANIC COMPLEXES

Frogs are susceptible to absorption of toxicants in water through all life stages. The aim of this study was to evaluate the effect of waterborn engeneering nanocomposites (NCs) – Co-NC and Zn-NC, correspondent concentrations of Co^{2+} (50 μg/L), Zn^{2+} (100 μg/L), or utilised polymeric substance (PS) on male frog Rana ridibunda during 14 days. Levels of vitellogenin and nuclear lesions (NL) in the blood, cytochrome P450-dependent monooxygenase (CYP450) and glutathione S-transferase (GST) as well as the distribution of Zn, Co, Cu, Cd in the liver were determined. Zn, Zn-NC and PS caused toxic manifestations: oesrogenic effect (Zn and PS), GST downregulation (Zn-NC), NL elevation (Zn-NC and PS). Co-NC only upregulated CYP450, while all other exposures inhibited it. The ratio (Cu+Co)/(Zn+Cd) in metallothionein was increased by the exposures to Co and Co-NC, whyle Zn, Zn-NC and PS increased it in other compounds. This remarkable selectivity distinguished frog from studied previously Carassius sp.

Keywords: cobalt, zinc, nanoscale composite, Rana ridibunda, vitellogenin, metallothionein, cytotoxicity

Frogs are susceptible to absorption of toxicants in water through all life stages. However, they represent the least studied for biochemical adaptations group of animals [1, 5]. Rapidly developing production and growing scales of the application of engineering metal-contained nanoscale materials (Me-NCs) in industry and medicine could cause their potential risks for human and wildlife, primarily for the aquatic ecosystems [2, 6]. For the present study we select the organic nanoscale composites (NCs) of cobalt (Co) and zinc (Zn), highly utilized in industry and medicine, to elucidate the ability of frogs to release these ions from NCs by the comparison of the effect of these NCs, free Co and Zn and correspondent organic polymeric substance (PS) per se. Owing to the reconstruction of environmentally relevant situations, concentrations of Co^{2+} and Zn^{2+} corresponding to the limits found in freshwater areas were applied [6].

Materials and methods

Male frogs Rana ridibunda were collected from a rural site and studied with permission of the Ministry of Environment Protection of Ukraine, No. 466/2013. One group was used as a control and five other groups were exposed to Co^{2+}- or Zn^{2+}-containing nanoscale polymeric composites (Co-NC, or Zn-NC), corresponding concentrations of Co (50 $\mu g \cdot L^{-1}$), or Zn (100 $\mu g \cdot L^{-1}$) or polymeric substance (PS) during 14 days in laboratory tanks [3,6]. Levels of Vtg-LP in the blood, cytochrome P450-dependent monooxygenase (CYP450 by ELISA) and glutathione S-transferase (GST) as well as the distribution of Zn, Co, Cu, Cd and nuclear lesions (NL (nuclear abnormalities (NA) and micronuclei (MN)) in the liver were determined. Characteristic of Co- and Zn-NCs and applied assays are given in [3, 6]. All statistical calculations were performed with Statistica v 8.0 and Excel programs for Windows-2000.

Results and discussion

The comparison of the exposed groups had shown (Fig. 1) that Zn, Zn-NC and PS caused plural toxic manifestations. The elevated Vtg-LP level in blood witnessed oesrogenic effect of Zn and PS. The activity of CYP450 was typically downewgulated (except of its elevation caused by Co-NC), and GST was downregulated by Zn-NC. Hense, the metabolic activity in general and ability to transform NCs was inhibited.

Zn was most abundant within the determined metals in the tissue and MTs (Table). The exposures caused the changes in the metal concentration and composition in the liver. The most typical feature of these changing was the avoidance of Zn and Cu accumulation. When the molar ratio of redox-active metals (Cu and Co) and redox-stable metals (Zn and Cd) was compared, the (Cu+Co)/(Zn+Cd) ratio in MTs was increased from 0.06 till 0.14–0.19 in Co-, Co-NC- and PS-groups and was not changed in Zn- and Zn-NC-groups. In opposite, in Zn-, Zn-NC- and PS-groups, this ratio increased from 0.08 till 0.10 – 0.14 in other tissue compounds and did not change in MTs.

