Chemosphere 191 (2018) 118–127

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

17β-Estradiol induces cyto-genotoxicity on blood cells of common carp (*Cyprinus carpio*)

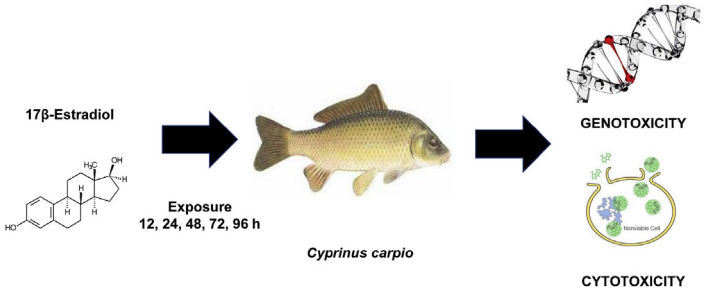
Luis Orozco-Hernández [a], Adriana Andrea Gutiérrez-Gómez [a], Nely SanJuan-Reyes [a], Hariz Islas-Flores [a], Sandra García-Medina [b], Marcela Galar-Martínez [b], Octavio Dublán-García [a], Reyna Natividad [c], Leobardo Manuel Gómez-Oliván [a, *]

[a] *Laboratorio de Toxicología Ambiental, Facultad de Química, Universidad Autónoma del Estado de México, Paseo Colón Intersección Paseo Tollocan s/n. Col. Residencial Colón, 50120, Toluca, Estado de México, Mexico*
[b] *Laboratorio de Toxicología Acuática, Departamento de Farmacia, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Unidad Profesional Adolfo López Mateos, Av. Wilfrido Massieu S/n y Cerrada de Manuel Stampa, Col. Industrial Vallejo, C.P. 007700, Ciudad de México, Mexico*
[c] *Chemical Engineering Lab., Centro Conjunto de Investigación en Química Sustentable UAEM-UNAM, Carretera Toluca-Atlacomulco Km 14.5, Unidad San Cayetano, Toluca, Estado de México, 50200, Mexico*

HIGHLIGHTS

- This study aimed to evaluate 17β-estradiol-induced cyto-genotoxicity in *Cyprinus carpio*.
- Increases in frequency of micronuclei, caspase-3 activity and TUNEL-positive cells were observed.
- The comet assay detected significant increases at 24 and 96 h with the 1 μg and 1 ng L^{-1} of E2.
- 17β-Estradiol induces cyto-genotoxicity on *C. carpio*.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 30 July 2017
Received in revised form
18 September 2017
Accepted 5 October 2017
Available online 6 October 2017

Handling Editor: Jim Lazorchak

Keywords:
17 β-Estradiol
Cyprinus carpio
Comet assay
Micronucleus test
TUNEL
Caspase-3

ABSTRACT

17β-Estradiol, a natural hormone present at high concentrations in aquatic ecosystems, affects and modifies endocrine function in animals. In recent years research workers have expressed concern over its potential effects on aquatic organisms; however, little is known about its capacity to induce genetic damage or the pro-apoptotic effects of such damage on fish. Therefore, this study aimed to evaluate 17β-estradiol-induced cyto-genotoxicity in blood cells of the common carp *Cyprinus carpio* exposed to different concentrations (1 ng, 1 μg and 1 mg L^{-1}). Peripheral blood samples were collected and evaluated by comet assay, micronucleus test, determination of caspase-3 activity and TUNEL assay at 12, 24, 48, 72 and 96 h of exposure. Increases in frequency of micronuclei, TUNEL-positive cells and caspase-3 activity were observed, particularly at the highest concentration. In contrast, the comet assay detected significant increases at 24 and 96 h with the 1 μg and 1 ng L^{-1} concentrations respectively. The set of assays used in the present study constitutes a reliable early warning biomarker for evaluating the toxicity induced by this type of emerging contaminants on aquatic species.

