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The chlorination behaviour and environmental fate of the antiretroviral drug nevirapine in South African surface water

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ABSTRACT

The wastewater treatment process, besides discharging pharmaceuticals into the environment, has been found to result in the formation of a variety of undescribed compounds. Here we investigate the laboratory scale chlorination of the commonly used anti-HIV drug Nevirapine, characterise its disinfection transformation products (DTPs), and using liquid chromatography with high resolution mass spectrometry, screen environmental surface water for these DTPs. Chlorination of Nevirapine was scaled up, fractioned by preparative chromatography and the fractions were tested *in vitro* for toxicity and anti-HIV activity. Nevirapine was found to be resistant to degradation at relevant chlorination levels, which may partially explain its ubiquitous presence in South African surface water. During simulated chlorination, a variety of DTPs with varying properties were formed, some of which were detected in the environment, close to wastewater treatment plants. Interestingly, some of these compounds, although not as toxic as Nevirapine, retained antiviral activity. Further purification and synthesis is required to fully characterise these novel molecules.

1. Introduction

Over the past two decades researchers have shown that pharmaceuticals and personal care products (PPCP) are released into water courses as a result of human use (Ternes et al., 2001; Peng et al., 2014). The effect these compounds, at low concentrations, have on human health or aquatic fauna and flora have yet to be determined fully (Petrie et al., 2014; Roden et al., 2015). Furthermore, the development and promotion of drug resistance in bacterial populations has been postulated (Kümmerer, 2009).

Besides releasing pharmaceuticals into the environment, these compounds have also been found to be modified as a result of wastewater treatment. The resulting disinfection transformation products (DTPs), many of which are undescribed, are then released into the environment, which further complicates the impact of PPCPs on the environment. The mechanism and type of transformation product formation is dependent on the type of disinfection utilised. It has been found that pharmaceuticals may be modified by ozonation (Zimmermann et al., 2011), chloramination (Zhai et al., 2014) and chlorination (He et al., 2013; Bulloch et al., 2015). Chlorination is one of the more popular methods used to disinfect wastewater and has therefore received the most attention in the literature. In South Africa water disinfection is commonly achieved using chlorine gas. The Department of Water Affairs and Forestry (DWAF) requires that discharged wastewater should contain zero faecal coliforms per 100 mL with the caveat that residual chlorine may not be higher than 0.25 mg/mL (Leopold and Freese, 2009).

Chlorination, as a mechanism to treat wastewater and drinking water, has been the method of choice for a long time since it is a cost effective and broad spectrum method of disinfection. In addition to this, chloramination of treated water ensures a longer

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duration of disinfection as chloramines have a longer half-life than free residual chlorine (Leopold and Freese, 2009).

Unfortunately chlorination has a number of drawbacks, such as the formation of disinfection by-products during the disinfection process. A large number of toxic compounds may be formed through the interaction between chlorine and dissolved organic matter. Compounds such as the trihalomethanes and the haloacetic acids have been identified in previous decades and are now strictly regulated (Richardson et al., 2007).

Very little is known about the chemical characteristics of pharmaceutical DTPs and their toxicity profiles cannot always be based on those of the parent compound. This was shown in the case of the chlorination of acetaminophen, which resulted in the production of the toxic compounds 1,4- benzoquinone and *N*-acetyl-p-benzoquinone imine (Bedner and MacCrehan, 2006).

Besides adding complexity to the potential toxicity profile, the biological activities of many of the degradation products are not known. The degradation products of antivirals or antibiotics may retain antimicrobial properties or even gain additional activities. Inroads into understanding the transformation of antibiotics are being made by various researchers and it has been found that while most antibiotics lose their activity during water disinfection, a few do form biologically active transformation products (Dodd et al., 2009; Escher and Fenner, 2011; Mestankova et al., 2012; Keen and Linden, 2013). In addition to understanding the chemistry behind their transformation, various technologies are in development to effectively remove pharmaceuticals and their disinfection transformation products from wastewater (Prasse et al., 2015). These technologies are however in their infancy and have yet to be adopted widely in "first world countries", let alone in developing countries such as South Africa.

Recent research on the prevalence of HIV-1 antiretroviral compounds (ARVs) in South African surface water has shown that Nevirapine occurs ubiquitously in the environment (Wood et al., 2015). The drug is a non-nucleoside reverse transcriptase inhibitor (NNRTI) that is commonly used to prevent mother to child transmission of HIV and features as a first-line regimen for treatment of HIV-1 infection (Mofenson, 2010; Coovadia et al., 2012). Prasse and colleagues have shown that the compound also occurs in European surface water and its presence is attributable to inefficient removal during wastewater treatment (Prasse et al., 2010).

