EVALUATION OF IMMUNE SYSTEM OF FEMALE Steatoda grossa (Theridiidae) SPIDER EXPOSED TO FOOD CONTAMINATED WITH COPPER

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Introduction

Functioning of the immune system can be impaired due to pro-oxidative nature of stressors, including metals. They can cause cell damages and reduce their viability leading to changes in the number and composition of hemocytes circulating in the hemolymph. Copper is an essential micronutrient, which can be found in the composition of many enzymes, and in spiders, it also constitutes a component of hemocyanin. Increased level of this metal in the organism may increase the production of reactive oxygen species. In this study, using a simple model of the food chain—medium with copper → *Drosophila hydei* fly → *Steatoda grossa* spider—it was examined whether and to what extent the metal administered via oral route changes the efficiency of the immune system and influences antioxidant potential of hemolymph, under immunostimulation conditions and in subjects not exposed to any additional stressors.

Methods

Studies were carried out using adult female *S. grossa* (Theridiidae) synanthropic spiders. Under environmental conditions, they are found mainly in buildings and in dark and sheltered areas where they build tangled, three-dimensional webs. The subjects were kept in a growth chamber under standard lighting (12L: 12D), temperature (L:24 C, D:18 C), and humidity (70% ± 10%) conditions. Three experimental groups were distinguished: control (C), spiders exposed to copper (Cu_II) for two weeks, and spiders exposed to metal for four weeks (Cu_IV). Control spiders were fed with *D. hydei* cultured using uncontaminated standard medium, while the other groups of spiders were provided with flies cultured using media supplemented with 0.234 mM $CuSO_4$. From each of these main groups, subjects were distinguished into subgroups that were injected with 5 µl of immunostimulatory phorbol-12-myristate-13-acetate (PMA) at a concentration of 0.01µg ml^{-1}. Hemolymph was collected by puncture made on the border of limbs attachment and prosoma followed by suspension in 100 µl PBS. The number of hemocytes in spiders' hemolymph was counted by Bürker hemocytometer, light microscope at 400× magnification, and their amount was expressed per ml hemolymph. The respiratory burst activity of hemocytes was analyzed with nitroblue tetrazolium (NBT) (Homa et al., 2013). Catalase activity (CAT; EC 1.11.1.6) was determined according to Orr (1970). Decomposition of H_2O_2 was monitored spectrophotometrically at 230 nm, with the unit of catalase activity defined as 1 µmol H_2O_2 decomposed per minute. Total antioxidant capacity (TAC) was assessed by the ABTS method (Re et al., 1999), using the reduction reaction of $ABTS^+$ cation radicals

derived from ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt)). Copper content in spiders' organisms and their victims was determined by atomic absorption spectrometry (AAS). Statistical analyzes were performed using the STATISTICA 12.0 software package (StatSoft, Inc. 2013).

Results

No accumulation of copper was observed in *S. grossa* after two and four weeks of ingestion of diets that contained *D. hydei* flies cultured using media supplemented with $CuSO_4$(Table 1).

Table 1. Mean (x) ± standard deviation (SD) of copper concentrations ($\mu g \cdot g^{-1}$ dry weight) in females of *S.grossa* and their prey, *D.hydei*, from the control (C) and Cu groups. Different letters (a,b) indicate heterogenous groups (Tukey's test, $p<0.05$).

Species	C	Cu II	Cu IV
S. grossa	12.6±0,96[a]	13.1±2,8[a]	13,2±2,1.[a]
D. hydei	18,1±1,3[a]	67.4±6.5[b]	

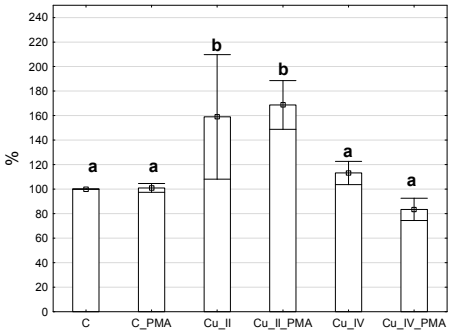

Figure 1. The respiratory burst activity (%). The same letters indicate homogenous groups (Tukey's test, $p< 0.05$)

Regardless of the duration of exposure to copper, catalase activity and TAC value in the hemolymph did not change significantly among infected and uninfected subjects compared to the control. Increased hemocytes, in response to PMA administration, were found only in the hemolymph of spiders from the control group (Figure1). In other groups, regardless of the duration of exposure to the metal, the number of hemocytes did not change significantly compared to the control. Two-week exposure to copper intensified respiratory burst activity in both immunostimulated subjects and in those with no PMA injection. After four weeks of exposure to metal, the least efficient ability of hemocytes to produce reactive oxygen species was demonstrated in immunostimulated spiders.

Conclusion

Low concentration of copper found in *S. grossa* indicates the possibility of removal of excess metal that enters the organism via food through feces. Copper at concentrations used in this study did not impair antioxidant efficiency, as confirmed by the level of CAT and TAC activity in hemolymph of the species investigated. Short-term exposure of the organism to increased copper concentrations via diet stimulated the immune system both under simulated invasion of pathogens and under the absence of infection as well. Increased exposure time to metal may reduce the effectiveness of *S. grossa* immune response under infectious conditions.

References

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