Effects of 5-fluorouracil and CdCl₂ in freshwater oligochaete limnodrilus udekemianus clapared (tubificidae) measured by comet assay

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Freshwater oligochaete Limnodrilus udekemianus is a cosmopolitan tubificid species often found in organic-rich sediment. Many of freshwater oligochaetes with their benthic, sediment borrowing way of life and low mobility directly interacts in the recycling of materials in water and sediment, in food chain structure, and therefore they are especially affected by the presence of xenobiotics in aquatic environment. This study is the first report of potential genotoxicity of base analog cytostatic drug 5-fluorouracil (5-FU) and CdCl₂ in L. udekemianus evaluated by alkaline comet assay. Oligochaetae were gained from laboratory culture grown on medical clay and dechlorinated tap water. In vivo treatments were performed as water-only (without sediment) short-term genotoxicity tests for 5-FU (96-h test) and CdCl₂ (72-h test). For both treatments groups of 50 worms were carefully chosen (similar size) and exposed to 5-FU (0.004, 0.04, 0.4, 4, 40 and 585 μM) and to CdCl₂ (0.04, 0.4, 4 and 40 μM). Negative control was done for each experiment in triplicate with groups of 50 worms in clean regional spring water. In both cases, treatments with 5-FU and CdCl₂, mortality of worms was not detected, although two highest concentrations (40 and 585 μM for 5-FU; 4 and 40 μM for CdCl₂) reduced the viability of the cells for more than 50%. Images of 50 nuclei per each concentration of test substance were analyzed with a fluorescence microscope and scored using analysis software (Comet Assay IV Image analysis system, PI, UK). Results were analyzed using Student test (t-test, statistica 6.0). Short-term exposure to 5-FU have shown statistically significant increase of DNA damage (p<0.05, t-test) in comparison with negative control. These results show that even smallest concentration of this cytostatic drug has great impact on DNA integrity. Preliminary results of exposure to CdCl₂ have not resulted in significant increase in DNA damage, although this agent is well known model mutagen. Reason for this may be the exposure period and/or ability of Tubificidae to accumulate heavy metals in the caudal region on their body making them, in this way, less sensitive to heavy metals exposure. Due to the cosmopolitan distribution and good knowledge of the general biology of the species belonging to genus Limnodrilus this taxa could be considered as effective test organisms in ecogenotoxicology. Even though statistically significant response was noticeable, further work and completion of the data sets is required for definite conclusion.