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Using the nucleolar biomarker and the micronucleus test on in vivo fish fin cells

V.V. Arkhipchuk*, N.N. Garanko

Laboratory of Biomarkers and Biotesting, Institute of Colloid Chemistry and Water Chemistry, Vernadsky Blvd. 42, Kiev 03142, Ukraine

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Abstract

This study was aimed at developing the nucleolar biomarker and the micronucleus test on in vivo fish fin cells for assessing water cytotoxicity and genotoxicity. Both biomarkers can be used either jointly or separately on fins of the same fish during the experiment. For studying the nucleolar characteristics, small pieces of the fin edge were cut several times during 30−180 min of fish exposure. For micronucleus testing, the fin tissue regenerating after its cutting was investigated after 2–5 days of fish incubation. Effects of copper (0.1 and 2.5 mg/L), cadmium (0.005 and 1.0 mg/L) ions and chloral hydrate (400 and 800 mg/L) solutions were studied on cells of common carp (*Cyprinus carpio* L.), crucian carp (*Carassius auratus gibelio* Bloch.), and Mozambique tilapia (*Tilapia* (*Sautherodon*) *mossambica*) using a set of nucleolar characteristics (the number of nucleoli per cell, the size of a single nucleolus, and the percentage of cells with heteromorphic paired nucleoli) and the frequencies of cells with micronuclei and double nuclei. Substantial changes in parameters of nucleolar activity of fin cells were found to be caused by cadmium and copper impact. In comparison to blood cells, gill and fin cells were more sensitive as demonstrated by their nuclear damages after the chloral hydrate influence. Fin cells were useful to determine periodically cytotoxic and genotoxic effects of organic and inorganic substances in the same individual fish without any disruption of its physiological functions.

Keywords: Chloral hydrate; Cytotoxicity; Fish fins; Genotoxicity; Heavy metals; Micronucleus test; Methods; Nucleolar biomarker

1. Introduction

Fish are frequently used test organisms for studying water toxicity, cytotoxicity, and genotoxicity. For example, at the cellular level the micronucleus test on various fish tissues is among the most widespread assessments of genotoxicity in water (Al-Sabti and Metcalfe, 1995; Hayashi et al., 1998). The micronucleus assay is a measure of subcellular processes such as induced chromosomal breaks (clastogenesis) or cell spindle malfunction (aneugenesis) (Reddy et al., 1995). Micronuclei are used as biomarkers of structural and quantitative chromosome damages, and thus can be applied to any proliferating cell population.

The idea of using the nucleolar biomarker for prompt assessment of water cytotoxicity was also proposed earlier. For instance, the set of nucleolar characteristics was successfully applied to estimation of the radiation exposure of fish embryos and the cytotoxicity of organic and inorganic substances for plants and invertebrates (Arkhipchuk, 1995a; Arkhipchuk et al., 2000a; Arkhipchuk and Garanko, 2002). Moreover, the combination of the nucleolar biomarker and the micronucleus test on cells of the same organism was proposed as an efficient tool for studying water cytotoxicity and genotoxicity (Arkhipchuk et al., 2000a).

The sacrifice of fish is usually required to study cytological effects on erythrocytes and cells of gills, liver, kidney, etc., whereas fin cells could be used for in vivo cytological investigations without causing any damage to the fish organism and its functioning. Results of our

*Corresponding author. Fax: +38 044 423 82 24.
E-mail address: arvic@nbi.com.ua (V.V. Arkhipchuk).

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studies demonstrated the possibility of using the fin edge as the source of cells for experiments involving a periodic sampling, such as when studying diurnal and seasonal rhythmicity, spawning cycle, and some ontogenetic processes (Arkhipchuk, 1999). Micronucleated fin cells were not studied in vivo before due to their low mitotic activity.

Heavy metals, particularly copper and cadmium, were extensively studied in various toxicological investigations (Straus, 2003; Brooks et al., 2004; Chowdhury et al., 2004). Heavy metals are interesting and noteworthy due to their strong impact on aquatic ecosystems and their bioaccumulation in hydrobionts. Cadmium and copper were tested in genotoxicity assays, with sometimes contradictory results (Sanchez-Galan et al., 1999; Ayllon and Garcia-Vazquez, 2000; Conners and Black, 2004). Both metals are also widely employed in studies of various cytotoxic effects (Butler and Roesijadi, 2001; Smith et al., 2001; Manzl et al., 2003).

