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Incidence of anticancer drugs in an aquatic urban system: From hospital effluents through urban wastewater to natural environment

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ABSTRACT

The presence of 10 anticancer drugs was studied along the entire urban water cycle -from hospital effluents through urban wastewater treatment plant till surface waters- and their potential environmental risk was assessed. Azathioprine, etoposide, docetaxel, paclitaxel, methotrexate, cyclophosphamide, tamoxifen and ciprofloxacin were detected in hospital effluent and in the urban influent of the sewage treatment plant although most of them were totally eliminated after WWTP. Only cyclophosphamide, tamoxifen and ciprofloxacin were found in both WWTP effluent and in the receiving river at a concentration range between nd-20 ng L^{-1}, 25–38 ng L^{-1} and 7–103 ng L^{-1} respectively. Tamoxifen and ciprofloxacin, commonly used for veterinary practices, were also detected in the river upstream the sewage discharge. In addition, they both were considered to pose a potential risk to the environment based on the levels found in the WWTP effluent together with their ecotoxicological impact in selected organisms.

1. Introduction

Cancer is one of the most concerning diseases in western countries and quite a lot of resources are devoted to investigate its treatment and eventually its cure. Despite the chemotherapeutic treatments have been improved in the last decades and are now more effective and patient specific, cancer is still one of the most harmful diseases worldwide. World Health Organization (WHO) has recently published a world health report where cancer is ranked as the second cause of death (21%) after cardiovascular illness (48%) and followed by respiratory diseases (12%) in the sector of non-communicable diseases; namely diseases caused by non-infectious and non-transmissible medical conditions (www.who.int). American Cancer Society has foreseen a total of 1.638.910 of new cases and 577.190 deaths in United States of America in 2012 whereas annual cancer mortality is expected to decrease only a 1% (Avendaño-López, 2012). Since the cancer incidence is increasing in the so-called “modern societies”, the consumption of anticancer drugs has consequently augmented in the last years.

Anticancer drugs have been shown to have potent cytotoxic, genotoxic, mutagenic, carcinogenic, endocrine disruptor and/or teratogenic effects in several organisms, since they have been designed to disrupt or prevent cellular proliferation, usually by interfering in DNA synthesis. Some ecotoxicological studies with anticancer drugs, on the other hand, such as in the case of for 5-Fluorouracil, have shown that the lowest observed-effect concentration (LOEC) in alga and bacterial assays (10 μg L^{-1}) was close to the concentration found in sewage effluents (Zounkova et al., 2007). In another example, LOEC obtained for Tamoxifen in freshwater fish was 5.6 μg L^{-1} being this concentration slightly higher than those found in wastewaters till the moment (Williams et al., 2007). Recent studies have revealed that mixtures of anticancer drugs in real samples possess an important toxicological effect comparing with the individual drug (Mater et al., 2014).

Although anticancer drugs seem to be equally released via hospital or domestic wastewater in previous studies (Ferrando-Climent et al., 2013), other authors have found that hospitals are, in general, the main source of some pharmaceuticals (Verlicchi et al., 2010, 2012). In any case, anticancer drugs have shown to be recalcitrant in wastewater: they are not removed by conventional wastewater treatments, and also have proven to be a challenge for the non-conventional technologies of water decontamination (Zhang et al., 2013). Therefore, there is a high probability that

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anticancer drugs reach the environment and their occurrence in wastewater, surface water and potential presence in drinking water is cause of concern (Kümmerer et al., 1997; Johnson et al., 2008; Liu et al., 2010; Booker et al., 2014).

Chemotherapy drugs are thus considered a group of emerging pollutants, which could be impacting the aquatic life in WWTP effluents receiving waters. Most of the studies till date report relatively high levels of these compounds in urban wastewaters (up to 146 ng L^{-1} for Cyclophosphamide and up to 42 ng L^{-1} for Tamoxifen) and some others have also found them (i.e. Tamoxifen) in natural waters up to 200 ng L^{-1}(Roberts and Thomas, 2006; Coetsier et al., 2009; Kosjek and Heath, 2011; Martín et al., 2011; Yin et al., 2010; Ferrando-Climent et al., 2013).

