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Activation of Apoptosis in the Midgut Epithelium of Spodoptera exigua Hübner (Lepidoptera: Noctuidae) Exposed to Various Cadmium Concentration

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Abstract : The digestive system of insects is composed of three distinct regions: fore-, mid- and hingut. The middle region (the midgut) is treated as one of the barriers which protects the organism against any stressors which originate from external environment, e.g. toxic metals. Such factors can activate the cell death in epithelial cells to preserve the entire tissue/organs against the degeneration. Different mechanisms involved in homeostasis maintenance have been described, but the studies of animals under field conditions do not give the opportunity to conclude about potential ability of subsequent generation to inherit the tolerance mechanisms. It is possible only by a multigenerational strain of an animal led under laboratory conditions, exposed to a selected toxic factor, present also in polluted ecosystems. The main purpose of the project was to check if changes, which appear in the midgut epithelium after Cd treatment, can be fixed during the following generations of insects with the special emphasis on apoptosis. As the animal for these studies we chose 5th larval stage of the beet armyworm Spodoptera exigua Hübner (Lepidoptera: Noctuidae), which is one of pest of many vegetable crops. Animals were divided into some experimental groups: K, Cd, KCd, Cd1, Cd2, Cd3. A control group (K) fed a standard diet, and was conducted for XX generations, a cadmium group (Cd), fed on standard diet supplemented with cadmium (44 mg Cd per kg of dry weight of food) for XXX generations. A reference Cd group (KCd) has been initiated: control insects were fed with Cd supplemented diet (44 mg Cd per kg of dry weight of food). Experimental groups Cd1, Cd2, Cd3 developed from the control one: 5 mg Cd per kg of dry weight of food, 10 mg Cd per kg of dry weight of food, 20 mg Cd per kg of dry weight of food. We were interested in the activation of apoptosis during following generations in all experimental groups. Therefore, during the 1st year of the experiment, the measurements were done for 6 generations in all experimental group. The intensity and the course of apoptosis have been examined using transmission electron microscope (TEM), confocal microscope and flow cytometry. During apoptosis the cell started to shrink, extracellular spaces appeared between digestive and neighboring cells, the nucleus achieved a lobular shape. Eventually, the apoptotic cells was discharged into the midgut lumen. A quantitative analysis revealed that the number of apoptotic cells depends significantly on the generation, tissue and cadmium concentration in the insect rearing medium. In the following 6 generations, we observed that the percentage of apoptotic cells in the midguts from cadmium-exposed groups decreased gradually according to the following order of strains: Cd1, Cd2, Cd3 and KCd. At the same time, it was still higher than the percentage of apoptotic cells in the same tissues of the insects from the control and multigenerational cadmium strain. The results of our studies suggest that changes caused by cadmium treatment were preserved during 6-generational development of lepidopteran larvae. The study has been financed by the National Science Centre Poland, grant no 2016/21/B/NZ8/00831.

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