Science of the Total Environment 650 (2019) 2284–2293

Contents lists available at ScienceDirect

Science of the Total Environment

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

Anti-cancer drugs in aquatic environment can cause cancer: Insight about mutagenicity in tadpoles

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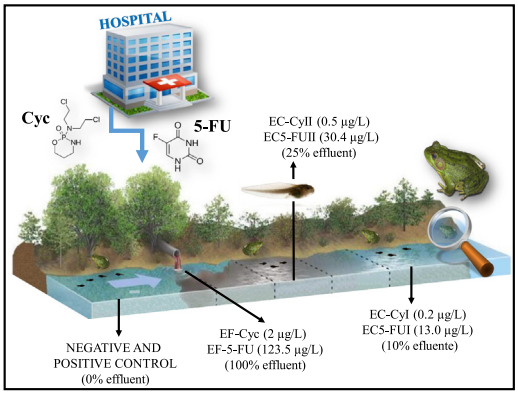
HIGHLIGHTS

- Toxicity of antineoplastic drugs in tadpoles
- Cyclophosphamide (Cyc) and 5-fluorouracil (5-FU) induced morphological changes in tadpole.
- Cyc and 5-FU cause mutagenic effects on *L. catesbeianus* tadpoles.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 13 May 2018
Received in revised form 30 September 2018
Accepted 30 September 2018
Available online 1 October 2018

Editor: Henner Hollert

Keywords:
Amphibian
Mutagenicity
Hospital effluent
Antineoplastic drug

ABSTRACT

Cyclophosphamide (Cyc) and 5-fluorouracil (5-FU) are two of the most used antineoplastic drugs (AD) in the world. However, their discharge in the environment became a yet-unknown environmental issue that has impact on some groups of animals, such as amphibians. We assessed tadpoles (*Lithobates catesbeianus*) exposed to environmental concentrations (EC) of Cyc and 5-FU to evaluate whether they can cause morphological and mutagenic changes in them. We defined the following groups: control, positive control (50 mg/L of Cyc), EC-Cyc-I (0.2 µg/L), EC-Cyc-II (0.5 µg/L), EF-Cyc (2.0 µg/L), EC-5-FU-I (13.0 µg/L), EC-5-FU-II (30.4 µg/L) and EF-5-FU (123.5 µg/L). EC groups presented predictive AD concentrations in 10% and 25% hospital-effluent dilutions in water. EF groups met gross hospital-effluent concentrations. Based on our data, ADs caused intestinal changes and influenced the interocular distance in tadpoles after 30-day exposure. We also observed the aneugenic and clastogenic effect of ADs due to the higher frequency of micronucleated and binucleated erythrocytes, and blebbed, multilobulated, notched and kidney-shaped nuclei in animals exposed to them. Based on such changes, we assume that Cyc and 5-FU can trigger malignant cell transformation processes, and cancer, in animals exposed to them, even at low concentrations. Our study is the first to describe that Cyc and 5-FU, spread in the environment, cause damages in non-target organisms opposite to their original end.

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https://doi.org/10.1016/j.scitotenv.2018.09.373
0048-9697/

1. Introduction

The agricultural revolution from the mid twentieth century (Gengenbach et al., 2018; Kansaga et al., 2018) boosted the agricultural production thanks to techniques such as culture rotation, seed diversification and balanced areas for livestock. However, we cannot forget that such increased agricultural activity led to declined biodiversity in many locations (Lanz et al., 2018; Kok et al., 2018; Decaëns et al., 2018) due to substantial landscape changes: habitat fragmentation, deforestation, changes in the quality of soils and water, among others.

These changes have stronger impact on groups of animals showing high dependence on specific ecosystems and low dispersion ability, such as amphibians. These animals also depend on aquatic and terrestrial environments, so they are vulnerable to changes in ecosystems (Becker et al., 2010; Bovo et al., 2018; Ribeiro-Jr et al., 2018). Habitat losses and changes mostly caused by continuous updates in agricultural practices and by broader land use have been important agents for amphibian population decline in many regions around the globe (Turvey et al., 2018; Pyron, 2018).

Although fertilizers and pesticides are directly and indirectly responsible for the smaller number of amphibians in the environment, nowadays we find a number of new pollutants capable of intensifying their biodiversity losses. These contaminants include emerging chemicals such as pharmaceutical products, endocrine disruptors, hormones, toxins, among others; as well as biological micropollutants (bacteria and viruses) in the soil, sediments, groundwater, industrial and municipal wastewater, aquaculture effluents, freshwater and marine ecosystems (Gavrilescu et al., 2015; Ribeiro et al., 2016; Poi et al., 2018). According to Sanchez and Egea (2018), many of these substances are discharged in the environment and cause widespread contamination in the soil and in water.

Medications stand out among emerging pollutants due to their ability to interact with non-target organisms and to cause unexpected issues (Rivera-Utrilla et al., 2013; López-Ortiz et al., 2018; Wilkinson et al., 2018; Gomes et al., 2018). Antineoplastic drugs (ADs) have been widely used in human treatments (Barata et al., 2018), and cyclophosphamide (Cyc) and 5-fluorouracil (5-FU) stand out among them, due to their broad use by this population (Besse et al., 2012; Jamieson et al., 2014). Both drugs act in cancer cells by incorporating themselves to the DNA and RNA. They inhibit the activity of the thymidylate synthase enzyme and alkalinize the DNA. Such process leads to damages and to eventual cell death (Longley et al., 2003; Perini et al., 2008). These agents also change the metabolism and morphology of cells, and it leads them to death (Besse et al., 2012). They have the potential to perform mutagenic, carcinogenic and teratogenesis (Kümmerer et al., 2016), so that their presence in aquatic media cannot be ignored.

