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Reactivity of vinca alkaloids during water chlorination processes: Identification of their disinfection by-products by high-resolution quadrupole-Orbitrap mass spectrometry

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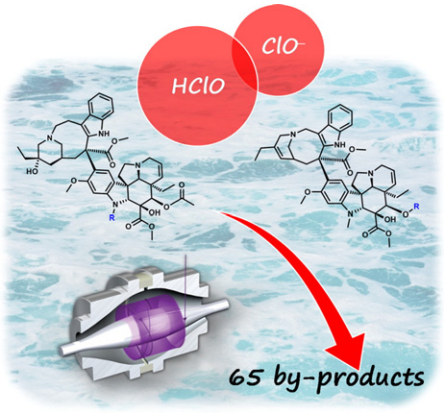
HIGHLIGHTS

- First study on the chlorination of vincristine, vinblastine and vinorelbine
- Vinblastine, vinorelbine and 4-*O*-deacetyl vinorelbine were quickly degraded.
- Sixty-five disinfection by-products were tentatively identified.
- Thirty disinfection by-products corresponded to chlorinated compounds.
- Disinfection by-products were also detected in wastewater chlorination experiments.

GRAPHICAL ABSTRACT



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ABSTRACT

Concerns about the presence of anticancer drugs in the environment are rapidly increasing mainly due to their growing use in the developed countries and their known cytotoxic effects. Vinca alkaloids are widely used in cancer therapy; however, very scarce information is available on their occurrence, environmental fate and toxicological effects on aquatic organisms. Even less attention has been paid to their potential transformation products, which can exert higher toxicity than the parent compounds.

Thus, in the present work, the reactivity of vincristine, vinblastine, vinorelbine and its metabolite 4-*O*-deacetyl vinorelbine during water chlorination processes has been investigated for the first time. Under the studied chlorination conditions, vincristine was fairly stable whereas vinblastine, vinorelbine and 4-*O*-deacetyl vinorelbine were quickly degraded. A total of sixty-five disinfection by-products were tentatively identified by ultra-high performance liquid chromatography coupled to high-resolution hybrid quadrupole-Orbitrap tandem mass spectrometry. Among them, twenty by-products corresponded to mono-chlorinated compounds, eight to di-chlorinated compounds and two to tri-chlorinated compounds, which may be of major environmental concern. Other disinfection by-products involved hydroxylation and oxidation reactions. Although the structures of these

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by-products could not be positively confirmed due to lack of commercial standards, their chemical formulas and product ions can be added to databases, which will allow their screening in future monitoring studies.

1. Introduction

Cancer has become one of the major causes of ill health and death globally, accounting for about 14.1 million new cancer cases, 8.2 million cancer deaths and 32.6 million people living with cancer in 2012 worldwide (Ferlay et al., 2013). The alarming rise in cancer incidence and mortality has driven the pursuit for anticancer agents to combat this disease.

Many effective anticancer drugs currently in use are plant derived. Thus, for instance, vinblastine (VBL) and vincristine (VCN) were first isolated from the *Catharanthus roseus* (Madagascar periwinkle), formerly *Vinca rosea* (Damen et al., 2010; Khazir et al., 2014). These compounds belong to the family of vinca alkaloids and are widely used, as single agents or in combination with other drugs, for the treatment of a wide variety of cancers, including non-small cell lung cancer, breast cancer, bladder cancer, lymphomas and leukemias (Damen et al., 2010; Kosjek et al., 2013). Their administration is only intravenous in their sulfate form and their mechanism of action consists in the binding to tubulin dimers, inhibiting assembly of microtubule structures and arresting mitosis in metaphase (Kosjek et al., 2013; Turci et al., 2003). This mechanism targets not only cancerous cells but all rapidly dividing cell types, which results in bone marrow suppression, gastrointestinal disorders and neurotoxicity (Checchi et al., 2003). So, efforts have been made to design new analogues with reduced toxicity, such as vinorelbine (VRB), a semisynthetic derivative of VBL used in the treatment of a wide range of cancer types including breast cancer and non-small cell lung cancer (Aapro et al., 2007; Damen et al., 2009). This drug inhibits the polymerization of tubulin dimers into microtubules, resulting in the disruption of mitotic spindle formation in dividing cells (Bourgeois et al., 2007) and, unlike VCN and VBL, it can be orally administrated (Damen et al., 2009).

VCN and VBL are potentially fetotoxic and embryotoxic (Al-Ahmad and Kümmerer, 2001), but so far there is no evidence of carcinogenicity in humans; therefore, the International Agency for Research on Cancer (IARC) has classified them as Group 3 carcinogens (not classifiable as to their carcinogenicity to humans) (IARC, last update 14.01.14). VRB has not been categorized yet.