* Corresponding author. Laboratorio de Toxicología Ambiental, Facultad de Química, Universidad Autónoma del Estado de México, Paseo Colón intersección Paseo Tollocan s/n. Col. Residencial Colón, Toluca, Estado de México, CP 50120, Mexico.
E-mail addresses: lmgomezo@uaemex.mx, lgolivan74@gmail.com (L.M. Gómez-Oliván).

https://doi.org/10.1016/j.chemosphere.2017.10.030
0045-6535/

1. Introduction

In recent years, water contamination has become a serious problem for public health and aquatic ecosystems due to domestic and industrial wastewater discharges which contain a large variety of environmental contaminants such as personal hygiene products, pharmaceuticals, and natural and synthetic hormones (Tetreault et al., 2011; Fang et al., 2012), all these are part of "emerging contaminants", a group of compounds which do not have a clear regulation to date regarding their handling and discharge into ecosystems (Kümmerer, 2001; Petrie et al., 2015; Richardson and Kimura, 2016). These substances are biologically active and although some are not considered persistent, they are introduced constantly, so they can represent a risk to ecosystem (Galar-Martínez et al., 2015). In particular, concern has been expressed over the presence of steroid hormones (estrogens, progestagens and androgens), because it has been reported that members of this group can affect and modify endocrine function in animals, and are therefore known as endocrine disrupting compounds (EDCs) (Matozzo and Marin, 2008; Bolong et al., 2009; Ma et al., 2016). These chemicals can include natural estrogens (e.g., 17β-estradiol), synthetic estrogens (e.g., 17α-ethinylestradiol), and estrogen mimics (e.g., bisphenol A, 4-nonylphenol) (Sackett et al., 2015).

Estrogens are used in hormone replacement therapy, most often to overcome the basic symptoms associated with menopause; however, their use has made evident diverse complications, including a higher risk of developing breast, endometrial and ovarian cancer (El Habachi et al., 2014). One of the most extensively studied estrogens in mammals, and probably the most common one, is 17β-estradiol, often abbreviated E2. It has two hydroxyl groups in its molecular structure and is a natural element formed in the body. Of the three forms of estrogen produced naturally by all vertebrates, E2 is the most potent one. It is the main estrogen secreted by the ovaries and is oxidized to estriol before being eliminated. E2 also undergoes reactions such as hydroxylation, reduction, oxidation by methylation, and conjugation by liver cytochrome P450 enzymes for subsequent elimination in urine. There are no reports of E2 conjugates released into the environment having direct biological activity, but they may be a storehouse of hormone precursors since they are transformed to E2 by bacterial action (Baronti et al., 2000; Yan et al., 2013a; Heffron et al., 2016).

EDCs enter the environment, through a variety of pathways such as municipal sewage, industrial wastewaters, landfill, agricultural run-off, wash-off from roadways and underground contamination. Detection of these compounds in water is at trace levels (μg L^{-1} or even ng L^{-1}) (Bolong et al., 2009), because standard methods of sewage treatment are not completely effective in removing such substances (Zhang et al., 2007). E2 is among the most potent EDC having the potential to exert effects at extremely low concentrations (Bowman et al., 2002). In Mexico, there is only one study that reports the presence of E2 at concentrations from 0.11 ng L^{-1} to 1.72 ng L^{-1} in Xochimilco wetland (Díaz-Torres et al., 2013). Studies of aquatic wildlife that are directly exposed to contaminants, often provide early indications of potential environmental problems. A few studies report E2 can produce different harmful effects, for example, is an endocrine disrupter at concentrations above 200 ng L^{-1} in *O. mykiss* (Marlatt et al., 2006), affect the reproduction of *O. javanicus* at concentrations greater than 16 ng L^{-1} (Imai et al., 2005), can decrease the capacity of cellular antioxidant systems in *P. aibuhitensis* at concentrations greater than 0.1 μg L^{-1} (Lv et al., 2016) and can induce immunomodulatory changes and neurotoxicity in different organisms (Thilagam et al., 2014). On the other hand, *in vitro* assays have shown that at low concentrations (1–10 ng L^{-1}) of E2 induce feminization in some species of male wild fishes (Routledge et al., 1998), and changes the hepatic gene expression of the vitellogenins and choriogenins in *P. flesus* (Williams et al., 2007). Other possible endocrine-disruption effects have been observed in fish, such as reduced ovary size, lower egg viability, and delayed sexual maturity (Munkttrick et al., 1992; Hontela et al., 1995). However, there are no reports of the cytotoxic effect of exposure to E2 on aquatic organisms, which is one of the objectives of this study.