Chloral hydrate has been widely used as a sedative/hypnotic drug in humans. The recommended dose for an adult as a sedative is 250 mg three times a day and as a hypnotic it is 500–1000 mg (Gilman et al., 1985). Moreover, millions of people are exposed to chloral hydrate on a daily basis because it is formed during the disinfection of drinking water with chlorine. Genotoxic effects of chloral hydrate have been revealed and discussed (Brunner et al., 1991; Wallin and Hartley-Asp, 1993).

The present study was aimed at developing the nucleolar biomarker and the micronucleus test on fish fin cells for in vivo analysis of cytotoxicity and genotoxicity of water samples of some organic and inorganic reference substances.

2. Materials and methods

2.1. Chemicals

Two heavy metals were studied as inorganic substances. Salt $CdCl_2$ (CAS No. 10108-64-2; produced by Reachim, Russia) was dissolved to yield Cd^{2+} ion concentrations of 0.005 and 1.0 mg/L, and salt $CuSO_4 \cdot 5H_2O$ (CAS No. 7758-99-8; produced by Reachim) was used to obtain Cu^{2+} ion concentrations of 0.1 and 2.5 mg/L in the solutions studied. The concentrations were selected based on results of preliminary assessments of toxicity of the metals by biotesting with the use of the WaterTox set of animal and plant bioassays (Arkhipchuk et al., 2000b). Cadmium and copper concentrations exhibiting both weak (LOEC or $IC_{20} = 0.005$ and 0.1 mg/L, respectively) and strong (EC/LC_{50} or $EC/LC_{100} = 1.0$ and 2.5 mg/L, respectively) toxic effects at the organismic level were studied.

The aqueous solution of chloral hydrate $CCl_3CH(OH)_2$ (CAS No. 302-17-0; produced by Merck, Germany) was studied in experiments as an organic substance. Concentrations analyzed (400 and 800 mg/L) were selected based on literature data and on original preliminary investigations on animal and plant cells, where these concentrations produced some quantity of micronuclei, on the one hand, and did not inhibit completely the mitotic cell activity, on the other hand.

2.2. Fish

All fish used in experiments with the nucleolar biomarker were constantly cultivated under artificial, controlled conditions ($20 \pm 2\,^{\circ}C$, a 16-h daylight cycle) in indoor pools of 400 L of well-aerated fresh water. We studied yearlings of two tetraploid fish species of the order Cypriniformes, common carp (*Cyprinus carpio* L.) and crucian carp (*Carassius auratus gibelio* Bloch.), and yearlings of one diploid species of the order Perciformes, Mozambique tilapia (*Tilapia* (*Sautherodon*) *mossambica*). In experiments with the micronucleus test, we used a group of yearling crucian carps (7–9 cm long) taken from an environmental water body (Kiev Region, Ukraine) and then cultivated for 8 months under the same laboratory conditions in 100-L aquariums. Common and crucian carps are typical representatives of the ichthyofauna of environmental waters of Ukraine, and tilapia is widely used in aquaculture.

2.3. Experimental design

For analysis of toxic effects, three specimens of each species were placed into separate 5-L aquariums containing the solutions studied. The exposure times were within 30–180 min when nucleolar characteristics were studied and within 2–5 days when the micronucleus test was applied on fish fin cells (at a temperature of $20 \pm 2\,^{\circ}C$, under a 16:8-h light–dark photoperiod). Epithelial cells were sampled for the nucleolar analysis from the edge of caudal fins, as indicated in Fig. 1A (small pieces of several square millimeters per each sampling). Fins have never been used earlier as in vivo source material for micronucleus testing, because the tissue does not demonstrate sufficient mitotic activity, considering that the micronucleus test registers consequences of chromosomal aberrations occurring during cell mitosis. For solving the problem (i.e., stimulation of cell division and, as a result, generating a sufficient number of mitotic cells for the micronucleus test) the fin tissue is damaged at the beginning of the experiment. For the three fish species used the fin edge was cut at a depth of 2–3 mm (this depends on the fish species, particularly their body and fin sizes). The tissue regeneration proceeds in the solution analyzed, so the formation of novel cells occurs under the studied impact. The newly regenerating tissue is used for further cytological analysis.