Table

Concentrations of zinc, cobalt, copper and cadmium in the liver, hepatic metallothioneins (nmol/g DW), (M±SD, n=8)

Parameters	C	Co	Co-NC	Zn	Zn-NC	PS
Cu-MT	3,3±0,4^{a}	7,1±0,7^{b}	2,4±0,2^{c}	7,6±0,8^{b}	3,9±0,3^{d}	33,6±3,9^{e}
Zn-MT	237,6±19,7^{a}	155,7±18,9^{b}	94,0±9,9^{c}	378,2±41,9^{d}	132,7±11,7^{b}	261,1±27,2^{a}
Cd-MT	0,9±0,1^{a}	6,5±0,6^{b}	1,9±0,2^{c}	2,7±0,3^{d}	3,0±0,3^{d}	0,9±0,1^{a}
Co-MT	13,4±1,5^{a}	18,2±1,5^{b}	15,2±1,4^{c}	20,9±2,1^{b}	6,8±0,6^{d}	20,8±2,1^{b}
Cu (other)	74,9±8,1^{a}	64,8±6,1^{a}	55,5±5,2^{b}	73,7±7,1^{a}	72,6±7,1^{a}	16,4±1,5^{c}
Zn (other)	2006±197^{a}	1923±167^{a}	1683±176^{b}	1142±111^{c}	1337±126^{d}	722±65^{e}
Cd (other)	10,7±1,2^{a}	8,6±0,9^{b}	21,3±1,7^{c}	19,6±2,0^{c}	14,8±1,5^{d}	7,1±0,8^{b}
Co (other)	100,1±9,3^{a}	125,8±11,2^{b}	73,0±6,9^{c}	84,2±7,1^{d}	127,1±13,2^{b}	64,0±6,2^{e}

The same letters indicate the values of biomarkers that do not differ significantly ($p > 0.05$)

The genotoxicity was detected in the exposures to Zn-NM and PS. In the case of Zn, the decrease of their values could be explained by the activation of apoptosis [6]. Pearson correlation analysis revealed the relationships between the studied characteristics across all experimental groups ($r > 0.4$; $p<0.05$). Characteristic of monooxygenases had the highest number of correlations, including positive correlations with the Vtg-LP, level of metals in the tissue (Zn, Cu, Co) and negative correlations with the markers of genotoxicity.