Accordingly, previous investigations have reported the teratogenic effects of the exposure to ADs, such as 5-FU, capecitabine, cisplatin, doxorubicin, etoposide and imatinib and their ability to inhibit the growth of *Xenopus laevis* embryos, the reproduction of *Daphnia magna* and *Ceriodaphnia dubia* (Parrella et al., 2014) and to change the mitotic indices in *Allium cepa* (Mišík et al., 2014). Therefore, the biological responses to such drugs can vary in different organisms and classes of vertebrates. Nothing is known about the effects from the exposure to these medications at environmentally relevant concentrations. The questions are: What would be the impacts of these emerging pollutants on amphibian populations? Can these pollutants, even at low concentrations, reinforce the decline of these animals?

Thus, the aim of the current study was to assess the mutagenic effect of Cyc and 5-FU on *Lithobates catesbeianus* tadpoles. Our hypothesis is that ADs, event at low, but environmentally relevant, concentrations, increase the frequency of micronuclei and of other nuclear abnormalities in circulating erythrocytes, besides the physical-morphological damages caused by them. Studies similar to ours are a milestone in the adoption of mitigating and remediating measures and contribute to broaden the knowledge about the impact of emerging pollutants on amphibian populations.

2. Material and methods

2.1. Animals and experimental design

The sample comprised 120 *Lithobates catesbeianus* tadpoles at the same development stage (25G). The specimens were purchased in a commercial nursery (Gameleira de Goiás, GO, Brazil). The species known as American bullfrog is distributed in natural environments in several countries. It lives in water bodies, feeds on the resources available in them and records high reproduction rates (Lowe et al., 2004). *L. catesbeianus* was chosen as experimental model, because its representatives were successfully used in studies about the environmental toxicology of several xenobiotics (Veronez et al., 2016; Rissoli et al., 2016; Montalvão et al., 2017; Montalvão and Malafaia, 2017; Amaral et al., 2018).

The experiment was conducted in laboratory at controlled temperature and light conditions (24 ± 2 °C; 12 h light/12 h dark, respectively). The models were allowed to acclimate to the laboratory conditions for 96 h and distributed in different experimental groups ($n = 15$, in each group) after their body biomass was counterbalanced (Table 1).

The animals were exposed to the treatments for 30 days, approximately 33.3 (<40%) of the exclusively aquatic life of the species. The complete metamorphosis of *L. catesbeianus* lasts from 75 to 90 days, depending on weather conditions and on other factors (Cribb et al., 2013). The adopted exposure time was ecologically relevant based on the exclusively aquatic lifetime of these animals.

The tadpoles were stored in 15-L polyethylene boxes (dimensions: 18 cm height × 34 cm width × 41 cm length). Each box had 13 L of dechlorinated water. No substrates were placed at the bottom of the boxes. Compressors were used to keep the water constantly oxygenated throughout the experimental period. Water in the boxes was 100% renewed every four days. Tadpoles fed twice a day on commercial feed for carnivorous fish. The feed recorded the following nutrient levels: 45% (minimum) crude protein (CP), 14% (minimum) ethereal

Table 1
Experimental group distributions based on exposure type.

Groups	Specifications
1. Control (C)	Boxes containing water with dechlorinated
2. Positive control (PC)	Water with 40 mg/L of cyclophosphamide – which is well-known by its mutagenic effect – (Genuxal®, Baxter Healthcare S/A, Sao Paulo, Brazil).
3. Cyclophosphamide (Cyc) at environmental concentration I (EC-Cyc-I)	Water with Cyc at hospital-effluent concentration, based on Steger-Hartmann et al. (1997) (2.1 μg/L), diluted in approximately 10% - which corresponds to 0.2 μg/L of it.
4. Cyc at environmental concentration II (EC-Cyc-II)	with Cyc at hospital-effluent concentration, based on Steger-Hartmann et al. (1997) (2.1 μg/L), diluted in approximately 25% - which corresponds to 0.5 μg/L of it.
5. Cyc effluent (EF-Cyc)	Water with 100% concentration of Cyc (2.1 μg/L) – overestimated hospital-effluent.
6. 5-Fluoruracila (5-FU) at environmental concentration I (EC-5-FU-I)	Water with 5-FU at hospital-effluent concentrations, based on Mahnik et al. (2007) (123.5 μg/L), diluted in approximately 10% - which corresponds to approximately 13.0 μg/L.
7. 5-FU at environmental concentration II (EC-5-FU-II)	Water with 5-FU at hospital-effluent concentrations, based on Mahnik et al. (2007) (123.5 μg/L), diluted in approximately 25% - which corresponds to approximately 30.4 μg/L.
8. 5-FU effluent (EF-5-FU)	Water with 100% of 5-FU (123.5 μg/L) – overestimated hospital-effluent.