In this work, the occurrence of anticancer drugs through the entire urban sewage system was performed to clearly assess their fate in sewage system as well as their input and potential risk onto the aquatic environment Ten selected anticancer drugs were measured during a sampling campaign in the effluent of the main hospital of Girona (north-east of Spain), in the influent and effluent of Girona wastewater treatment plant (WWTP) (which receives hospital loads) and also in the river Ter where the WWTP discharges the treated water. Target anticancer drugs, selected due to their importance, consumption, inherent cytotoxic activity, and potential risk to the environment were Azathioprine (AZA), Cyclophosphamide (CY), Ciprofloxacin (CIP), Docetaxel (DOC), Etoposide (ETO), Ifosfamide (IF), Methotrexate (MTX), Paclitaxel (PAC), Tamoxifen (TAM) and Vincristine (VIN). Finally, the risk that these compounds can pose to the environment was assessed based on the results derived from their occurrence in the wastewater effluents as well as their ecotoxicological effects described in the literature.

2. Material and methods

Ciprofloxacin HCl, Cyclophosphamide, Ifosfamide, Methotrexate, Azathioprine, Etoposide, Docetaxel, Paclitaxel, Vincristine Sulfate and Tamoxifen Citrate were purchased by European Directorate for the Quality of Medicines and Healthcare (EDQM) Reference Standards (Strasbourg, France). Isotopically labeled compounds, used as internal standards, [$^{2}H_4$]-Cyclophosphamide, [$^{13}C_6$]-Tamoxifen Citrate, [$^{2}H_3$]-Etoposide, [$^{2}H_3$]-Methotrexate, [$^{2}H_3$]-Vincristine Sulfate, [$^{13}C_4$]-Azathioprine were purchased from Toronto Chemical Research Inc. (Canada) and [$^{2}H_8$]-Ciprofloxacin from EDQM Reference Standards (Strasbourg, France). HPLC-grade Water and HPLC-grade acetonitrile and water (LiChrosolv) were supplied by Merck (Darmstadt, Germany). Reagents like Formic acid 98% (HCOOH) were provided by Scharlab (HPLC-grade) and the NH_3 30% by Panreac. Ethylenediaminetetraacetic Acid Disodium Salt 0.1 M solution (SV) was provided by Panreac.

2.1. Samples and standards preparation

Individual stock standard solutions of each target compound were prepared on a weight basis in methanol at 1 mg mL^{-1} and kept frozen at −20 °C. A mixture of all pharmaceutical standards was prepared by appropriate dilution of individual stock solutions. Stock solutions of internal standards were also prepared in methanol and were stored at −20 °C. A mixture of these internal standards was also prepared by diluting the individual stock solution in methanol.

Calibration standard solutions were prepared based on methodology previously developed (Ferrando-Climent et al., 2013) using a matrix match approach by appropriate dilution in extracted samples of the stock solution of target compounds.

2.2. Sampling campaign

Girona (north-east of Spain), the urban area selected for this study, has approximately 96,000 habitants and the main hospital of the region is located also in this municipality: Dr. Josep Trueta Hospital, which counts with around 400 beds, receives indeed most of the oncologic patients of this area. Municipal wastewater treatment plant (WWTP) of Girona, receives the urban wastewater from the main city and also from diverse municipals nearby (Salt, Sarrià de Ter, Sant Julià de Ramis, Aiguaviva, Vilablareix and Fornells de la Selva). Not only domestic sewage water but also wastewater from different sources: health centres (including the Dr. Josep Trueta hospital), industrial zone, etc. are discharging in the WWTP. Wastewater volume processed by this WWTP is estimated between 40,000 and 50,000 m^3/day (data provided by TRARGISA, trading company which manages the plant).

The sampling campaign was performed along consecutive months: November, December and January. Water was collected from hospital effluent through wastewater influent and effluent, till surface water, from Ter River at 500 m upstream and downstream of the wastewater treatment plant as presumably non-impacted and impacted sampling points respectively (Fig. 1).

The hospital effluent and the influent of the WWTP were collected within the same day while the wastewater effluent samples were collected the day after, together with the surface water samples, taking into account the hydraulic retention time (27 h) of WWTP.

Samples from the river, were taken 500 m upstream and downstream of the discharge of WWTP into the river. Both samples were taken same day that WWTP effluent was sampled. Samples were taken for specific days (work days), at same hours (morning) according with the urban sewage timings.

