CYTOSTATICS AS EMERGING POLLUTANTS IN AQUATIC ENVIRONMENTS - RISK ASSESSMENT BASED ON GENOTOXIC EFFECTS IN HAEMOCYTES OF FRESHWATER MUSSELS

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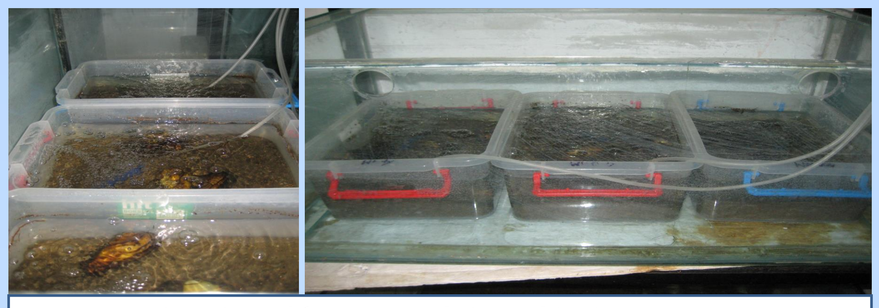
INTRODUCTION

Pharmaceutical compounds have begun to be considered as dangerous environmental pollutants, due to their widespread occurrence in wastewaters and their potential hazard towards the aquatic ecosystems. The comet assay was used to study acute impacts of most used cytostatic drugs on the haemocytes of widely distributed freshwater mussels *Unio pictorum and Unio tumidus*.

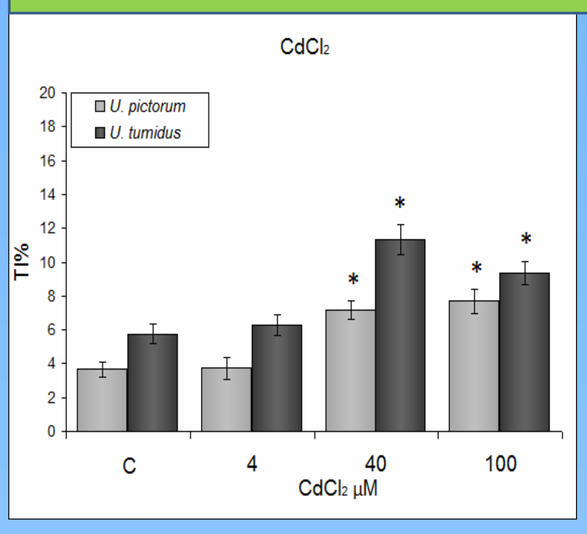
MATERIAL AND METHODS

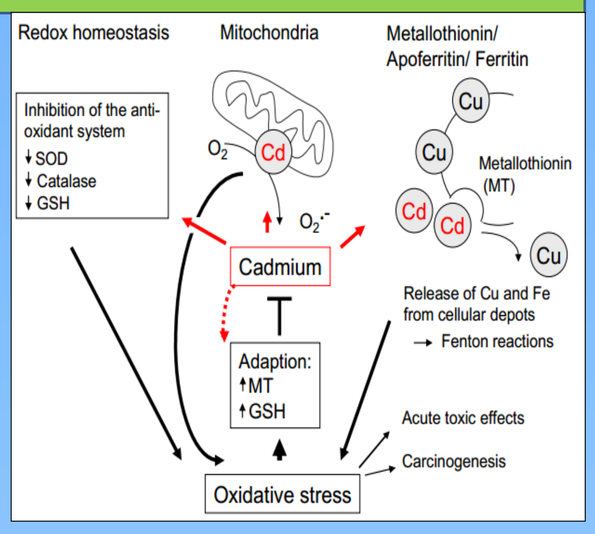
After collection from unpolluted location, the mussels were held on accommodation for 10 days in controlled laboratory conditions before exposure to cytostatics. Groups of 5 mussels were exposed to 5-Fluorouracil, (0.04 – 40 μM), Cisplatin (0.004 – 4 μM), Etoposide (4, 40 and 100 μM) and Imatinib msesylate (0.04 - 40 μM). For positive control treatment with Cd was used (4, 40 and 100 μM), while as negative control mussels were held in control aquarium with clean water. Exposure was performed for 72h in aquaria.

