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Identification of transformation products of antiviral drugs formed during biological wastewater treatment and their occurrence in the urban water cycle

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ABSTRACT

The fate of five antiviral drugs (abacavir, emtricitabine, ganciclovir, lamivudine and zidovudine) was investigated in biological wastewater treatment. Investigations of degradation kinetics were accompanied by the elucidation of formed transformation products (TPs) using activated sludge lab experiments and subsequent LC-HRMS analysis. Degradation rate constants ranged between 0.46 L d^{-1} g_{SS}^{-1} (zidovudine) and 55.8 L d^{-1} g_{SS}^{-1} (abacavir). Despite these differences of the degradation kinetics, the same main biotransformation reaction was observed for all five compounds: oxidation of the terminal hydroxyl-moiety to the corresponding carboxylic acid (formation of carboxy-TPs). In addition, the oxidation of thioether moieties to sulfoxides was observed for emtricitabine and lamivudine.

Antiviral drugs were detected in influents of municipal wastewater treatment plants (WWTPs) with concentrations up to 980 ng L^{-1} (emtricitabine), while in WWTP effluents mainly the TPs were found with concentration levels up to 1320 ng L^{-1} (carboxy-abacavir). Except of zidovudine none of the original antiviral drugs were detected in German rivers and streams, whereas the concentrations of the TPs ranged from 16 ng L^{-1} for carboxy-lamivudine up to 750 ng L^{-1} for carboxy-acyclovir. These concentrations indicate an appreciable portion from WWTP effluents present in rivers and streams, as well as the high environmental persistence of the carboxy-TPs. As a result three of the carboxylic TPs were detected in finished drinking water.

1. Introduction

Despite the importance in the treatment of a variety of diseases including infections of hepatitis B, influenza and HIV, the occurrence and fate of antiviral drugs in the urban water cycle has been much less investigated than other pharmaceuticals such as antibiotics, analgesics or antihypertensive drugs (Evgenidou et al., 2015; Luo et al., 2014). However, studying their fate in the urban water cycle is important for two main reasons. First, if viruses are exposed to antiviral drugs, resistance of viruses can occur in analogy to antibiotic resistances of bacteria (Gillman et al., 2015; WHO, 2014). These antiviral drug resistances prevent an effective treatment of viral infections. Resistances were identified during the treatment of several viral infections with antiviral drugs: herpes-simplex (HSV), cytomegaly (CMG), hepatitis B (HBV) (Strasfeld and Chou, 2010), influenza (Hayden and de Jong, 2011) and human immunodeficiency (HIV) (WHO, 2014).

Second, antiviral drugs and TPs may have (eco-)toxicological impacts. Especially nucleosidic reverse transcriptase inhibitors (NRTIs) might cause adverse effects in mitochondria (Kakuda, 2000; Venhoff et al., 2007), since their nucleosidic structure can be integrated in DNA or RNA-strains. For the NRTI zidovudine cancerogenic toxicity to female rodents has been observed (Ayers et al., 1996). In addition, it was recently shown that carboxy-acyclovir, the main biodegradation product of the herpes drug acyclovir as well as COFA, the main oxidation product formed during advanced wastewater treatment with ozone, exhibit an increased toxicity towards *Daphnia magna* and green algae, respectively (Schlüter-Vorberg et al., 2015).

Oseltamivir and its metabolite oseltamivir carboxylate (60 ng L^{-1}) were the first antiviral drugs detected in rivers and streams (Söderström et al., 2009). Since then, a number of other

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antiviral drugs have been detected in the aquatic environment including acyclovir, abacavir, lamivudine, nevirapine and zidovudine (Peng et al., 2014; Prasse et al., 2010).

Due to the higher prevalence of HIV infections in Africa, elevated concentrations of antiviral drugs were detected in several surface waters, reaching concentrations up to 9 μg L^{-1} for zidovudine (K'Oreje et al., 2012; Wood et al., 2015), despite high removal rates during biological treatment in WWTPs (abacavir: >99%, acyclovir: 98%) (Prasse et al., 2010). The transformation of oseltamivir and its metabolite oseltamivir-carboxylate was investigated by Accinelli et al. (2010) in contact with water, sediments and activated sludge indicating that 80% of incubated oseltamivir carboxylate was mineralized to CO_2. Prasse et al. (2011) revealed the transformation pathways of acyclovir and penciclovir, including the identification and quantification of the stable TP carboxy-acyclovir. The latter one was detected in small rivers and streams with concentrations of 3.2 μg L^{-1} and in finished drinking water up to 40 ng L^{-1}.

Emtricitabine (EMT), ganciclovir (GCV) and lamivudine (LMV) were metabolized to a small extent in the human body (10–30%) (Cihlar and Ray, 2010), whereas abacavir (ABV) and zidovudine (ZDV) (Cihlar and Ray, 2010) were predominantly metabolized to their glucuronide-adducts. Carboxy-abacavir (ABV-COOH) is a further metabolite (Cihlar and Ray, 2010), formed in proportions of 15%. All antiviral drugs and corresponding metabolites are renally excreted.

The objective of the present study was to elucidate the TPs and the transformation pathways of 4 NRTI drugs (abacavir, emtricitabine, lamivudine, zidovudine) and one thymidine kinase inhibitor (ganciclovir) (Table 1) in biological wastewater treatment. In addition, the occurrence of antiviral drugs and their TPs in the urban water cycle and the elimination in advanced (waste-)water treatment using ozonation and activated carbon filtration was investigated.