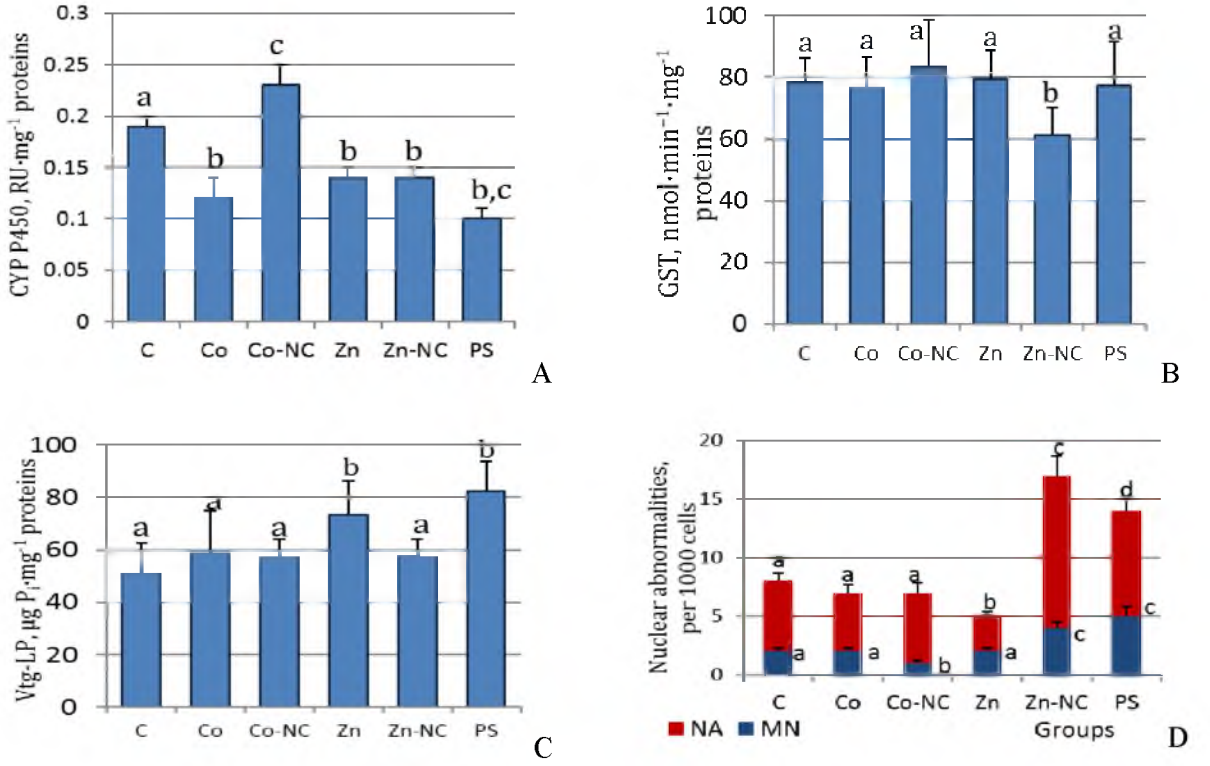

Fig. 1. Biomarkers in the liver (A, B) and plasma (C, D) of frog Rana sp., loaded by Co- and Zn-NCs and their constituents, (M±SD, n=8). A, CYP450 activity; B, GST activity; C, Vtg-LP level; D, Cells with nuclear abnormalities (NA) and micronuclei (MN). The same letters indicate the values of biomarkers that do not differ significantly ($P > 0.05$)

To generalise, specific effects of each exposure proved the low ability to release metals from NCs in frog. Low CYP450 activity confirmed this conclusion. Moreover, the opposite influences on vitellogenesis and metal distribution demonstrated low toxicity of Co-contained substances and high vulnerability of frogs to Zn, Zn-NC and PS. These data are well adjusting with our previous studies of redox-active copper and redox-stable Zn on frog [1, 4] demonstrating unusual for most animal models regularities. For example, the comparison of the responses of three aquatic species in the case of Vtg shows different sensitivity for Co-related exposures: whereas in frog this activity was nonsensitive to the effect of Co, in the mollusk vitellogenesis was activated by Co and in fish – by Co-and Co-NC.

Conclusion

High specificity and sensitivity of frog to trace metals and their nanocomposites was indicated. Biomarkers of endocrine activity, biotransformation and toxicity withstood with hepatic metal distribution.

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ВІТЕЛОГЕНЕЗ ТА БІОТРАНСФОРМАЦІЙНА АКТИВНІСТЬ У ЖАБ ЗА ВПЛИВУ КОБАЛЬТУ, ЦИНКУ ТА ЇХ ОРГАНІЧНИХ КОМПЛЕКСІВ

Жаби сприйнятливі до абсорбції токсикантів з води протягом всіх життєвих стадій. Метою цього дослідження стало оцінити вплив промислових нанокомпозитів (NC) – Co-NC та Zn-NC, відповідних концентрацій Co^{2+} (50 мкг/дм^3), Zn^{2+} (100 мкг/дм^3), або полімерного матеріалу (PS) на самців жаби Rana ridibunda протягом 14 діб. Визначали рівень вітелогеніну та ядерні ушкодження (NL) у крові, активність цитохром P450-залежної монооксигенази (CYP450) та глутатіон S-трансферази (GST), а також розподіл Zn, Co, Cu, Cd у печінці. Zn, Zn-NC та PS викликали токсичні прояви: ксеноестрогенну дію (Zn та PS), пригнічення GST (Zn-NC), збільшення кількості NL (Zn-NC та PS). Активність CYP450 зростала лише за дії Co-NC, тоді як інші чинники пригнічували її. Дія Co та Co-NC призводила до збільшення співвідношення кількості (Cu+Co)/(Zn+Cd) у металотіонеїнах, тоді як Zn, Zn-NC та PS викликали його збільшення у інших компонентах тканини. Ця визначна чутливість відрізняла реакції жаби від риби Carassius sp., дослідженої попередньо.