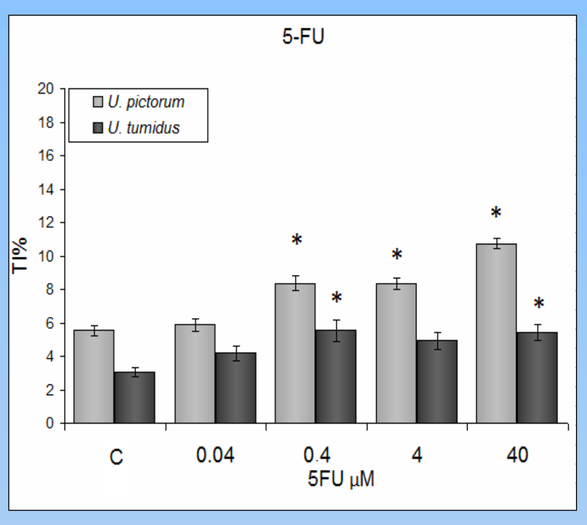


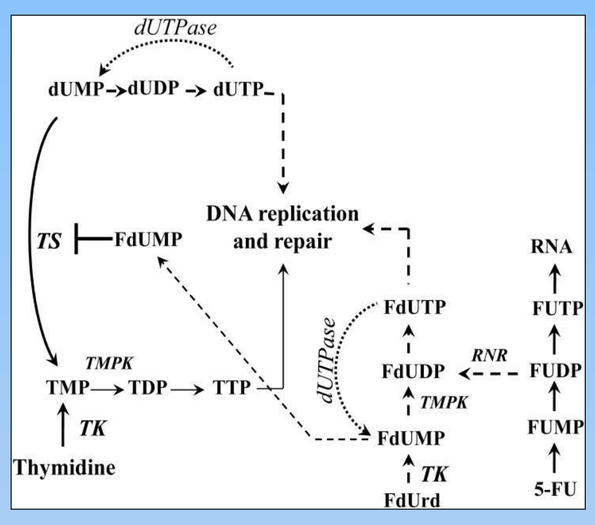
Aquaria used for accommodation and *in vivo* exposure