Vankova and co-workers showed that Nevirapine has low biodegradability in a closed bottle system (Vanková et al., 2010). Although this theoretical finding addresses the compound's ubiquitous presence in South African surface water (Wood et al., 2015), it does not describe how the compound reacts during wastewater treatment, if at all.

The antiretroviral class of compound has not been studied extensively in surface water across the world. This is most likely due to the regional prevalence of HIV. In addition to this, no research, to our knowledge, concerning the transformation behaviour of these drugs during the disinfection process has been described.

South Africa utilises more ARVs per capita than any other country in the world (WHO, 2013) which indicates that high amounts of these compounds would enter wastewater treatment works (WWTWs), that were not designed to remove pharmaceuticals. In addition to ineffective WWTWs, improper sanitation and illegal sewage release should also be considered. These factors, as well as the reduced expected dilution, in a water scarce region such as South Africa, lead one to expect that ARVs and their degradation products should be prevalent in the environment.

Here the reactivity of the antiretroviral drug Nevirapine to chlorine, in the form of sodium hypochlorite, is qualitatively studied. The degradation products that are formed as a result of chlorination are described and related to environmental water samples collected in South Africa. We proceed to show that although these disinfection transformation products of Nevirapine are not toxic, they may have the same or similar biological activity as the parent molecule. The environmental impact of releasing active, undescribed molecules from WWTWs has yet to be determined.

2. Materials and methods

2.1. Chemical reagents

Nevirapine was purchased from the United States Pharmacopeia, through Industrial Analytical (Johannesburg, South Africa) and stock solutions (1 mg/mL) were made up in methanol and stored at −20 °C until use. LC-MS grade water, methanol and dimethyl sulfoxide (DMSO) were purchased from Lab-Scan (Gliwice, Poland). Sodium hypochlorite from Merck (Johannesburg, South Africa), 10–14%, was diluted in water to 0.4 M and the concentration was found to be stable over time by iodometric titration. Monobasic and dibasic potassium phosphate (Merck) were used for buffering Nevirapine and NaOCl solutions to a final concentration of 10 mM. Ammonium Chloride, sodium thiosulphate and ascorbic acid were purchased from Radchem (Johannesburg, South Africa), formic acid from Sigma-Aldrich (Johannesburg, South Africa) and 20 mL borosilicate amber vials with PTFE caps from Macherey-Nagel (Düren, Germany). Pharmaceutical Nevirapine was obtained from Aspen (Johannesburg, South Africa) and utilised for large scale experimentation to reduce costs. Water and acetonitrile, each with 0.1% formic acid were obtained from Burdick & Jackson (Muskegon, USA). All buffers and reagents were formulated using LC-MS grade water (non-South African origin).

2.2. Chlorination reactions

Nevirapine (20 μg/mL) diluted in either LC-MS grade water, 10 mM phosphate buffer pH 5.8 or 10 mM phosphate buffer pH 8 was combined in equal volumes with NaOCl diluted in either of the aforementioned solvents (to yield 50, 100, 200 or 500 μM NaOCl) and stirred at room temperature (20 °C + −1 °C). Aliquots were taken from the reaction at 1, 5, 10, 20, 30, 60 and 120 min and then again at 24 h. Aliquots were analysed by HPLC-UV, UHPLC-QqQ and UHPLC-QTOF.

To identify an effective quenching agent, sample fractions (1 mL) for each time course were added to either sodium thiosulphate, ascorbic acid or ammonium chloride to yield a twofold molar excess (compared to NaOCl concentration), analysed by LC-MS plug injection and compared to unquenched data. In order to generate the most accurate data for a particular time point, unquenched reactions were incubated in the LC autosampler. Plug injections were performed using an Agilent 1290 series UHPLC coupled to an Agilent 6460 triple quadrupole (Agilent). Mobile phases consisted of water (A) and acetonitrile (B) both with 0.1% formic acid, held at 50% B at a flow rate of 0.4 mL/min. Sequential 15 μL plug injections (no column) of a sample incubated on the LC-MS autosampler, held at (20 °C + −1 °C), were analysed by mass spectrometry in MS2 scan mode by positive electrospray ionisation. Source conditions: gas temperature, 250 °C; gas flow, 8 L/min; nebulizer, 35 psi; sheath gas temperature, 300 °C; sheath gas flow, 10 L/min; capillary voltage, 3000 V and nozzle voltage, 0 V.

For kinetics studies Nevirapine (4 μM) diluted in either LC-MS grade water, 10 mM phosphate buffer pH 5.8, 10 mM phosphate buffer pH 8 or WWTW effluent from the Zeekoegat plant was combined in equal volumes with NaOCl diluted in either of the aforementioned solvents (to yield 2 μM Nevirapine and 20 μM