Ключові слова: *кобальт*, *цинк*, *нанорозмірний композит*, Rana ridibunda, *вітелогенін*, *металотіонеїн*, *цитотоксичність*

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ВИТЕЛЛОГЕНЕЗ И БИОТРАНСФОРМАЦИОННАЯ АКТИВНОСТЬ ЛЯГУШКИ ПРИ ВОЗДЕЙСТВИИ КОБАЛЬТА, ЦИНКА И ИХ ОРГАНИЧЕСКИХ КОМПЛЕКСОВ

Лягушки восприимчивы к абсорбции токсикантов из воды на протяжении всех жизненных стадий. Целью этого исследования стало оценить влияние промышленных нанокомпозитов (NC) – Co-NC и Zn-NC, соответствующих концентраций Co^{2+} (50 мкг/дм^3), Zn^{2+} (100 мкг/дм^3), или полимерного материала (PS) на самцов лягушки Rana ridibunda на протяжении 14 суток. Определяли уровень вителлогенина и ядерне повреждения (NL) в крови, активность цитохром P450-зависимой монооксигеназы (CYP450) и глутатион S-трансферазы (GST), а также распределение Zn, Co, Cu, Cd в печени. Zn, Zn-NC и PS вызывали токсические проявления: ксеноэстрогенный эффект (Zn и PS), угнетение GST (Zn-NC), увеличение числа NL (Zn-NC и PS). Активность CYP450 возростала только при действии Co-NC, тогда как другие воздействия угнетали ее. Действие Co и Co-NC приводило к увеличению соотношения количесттва (Cu+Co)/(Zn+Cd) в металлотионеинах, тогда как Zn, Zn-NC и PS вызывали его увеличение в других компонентах ткани. Эта исключительная чувствительность отличала реакции лягушки от рыбы Carassius sp., изученной предварительно.

Ключові слова: *кобальт*, *цинк*, *наноразмерный композит*, Rana ridibunda, *вителлогенин*, *металлотионеин*, *цитотоксичность*

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ENDOCRINE DISRUPTION AND CYTOTOXICITY IN BIVALVE MOLLUSK UNDER THE COMPLEX EXPOSURE TO ZINC NANOOXIDE

Despite metal-contained nanoparticles (n) represent novel kind of environmental pollution, their toxicity to aquatic habitats is unclear with regards to multiple stress exposures. In present study, male Unio tumidus were exposed for 14 days to n-ZnO (3.1 μM), Zn^{2+} (3.1 μM), Ca-channel blocker nifedipine (Nfd, 10 μM), combinations of n-ZnO and Nfd or n-ZnO and thiocarbamate fungicide Tattoo (Ta, 91 μg L^{-1}) at 18°C, and n-ZnO at 25°C. Activities of vitellogenesis (Vtg), stress and detoxification systems (Mn- and Cu,Zn-superoxide dismutase, metallothionein (MT), EROD), nuclear lesions (NL) and choline esterase (ChE) activity) were analysed. Vtg and NL were elevated in most exposures. N-ZnO per se did not provoke any other changes of indices unlike Zn. In combine exposures, the activation of stress-responses, EROD and phenoloxidase activities were detected. Nfd caused the most prominent modulations of n-ZnO effect on Vtg and MT. Only Zn and Nfd caused ChE depletion.

Keywords: bivalve mollusk, zinc nanooxide, heating, nifedipine, thiocarbamate, combine exposure, biomarker

Nanoparticle toxicity is a growing concern in freshwater habitats; however, understanding of the nanoparticle effects on aquatic organisms is impeded by the lack of the studies of the nanoparticles effects in the environmentally relevant context of multiple stress exposures. Metal-contained nanoparticles can be transformed in aquatic organisms by reactions with biological macromolecules,