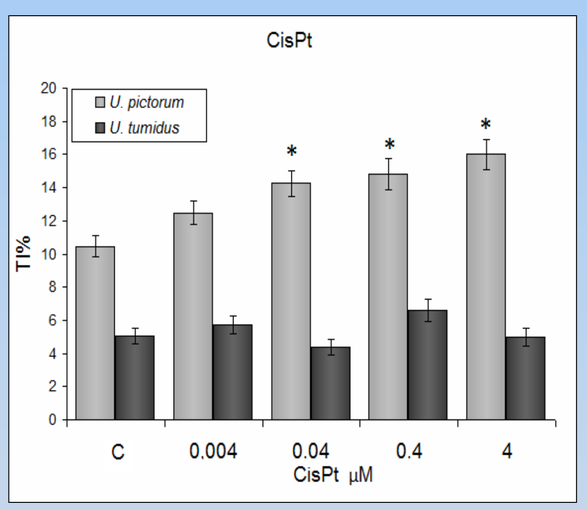
RESULTS

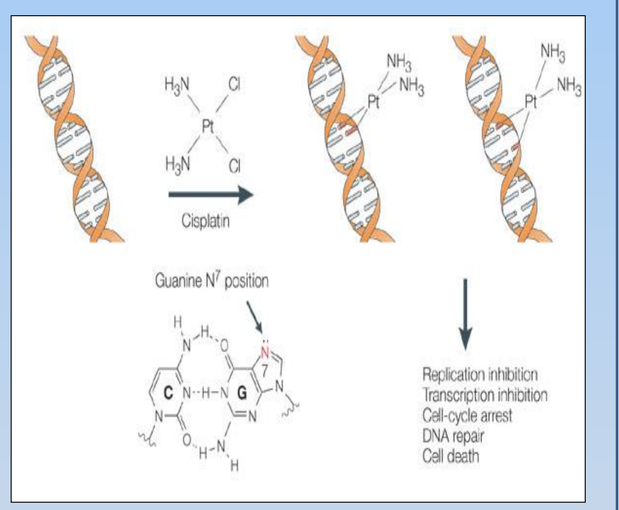


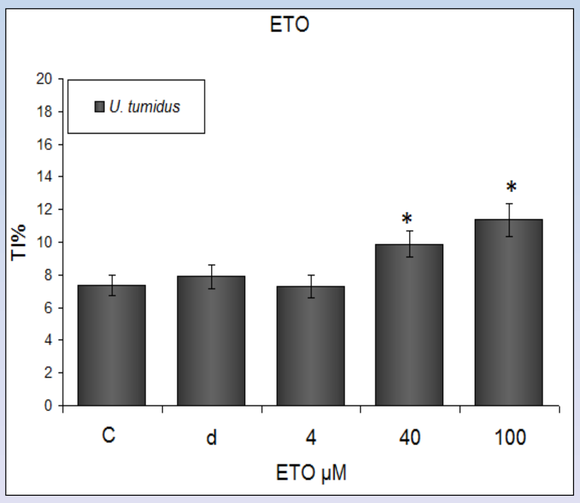


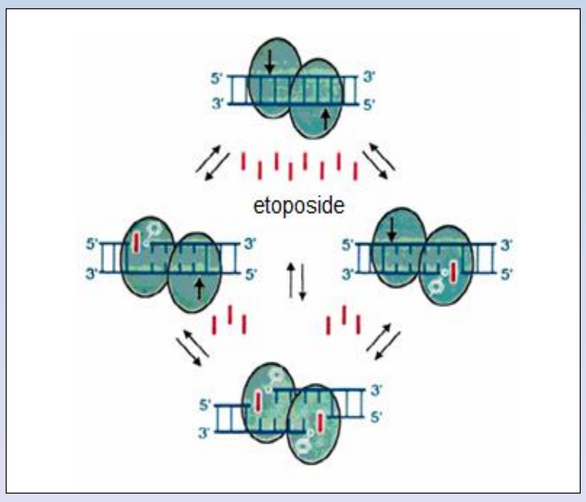


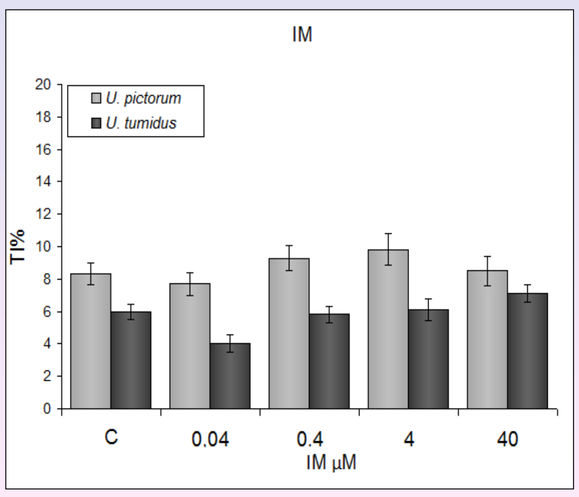


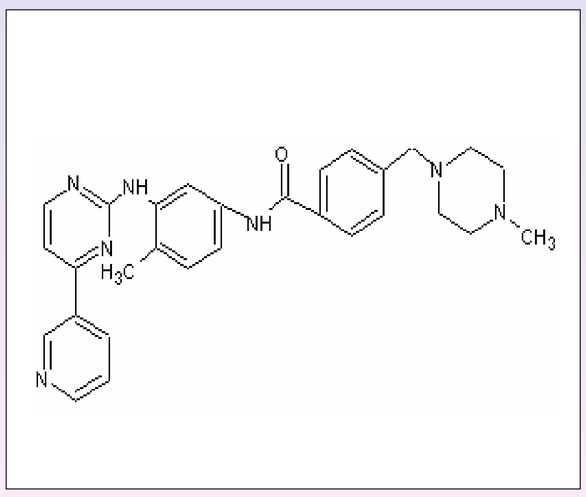






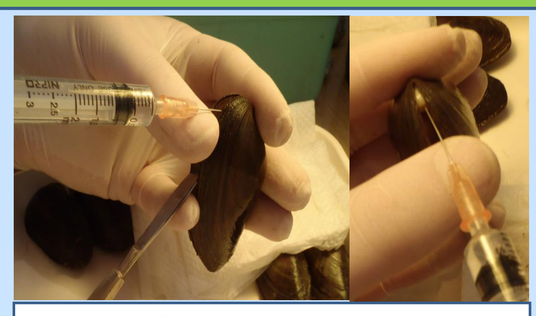






Haemolymph collection

Mussel was placed on soft tissue paper; blade of scalpel was inserted between mussel's shells and mussels were gently open for about 1 cm to allow paper to drain all water caught in mussel's shell. Haemolymph was collected from the adductor muscle using hypodermic syringe, to obtain at least 1 ml of sample (Fig. 1). Haemocyte viability was determined by the differential acridine orange/ethidium bromide (AO/EB) staining.

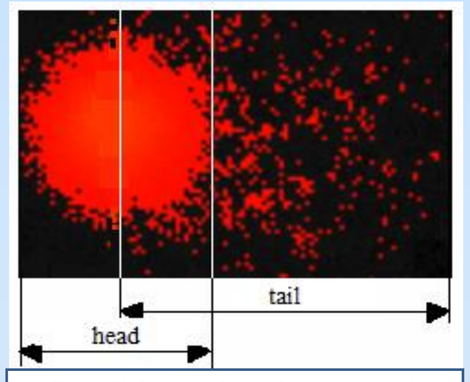


Haemolymph collection

COMET ASSAY

Comet assay is one of the major tools for the level of DNA damage assessment in ecogenotoxicology. It is based on single cell gel electrophoresis. In our experiments we performed alkaline version of comet assay described by Singh et al. (1988).

Images of 250 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). Tail intensity was chosen as relevant measure of DNA damage.



"Comet" formed from damaged nuclei

$CdCl_2$

Cadmium is well known genotoxic agent which induces DNA damage by oxidative stress. Therefore, treatment with cadmium was chosen as positive control.

In comparison with negative control, statistically significant increase of DNA damage was noticed after *in vivo* treatment with $CdCl_2$.

5-Fluorouracil (5-FU)

FdUMP prevents the conversion of dUMP to the sole de novo source of dTMP depleting the cell of dTTP for use in DNA replication and repair. This leads to the use of dUTP in place of dTTP during DNA synthesis, and subsequent DNA fragmentation due to extensive uracil excision in newly synthesized DNA or repeated futile repair attempts in the presence of a high dUTP/ dTTP ratio

