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Acute and chronic toxicity of six anticancer drugs on rotifers and crustaceans

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HIGHLIGHTS

- Acute and chronic toxicity of cytostatics on rotifers and crustaceans were assessed.
- Cisplatin and 5-fluorouracil had the highest chronic toxicity on all test organisms.
- This study contributes to cytostatic environmental risk evaluation.

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ABSTRACT

The growing use of cytostatic drugs is gaining relevance as an environmental concern. Environmental and distribution studies are increasing due to the development of accurate analytical methods, whereas ecotoxicological studies are still lacking. The aim of the present study was to investigate the acute and chronic toxicity of six cytostatics (5-fluorouracil, capecitabine, cisplatin, doxorubicin, etoposide, and imatinib) belonging to five classes of Anatomical Therapeutic Classification (ATC) on primary consumers of the aquatic chain (*Daphnia magna*, *Ceriodaphnia dubia*, *Brachionus calyciflorus,* and *Thamnocephalus platyurus*). Acute ecotoxicological effects occurred at concentrations in the order of mg L^{-1}, higher than those predicted in the environment, and the most acutely toxic drugs among those tested were cisplatin and doxorubicin for most aquatic organisms. For chronic toxicity, cisplatin and 5-fluorouracil showed the highest toxic potential in all test organisms, inducing 50% reproduction inhibition in crustaceans at concentrations on the order of μg L^{-1}. Rotifers were less susceptible to these pharmaceuticals. On the basis of chronic results, the low effective concentrations suggest a potential environmental risk of cytostatics. Thus, this study could be an important starting point for establishing the real environmental impact of these substances.

1. Introduction

For years the scientific community has been studying the presence and effects of pharmaceuticals in the aquatic environment, but only in the last few years, the focus of scientific concern about anticancer drugs has been growing due to their ever-increasing use (Johnson et al., 2008). Chemotherapy is growing because the incidence rates of some cancers are increasing (US National Institute of Health, www.cancer.gov) and higher doses of antineoplastic agents are being prescribed for the enhanced ability to control their side effects (Suhail et al., 2012). Additionally, treatment is moving towards the administration of a combination of more drugs (Shi et al., 2012). Furthermore, chemotherapy has been changing from in-patient to out-patient cancer treatment (Lenz et al., 2007), with higher environmental concern over the presence of cytostatics not only in hospital effluents, but also in municipal wastewater treatment plant effluents at concentrations from ng to μg L^{-1} as shown in Table 1. Anticancer drugs can be excreted as parent compounds or as one or more metabolites and, once in the water, they can undergo biotic and/or abiotic transformations into different compounds that can be more persistent and more toxic than the parent compounds (Mompelat et al., 2009).

The concern is that cytostatic drugs interfere with the structure and functions of DNA and affect not only target cells, but also non-tumoral cells. Generally, these drugs are present at low concentrations in the environment, concentrations below those of other pharmaceutical classes. However, each living organism may potentially be affected by their peculiar molecular mode of action and by the fact that they are expected to exert effects at very low concentrations. The development of accurate analytical methods has allowed the detection of the most abundant anticancer agents in aquatic systems, such as 5-fluorouracil (5-FU), ifosfamide, and cyclophosphamide (Kovalova et al., 2009; Kosjek et al., 2013;

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Table 1
Occurrence and predicted environmental concentration, refined by excretion rates, of cytostatic pharmaceuticals in aquatic systems.

Cytostatic	Matrix	Concentration detected	Refined PEC	Ref.
5-FU	Hospital effluent	<5.0–27 ng L^{-1}	–	Kovalova et al. (2009)
	Hospital effluent	20–122 μg L^{-1}	–	Mahnik et al. (2004)
	Hospital effluent	<8.6–124 μg L^{-1}	–	Mahnik et al. (2007)
	Hospital wastewater	–	2.03 μg L^{-1}	Hartmann et al. (1998)
	Municipal wastewater	–	<23 ng L^{-1}	Tauxe-Wuersch et al. (2006)
	Surface water	–	2.65 ng L-1	Straub (2009)
	Wastewater influent	–	44.8 ng L^{-1}	Straub (2009)
	Surface water	–	7.91 ng L^{-1}	Besse et al., 2012
	Hospital wastewater	35–92 ng L^{-1}	–	Kosjek et al. (2013)
	Municipal wastewater	4.7–14 ng L^{-1}	–	Kosjek et al. (2013)
CAP	Surface water	–	3.52 ng L-1	Besse et al. (2012)
	Wastewater influent	8.2–27 ng L^{-1}	–	Negreira et al. (2013)
CisPt as Pt compound	Hospital influent	3–250 μg L^{-1}	–	Lenz et al. (2007)
	Hospital effluent	2–150 μg L^{-1}	–	Lenz et al. (2007)
DOX	Hospital effluent	0.1–0.5 μg L^{-1}	–	Mahnik et al. (2006)
	Hospital effluent	<10 ng L^{-1}	–	Yin et al. (2010)
	Hospital effluent	<0.26–1.35 μg L-1	–	Mahnik et al. (2007)
	Surface water	–	0.19 ng L^{-1}	Besse et al. (2012)
	Wastewater influent	4.5 ng L^{-1}	–	Martin et al. (2011)
ET	Hospital effluent	6–380 ng L^{-1}	–	Yin et al. (2010)
	Hospital effluent	110–600 ng L^{-1}	–	Catastini et al. (2008)
	Surface water	–	0.87 ng L^{-1}	Besse et al. (2012)
	Wastewater effluent	3.4 ng L^{-1}	–	Martin et al. (2011)
	Wastewater influent	15 ng L^{-1}	–	Martin et al. (2011)
IM	Surface water	–	4.99 ng L^{-1}	Besse et al. (2012)