2. Materials and methods

2.1. Chemicals and standards

Abacavir (ABV), acyclovir (ACV), methanol (LC-grade), formic acid (for MS) and ammonium formate (for MS) were purchased from Sigma-Aldrich (Seelze, Germany). Descyclopropyl-abacavir (ABV-desCP), carboxy-abacavir (ABV-COOH), zidovudine (ZDV), carboxy-zidovudine (ZDV-COOH), lamivudine (LMV), lamivudine-S-oxide (LMV-S-oxide), lamivudine (LMV-COOH), emtricitabine (EMT), emtricitabine-S-oxide (EMT-S-oxide), carboxy-emtricitabine (EMT-COOH), ganciclovir (GCV), entecavir-$^{13}C_2$,^{15}N (ECV-$^{13}C_2$,^{15}N), acyclovir-d_4 (ACV-d_4), abacavir-d_4 (ABV-d_4), zidovudine-d_3 (ZDV-d_3), lamivudine-^{13}C-$^{15}N_2$ (LMV-^{13}C,$^{15}N_2$), emtricitabine-^{13}C-$^{15}N_2$ (EMT-^{13}C,$^{15}N_2$), ganciclovir-d_5 (GCV-d_5) were purchased from Toronto Research Chemicals (Toronto, Canada). Carboxy-acyclovir (ACV-COOH) was obtained from earlier lab-experiments (Prasse et al., 2011). Dimethylsulfoxide (Uvasol) was obtained from Merck (Darmstadt, Germany). Purified water was generated from a Merck Millipore Milli-Q-Integral 3-System (Darmstadt, Germany). Stock solutions of target analytes and surrogate standard solutions were prepared as listed in Table S4 and stored in the dark at −25 °C. Stock solutions containing all analytes were prepared in methanol at 10, 1, 0.1 and 0.01 mg L^{-1}, for surrogate standards at 1 mg L^{-1} and were stored at 4 °C.

2.2. Lab-scale experiments with activated sludge to identify transformation products

2.2.1. Transformation kinetics at environmental concentrations

Lab-scale experiments with activated sludge were performed to determine biological degradation rate constants. Activated sludge was obtained from WWTP Koblenz-Wallersheim. WWTP parameters are provided in Table S1. Amber glass bottles (500 mL) were filled with 60 mL of activated sludge (total suspended solids (TSS): 4 g_{ss} L^{-1}). To minimize sorption effects the sludge was diluted 1:5 with 240 mL of WWTP effluent. The lab-scale batch systems were continuously stirred and aerated with a mixture of CO_2 and air (1:20) to maintain aerobic conditions and a constant pH (7.2 ± 0.1). After an equilibration time of 30 min the analytes were spiked at a concentration of 10 μg L^{-1}.

All experiments were performed in triplicates. Samples were taken before spiking (t_{blank}), directly after spiking (t_0) and at defined time intervals over the course of the experiment (up to 7 days). All water samples were filtered through 0.45 μm syringe filters (regenerated cellulose filters, Spartan, Whatman) and were stored in the freezer (−25 °C) until analysis via LC-MS/MS. Sterile control experiments (autoclaved two times for 15 min at 121 °C) were used to distinguish between abiotic and biotic reactions. The rate constants k_{biol} and the transformation half-lives were calculated using

$$k_{biol} = -\frac{ln\left(\frac{c_t}{c_0}\right)}{t \cdot X_{SS}} \quad (1)$$

$$t_{\frac{1}{2}} = \frac{ln(2)}{k_{biol} \cdot X_{SS}} \quad (2)$$

where c [ng L^{-1}] is the concentration of the analyte, t [d] is the reaction time, k_{biol} [L g_{ss}^{-1} d^{-1}] is the biological rate constant, X_{ss} [g_{ss} L^{-1}] is the suspended sludge concentration in the batch system and $t_{1/2}$ [d] is the degradation half-life.

2.2.2. Identification of transformation products

For the identification of TPs the same experimental setup was used as described above, but the sludge dilution was increased to 1:10 with ground water and analytes were spiked at a concentration of 50 mg L^{-1}. Water samples were taken directly after spiking (t_0) and at defined times during the experiments. All water samples were filtered through 0.45 μm syringe filters (regenerated cellulose filters, Spartan, Whatman) and were stored in the freezer (−25 °C) until analysis via LC-HRMS.

2.3. LC-HRMS

For identification of formed TPs an Accela HPLC-system (Thermo Scientific, Bremen, Germany) coupled with a LTQ Orbitrap Velos mass spectrometer (Thermo Scientific, Bremen, Germany) was used. The chromatographic separation was achieved with a Synergi Hydro RP (4 μm, 150 × 3 mm i.d.) column (Phenomenex, Aschaffenburg, Germany) coupled with a SecurityGuard AQ-C18 (3 mm i.d.) guard column (Phenomenex, Aschaffenburg, Germany). Aliquots of 20 μL of each sample were injected into the LC-HRMS-system. 5 mM ammonium formate (A) and methanol (B) were used as mobile phases at a flow rate of 450 μL min^{-1} with the following gradient: 0 min 100% A, 10 min 100% A, 30 min 30% A, 32 min 100% A, 40 min 100% A. The mass spectrometer was used in positive electrospray-ionization-mode (ESI+). Further MS^n-experiments were conducted with a mass range of 50–400 m/z at a resolution 30,000. The MS^n-spectra were recorded via collision induced dissociation (CID) and higher energy collision dissociation (HCD).