Statistically significant increase of DNA damage was recorded *in vivo* experiments with different concentration of 5-FU.

Cisplatin (CisPt)

Cisplatin is alkylating-like drug, cisplatin undergoes hydrolysis, producing the highly reactive charged platinum complex which binds to DNA through the N7 atom of guanine base. In *in vivo* exposure significant increase of DNA damage was observed only in haemocytes of *U. pictorum*.

Etoposide (Eto)

Etoposide is inhibitor of topoisomerase II. Depending if only one or both of subunits of toposiomerase II are inactivated it creates single and double strand brakes. Significant increase in DNA damage was observed for concentrations 40 and 100 μM. Treatment was performed only on *U. tumidus*.

Imatinib mesylate (IM)

IM works by preventing a tyrosine kinase enzyme, from phosphorylating subsequent proteins and initiating the signalling cascade necessary for cancer development, thus preventing the growth of cancer cells and leading to their death by apoptosis. So far, data related to genotoxicity of this drug are not available. IM did not induce significant increase of DNA damage in tested concentration range.

CONCLUSION

Our results indicated significant increase of DNA damage in haemocytes during treatment in selected concentration ranges for all cytostatics with the exception of Imatinib mesylate. Detected LOEC values were as following: 5-Fluorouracil – 52 μg/L, Cisplatin 12 μg/L and Etoposide – 24 mg/L.

However, all effective concentrations of 5-FU, CP and ETO are higher than ones measured in surface water but still far below PNEC values which are currently used for the environmental risk assessment. This indicates that acute toxicity data might not be sufficient for prediction of adverse effects of substances, and that genotoxicity data should be also considered for the risk assessment.