All the samples were collected in amber glass bottles which were pre-rinsed with Milli-Q water. They were vacuum filtered through 1 μm glass fiber filters followed by 0.45 μm nylon membrane filters (from Whatman, Teknokroma, Barcelona, Spain). The samples were kept frozen at −20 °C in amber PET containers, a period always inferior than 1 month, until their analysis based on the stability studies described at Ferrando-Climent et al. (2013), that establishes that 1 month is the maximum time that the target cancer drugs can be stored in those conditions before some degradation or lost of contaminants is observed.

General physico-chemical parameters of each sample as temperature, pH, oxygen amount and conductivity were measured *in situ* during sampling. A portable pH-meter (Crison; Model GLP 21) and an oximeter (YSI; model ProODO handlhed) were used for this purpose.

2.3. Toxicity assay

Toxicity of each sample was perform using the bacterial bioluminescence assay from Microtox™ (Carlsbad, CA, USA) based on the ISO 11348-3 standard protocol (UNE-EN ISO 11348-3:2007, 2009). Quantitative information about the toxicity of the samples was obtained by calculating the toxicity in terms of EC_{50} and toxicity units ($TU = 100/EC_{50}$). In case of testing the toxicity real solutions it is not possible to present results based on units such the concentration (ng/L) since there are many known and unknown contaminants in the sample. Therefore, Microtox results obtained are derived from the real sample as the most concentrated solution (45%) and its consequent dilution following the microtox protocol. A wide range of dilutions of each sample were measured using saline solution where the initial volume was 2.5 mL (45, 22.5, 11.25 and 5.63% of sample dilution), inhibition curves were performed, and the corresponding 50% effective concentrations (EC_{50}) were calculated. The analysis was carried out tempered at 15 °C. To enhance test performance, the salt contain was adjusted in order to reach 2% of salinity in sample. Bacterial reagents were reconstituted just prior to analysis and the pre-incubation times used before luminescence measurements were those given in microtox protocols. The concentration of toxicant in the test that caused a 50% reduction in light (inhibition = 50%) after exposure for 15 and 30 min.

2.4. Sample pre-treatment

The analytical methodology previously developed by Ferrando-Climent et al. (2013) was applied for sample pretreatment. A suitable volume of the chelating agent EDTA was added to all of them to a final concentration of 0.1% (g solute g^{-1} solution), as it is well known that it improves the extraction of some antibiotics such ciprofloxacin(Cha et al., 2006; Gros et al., 2012; Hernandez et al., 2007). Preconcentration of samples was performed by solid phase extraction (SPE) by the automatic extraction system GX-271 ASPEC™ (Gilson, Villiers le Bel, France). 50 mL of each sample was loaded at 1 mL min^{-1} in the Oasis HLB (200 mg, 6 mL) cartridge previously conditioned using 5 mL of methanol followed by 5 mL 0.1% formic acid solution at 2 mL min^{-1}. Elution was performed with 10 mL at a flow rate of 2 mL min^{-1}, using pure methanol. The extract was evaporated under gentle nitrogen stream using a Reacti-Therm 18824 System (Thermo Scientific) and reconstituted with 500 μL of methanol-water (10:90, v/v). Finally, internal standard mix to compensate possible matrix effect was added in the sample extract for internal standard calibration reaching a concentration of 10 μg L^{-1}.

2.5. UPLC-QqLit method

Chromatographic separation was carried out with a Ultra-Performance liquid chromatography system (Waters Corp. Mildford, MA, USA) equipped with a binary solvent system (Mildford, MA, USA) and a sample manager, using an Acquity HSS T3 column (50 mm × 2.1 mm i.d. 1.7 μm particle size; Waters Corp. Mildford, MA, USA) under positive electrospray ionization (PI). The UPLC instrument was coupled to 5500 QqLit, triple quadrupole–linear ion trap mass spectrometer (5500 QTRAP, Applied Biosystems, Foster City, CA, USA) with a Turbo V ion spray source. All transitions were recorded by using Multiple Reactive Monitoring Mode (MRM) and the data were acquired and processed using Analyst 2.1 software. Analytical parameters as limits of detection and quantification are shown in previous work (Ferrando-Climent et al., 2013).

2.6. Risk assessment

In order to assess the risk that the presence of these cancer drugs can pose into the environment, “Risk Characterization Ratio” (RCR) was calculated for each compound. RCR are calculated according to the EU guidelines (93/67/EEC, 2003) as the ratio