DNA DAMAGE AND REPAIR EFFICIENCY COMET ASSAY IN INTENSIVELY REARED SEA BREAM (*SPARUS AURATA*), EXPOSED TO HEAVY METALS

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Abstract

The aim of this study was the detection of DNA damage and repair efficiency, in hepatocytes of sea bream (*Sparusaurata*), under copper (Cu^{++}) and zinc (Zn^{++}) experimental exposure. The results illustrated a different cellular behaviour between metals' exposure, implying variant response mechanisms to stress. Moreover, the contribution of repair processes in DNA fragmentation of hepatocytes is discussed.

Keywords: Aquaculture, Ecotoxicology, Metals, Pollution, Trace Elements

Introduction

Zinc and copper are natural elements occurring in aquatic ecosystems. On trace amounts within a narrow optimal concentration range, they are essential to living organisms. However, metal concentrations can become toxic to hydrobionts due to anthropogenic load on hydrosphere. Both zinc and copper are common metal cofactors conducting an important role in a plethora of fundamental biological processes [1] especially in the metabolic pathway. The scope of the present work was to study sublethal metal genotoxicity in gilthead sea bream (*Sparus aurata)* hepatocytes. The selected concentrations were directly comparable to marine pollution levels.

Materials and Methods

Three hundred and twenty specimens of gilthead sea bream, *Sparus aurata*, (70±10g) were provided from a local breeding farm in northern Evoikos Gulf. They were acclimatized for one month at the University facilities in 500L tanks. Different replicates of fish were exposed to 0.1 and 0.5ppm of $CuSO_45H_2O$ and 0.2 and 1.0ppm of $ZnCl_2$ for 24 and 96 hours. Livers were dissected from three specimens per treatment. Hepatocytes' isolation protocol was conducted according to [2]. Cell counting was carried out using a Neubauer haemocytometer and their viability was determined with eosin staining. Cell suspensions were divided into two aliquots, referring for the direct damage assessment (first assay) and the estimation of repair efficiency (second assay). DNA fragmentation was assessed using a slightly modified protocol of the alkaline comet assay [3]. In order to estimate the involvement of repair mechanisms in DNA fragmentation, 1ml of isolated hepatocytes was placed in a 5ml culture medium and was incubated for 2h in room temperature (metal-free treatment). Two microscope slides per specimen and 100 cells per slide were analyzed for both assays. Comet-shape structure was examined using a fluorescent microscope and was scored with the open-source image analysis package CASP [4].DNA damage was evaluated by computing the tail moment (TM) parameter [5]. Kruskal-Wallis tests were adopted for the statistical analysis [6].

Results and Discussion

TM parameters are presented in Fig. 1. Significant DNA fragmentation was detected on specimens exposed to both zinc and copper, compared with the control ones. Incubated hepatocytes in the metal free medium showed higher DNA fragmentation compared to those directly assayed. This significant difference in the TM values denotes an increased repair activity leading to substitution of the damaged DNA segments [7]. DNA fragmentation of specimens exposed to copper for either 24 or 96h revealed a significant increase in the higher concentration in both assays. Regarding the incubation in the metal-free medium, specimens' exposure to low copper concentration was non-significant in comparison with the control ones. The fact that a time response to copper exposure was detected only in the second assay could be explained by a delayed activation of the detoxification mechanisms [8]. On the other hand, specimens exposed to the high zinc concentration presented maximum DNA fragmentation in either 24 or 96h, indicating elevated genotoxicity. Furthermore, a significant time response to zinc exposure, similar to copper exposure, was observed in the metal-free medium incubation. Only this time there was a significant decrease rather than the observed increased pattern in the TM values regarding copper exposure. A possible explanation is that exposure to high zinc concentrations directly affects DNA repair mechanisms. The different assayed cellular behaviour pattern between specimens exposed to copper and zinc could be explained by variant response mechanisms to each metal [8]. Fish exposure to copper seems to instantly activate detoxification mechanisms. Adversely, zinc seems to inhibit their DNA repair activity.

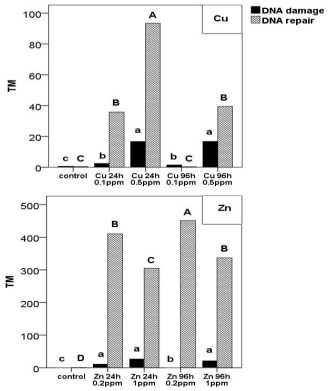

Fig. 1. Median TM values for all treatments. Significancies among bars are noted with different letters (*P<0.05*)

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