5-Fluorouracil (5- FU), capecitabine (CAP), cisplatin (CisPt), doxorubicin (DOX), etoposide (ET) and imatinib (IM).

Negreira et al., 2013), those occurring at lower concentrations, such as doxorubicin (DOX), vincristine, and etoposide (ET), and new compounds, such as imatinib mesylate (IM), temozolomide, and capecitabine (CAP) (Besse et al., 2012; Negreira et al., 2013).

Though the number of studies on the detection of anticancer agents in the environment is increasing, studies on the ecotoxicological effects of these compounds and the associated risk to human health due to their presence in the aquatic environment are lacking (Xie, 2012). Therefore, the aim of the present study was to investigate the toxicity of six cytostatics belonging to the five classes of the World Health Organization (WHO) Anatomical Therapeutic Classification (ATC) scheme, on different organisms in the aquatic chain.

5-FU and CAP are pyrimidine analogues characterized as antimetabolites. This class of drugs inhibits DNA polymerase and induces cell cycle arrest and apoptosis. CAP is the pro-drug of fluorouracil and rapidly metabolizes to the active 5-FU (Straub, 2009). Cisplatin (CisPt) is an inorganic platinum agent belonging to the class of platinum-derived drugs. These platinum compounds form highly reactive platinum complexes that bind to nucleophilic groups in DNA, inducing DNA cross-links and DNA-protein cross-links, resulting in apoptosis and inhibition of cell growth (Kartalou and Essigmann, 2001). CisPt has been classified by the International Agency for Research on Cancer (IARC) as a presumable carcinogen in humans (group 2A), whereas ET, a topoisomerase II inhibitor belonging to the class of mitotic inhibitors, has already been classified by IARC as a carcinogen in humans (group 1). DOX is characterized as a cytotoxic antibiotic (anthracycline class). Anthracyclines interact with DNA, intercalating between two base pairs to block DNA replication and prevent DNA relegation by stabilizing topoisomerase II (Xie, 2012). Other mechanisms of action are controversial despite the drugs' extensive clinical utilization (Minotti et al., 2004). Imatinib mesylate is a selective tyrosine kinase inhibitor belonging to the new class of kinase inhibitors. Tyrosine kinases play a critical role in the modulation of growth factor signalling. Activated forms of these enzymes can cause increased tumor cell proliferation and growth, induce anti-apoptotic effects, and promote angiogenesis and metastasis (Blume-Jensen and Hunter, 2001).

In order to evaluate the potential ecotoxicological effects of the six cytostatics described above, acute and chronic toxicity assays were carried out on primary consumers of the freshwater aquatic chain. Our results could be utilized for the evaluation of the potential environmental risk from these compounds as only limited data currently exists. The stability of compounds in stock solutions and test solutions was also investigated in order to establish possible differences between nominal and actual concentrations.

2. Materials and methods

2.1. Test compounds

5-FU (CAS: 51-21-8), CisPt (CAS: 15663-27-1), ET (CAS: 33419-42-0), and DOX (CAS: 25316-40-9) were supplied by Sigma–Aldrich (Milano, Italy). CAP (CAS: 154361-50-9) and IM (CAS: 220127-57-1) were supplied by Santa Cruz Biotechnology (Santa Cruz, CA, USA).

2.2. Chemical analysis

For 5-FU, CAP, ET, and IM, analytical HPLC was carried out using a Varian 1200 Series HPLC system equipped with a Varian 1200 G1311A quaternary pump, Varian 1200 G1329A auto sampler, and Varian 1200 G1314B UV–Vis detector. Chromatographic analyses were performed on a LUNA RP-18 column (5 μm, 250 × 4.6 mm i.d., Phenomenex) assembled with a pre-column SecurityGuard™ HPLC system consisting of a Max RP guard cartridge (4.0 × 2.0 mm, Phenomenex). An aliquot of each sample (1 mL) was transferred into a HPLC vial and 20 μL injected. Chromatograms were integrated using Agilent Chemstation software (A6.03.05).

5-FU, CAP, ET, and IM were identified by comparing retention times with authentic standards and quantified using standard calibration curves. A total of 20 μL of each standard dilution in 1:1 methanol:acetonitrile was used to prepare 5-point calibration curves, which were linear in the analytical ranges (0.1–1000 mg L^{-1} for 5-FU, 1–1000 mg L^{-1} for CAP, 10–1000 mg L^{-1} for ET and IM).