After administration, these compounds are often metabolized into more biologically reactive metabolites. For instance, de Graeve et al. (2008) studied the metabolism pathways of VRB and identified a total of 17 metabolites that disappeared rapidly from blood, with the only exception of 4-*O*-deacetyl vinorelbine (dea-VRB), which could be detected in plasma (Puozzo et al., 2007; Ragot et al., 2001) and urine (Jehl et al., 1991).

Cytostatic drugs and their metabolites enter into the aquatic environment mainly through excretion of urine and feces of patients into the public sewer system, and they can eventually reach drinking water if they are not biodegraded or eliminated during the wastewater treatment processes. The number of studies investigating and reporting the presence of vinca alkaloids in water samples is rather low (Kosjek and Heath, 2011). Thus, VCN has been found in only two samples of wastewater influent and hospital effluent at levels of 22.9 and 49 ng L^{-1}, respectively (Ferrando-Climent et al., 2013), whereas VRB was detected in wastewater effluent at levels between 9.1 (Martin et al., 2011) and 170 (Martín et al., 2014) ng L^{-1}. To the best of our knowledge, there are not yet data available on the presence of VBL and dea-VRB in the environment. Very little is also known about the potential transformation of these compounds in the aquatic environment into other products, which might be even more toxic than the parent drugs. In a previous work of our research group, VRB was determined to be stable in wastewater for at least three months at room temperature, whereas VBL and VCN were rapidly degraded in just a few days (Negreira et al., 2014). Al-Ahmad and Kümmerer (2001) reported the biodegradation of VCN and VBL, reaching 30% and 10%, respectively, after 28 days. Up to now, other processes such as ozonation and chlorination have not received any attention (Zhang et al., 2013).

Chlorine is globally the most used chemical oxidant in wastewater treatment plants (WWTPs) as well as in the pretreatment of hospital effluents prior to their discharge into the public sewer system (Verlicchi et al., 2010; Zhang et al., 2013), mainly because of its low cost. Since aqueous chlorine is not an oxidant strong enough to completely mineralize many anthropogenic compounds, numerous disinfection by-products (DBPs) may be formed due to oxidation/substitution reactions (Deborde and von Gunten, 2008; Negreira et al., 2015a), not only during the wastewater treatment processes, but also due to the discharge of chlorinated waters (drinking water) or bleach. In spite of the high toxicity of vinca alkaloids drugs, their potential reaction with aqueous chlorine has not yet been studied.

In the present work, the reactivity of VCN, VBL and VRB and its metabolite dea-VRB during water chlorination processes has been investigated in order to better understand their fate and potential risks to the aquatic environment. Formed DBPs were tentatively identified by ultra-high performance liquid chromatography (UHPLC) coupled to a high-resolution hybrid quadrupole-Orbitrap mass spectrometer Q-Exactive. Time-course profiles of the studied compounds and their DBPs were obtained by analysis of different reaction time aliquots. The formation of DBPs was also investigated in a real wastewater sample.

2. Material and methods

2.1. Standards and solvents

Standards of the cytostatic compounds: vinblastine sulfate (VBL), vincristine sulfate (VCN), vinorelbine ditartrate (VRB), and 4-*O*-deacetyl vinorelbine (dea-VRB) and their isotopically labeled standards: vincristine-d_3 sulfate (VCN-d_3), vinorelbine-d_3 ditartrate (VRB-d_3) and 4-*O*-deacetyl vinorelbine-d_3 (dea-VRB-d_3) were obtained from Santa Cruz Biotechnology (Heidelberg, Germany) at the highest available purity (>99%). Individual stock solutions of each compound (ca. 1000 mg L^{-1}) were prepared in dimethyl sulfoxide (DMSO) and stored in the dark at −20 °C. Different working standard solutions were made by appropriate dilution in DMSO (100 and 10 mg L^{-1}), ultrapure water and wastewater. Calibration standards (from 0.001 to 1 mg L^{-1}) were freshly prepared in ultrapure water on the day of the analyses.

All solvents were of HPLC grade and all chemicals were of analytical reagent grade. Formic acid (98–100%), hydrochloric acid (HCl, 37%), methanol and ultrapure water were purchased from Merck (Darmstadt, Germany). DMSO and ascorbic acid (>99%) were acquired from Sigma (Milwaukee, WI, USA), whereas sodium hydroxide was from Carlo Erba (Sabadell, Spain). A 10% (w/v) solution of sodium hypochlorite was purchased from Panreac (Barcelona, Spain). This solution was stored at 4 °C and its exact concentration was assessed using the N,N-diethyl-p-phenylenediamine method with photometric detection (Clesceri et al., 1998). The photometer (model HI 96710) was obtained from Hanna instruments (Guipuzkoa, Spain).

2.2. Safety considerations on cytostatic drugs handling

Due to their toxicity, manipulation of cytostatic drugs needs of severe safety measures in order to guarantee the best possible protection