Genotoxicity is toxicity against cell's genetic material affecting its integrity (DNA damage) and may lead to mutagenicity and carcinogenicity in some circumstances (Nesslany and Benameur, 2014). The genotoxic effects of physical and chemical agents/pollutants can be monitored using a broad range of both *in vivo* and *in vitro* biomarker assays (Kumar et al., 2017). A biomarker is a cellular, molecular, genetic or physiologic response altered in an organism or population in response to a chemical stressor (Costa et al., 2010). In a previous study it was reported that E2-induced oxidative damage (Gutiérrez-Gómez et al., 2016) which could be associated with genotoxicity (Maria et al., 2008). From an ecological viewpoint, genetic damage may lead to hereditary mutations compromising the physical condition integrity of fish populations and their capacity to confront stress (Brown et al., 2009). Also, damage to the DNA of aquatic animals has been associated with reduced growth, abnormal developed and reduced survival of embryos, larvae and adults (Lee and Steinert, 2003; Reinardy et al., 2013). Analysis of DNA changes in aquatic organisms has proved an effective method for evaluating water body contamination, even when compounds are present at very low concentrations, as in the case of E2 (Frenzilli et al., 2009). Among the genotoxicity tests, comet assay and micronucleus (MNi) test are recognized due to their robustness and sensitivity to evaluate DNA damage. The MNi test measures a small subset of unrepaired DNA strand breaks, whereas the comet assay measures strand breaks and labile sites that may be removed subsequently by the DNA repair system, both studies complement each other and their methodology is easy and does not require sophisticated equipment (Końca et al., 2003; Heuser et al., 2008). On the other hand, certain genotoxic agents also induce cytotoxicity (Selvi et al., 2013), defined as the pre-lethal changes and events which occur in cells prior to necrosis (Vasquez, 2012). Lesions to DNA and inefficient repair mechanisms are crucial in the un-leashing of apoptosis (Roos and Kaina, 2006), which is a fundamental biochemical pathway of cellular death characterized by diverse morphologic changes and has a major role in the maintenance of tissue homeostasis (Simoes et al., 2013). There are two main pathways of apoptosis: extrinsic and intrinsic. The extrinsic pathway is produced in response to activation of death receptors on the cell surface, while the intrinsic pathway arises in response to extracellular signaling and internal lesions such as DNA damage (Fadeel and Orrenius, 2005). From a mechanistic perspective, apoptosis may be expected to contribute to the elimination of cells with some kind of damage, particularly that of premutagenic/mutagenic lesions (Decordier et al., 2002). An assay that relies on detection of DNA strand breaks *in situ* by labeling them with fluorochromes has been developed to identify and quantify apoptotic cells by fluorescence microscopy. The assay is commonly called TUNEL, the acronym of Terminal deoxynucleotidyl transferase-mediated d-UTP Nick End Labeling (Darzynkiewicz et al., 2008). TUNEL assay is certainly one of the more simple, reliable, objective, and cost-effective methods available for assessing DNA damage (Sharma et al., 2013).

The common carp *Cyprinus carpio*, a freshwater fish, has been introduced in Mexico in 80% of freshwater bodies and has become an economically and ecologically important specie (Galar-Martínez et al., 2015), and has been extensively used to evaluate the cytotoxicity and genotoxicity of substances in the aquatic environment (García-Nieto et al., 2014) and is therefore a good bioindicator for