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Degradation of fluoroquinolone antibiotics during ionizing radiation treatment and assessment of antibacterial activity, toxicity and biodegradability of the products

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ABSTRACT

This work aimed at investigating the ionizing radiation induced degradation of two fluoroquinolone antibiotics: norfloxacin and ciprofloxacin. At 0.1 mmol dm^{-3} concentration a low dose, 2 kGy was sufficient to degrade the initial molecules. However, despite of the high removal efficiency the degrees of both the mineralization and the oxidation were low, ~10% and ~25%, respectively. (The difference between the results obtained in norfloxacin and ciprofloxacin solutions was not statistically significant.) Broth microdilution tests carried out on *Staphylococcus aureus* evidenced removal of antibacterial activity in samples irradiated with 2 kGy. Acute toxicity determined on *Vibrio fischeri* bacteria showed increased toxicity at low doses indicating that the early degradation products were more toxic than the initial molecules. The results of biodegradation experiments performed in activated sludge have shown that the degradation products have become available to the metabolic processes of the microorganisms.

1. Introduction

Biological treatment is the most widely used large scale method for the removal of organic content from municipal wastewater. This technology uses a complex mixture of microorganisms for pollutant degradation. These microorganisms decompose xenobiotics, like antibiotics, with low efficiency. Inefficient biological treatment leads to deterioration of wastewater effluent quality and continuous discharge of pollutants to surface waters (Kümmerer, 2001; Rivera-Utrilla et al., 2013). Contamination of natural waters by active antibacterial substances is a growing environmental issue of a global concern (Kümmerer, 2009). Even trace amounts of antibiotics induce development of antibacterial resistance by exerting selective pressure on environmental bacteria (Allen et al., 2010; Wang et al., 2015). Fluoroquinolone antibiotics are a group of persistent pollutants that are regularly detected in surface waters (Adachi et al., 2013; He et al., 2014).

To improve the removal efficiency of pharmaceuticals from wastewater, numerous advanced oxidation processes (AOP) are under development or testing. AOP benefit from the reactions of highly reactive hydroxyl radicals (•OH) that are produced in various ways, depending on the technique applied (von Sonntag, 2008). The transformation products formed during AOP may be more biodegradable than the starting molecules (De Bel et al., 2009). Toxicity testing pointed out formation of harmful products as the inhibitory effects to *Pseudokirchneriella subcapitata* and *Vibrio fischeri* increased following UV treatment or sonolysis (De Bel et al., 2009; Yuan et al., 2011). Regarding antibacterial activity, somewhat controversial data have been reported. Paul et al. (2010) found that antibacterial activity decreased following photolytic and photocatalytic decomposition and assumed that the degradation products did not show antibacterial activity, while De Witte et al. (2010) claimed that degradation products had antibacterial activity after ozonation. High-energy ionizing radiation treatment, an AOP technique, has very limited literature regarding the degradation of fluoroquinolones. In case of gamma irradiation, it was shown that 0.4 kGy dose leads to more than 80% removal efficiency at low (0.01 mmol dm^{-3}) initial concentration (Sayed et al., 2016). In another study Cho et al. (2014) used electron beam irradiation for the

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removal of 0.3 mmol dm^{-3} ciprofloxacin lactate. In this case, due to the higher concentration, 10 kGy dose was needed for complete removal of the initial compound.

In the present study the radiolytic degradation of fluoroquinolone antibiotics was investigated in a complex approach using dilute aqueous solutions of norfloxacin and ciprofloxacin. The removal efficiency was quantified by the changes in parent molecule concentration (liquid chromatography tandem mass spectrometry, LC-MS/MS) and also by measuring the degree of mineralization (total organic carbon content, TOC) and oxidation (chemical oxygen demand, COD). The antibacterial activity was characterized using *Staphylococcus aureus* bacterial strains, whilst the acute toxicity was evaluated on *Vibrio fischeri* bacteria. The biodegradation was assessed in solutions inoculated with activated sludge.

2. Experimental

2.1. Materials

Ciprofloxacin (CIP, 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid), norfloxacin (NOR, 1-ethyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid) (Fig. 1) and all other chemicals were purchased from VWR International Ltd. The liquid dried *Vibrio fischeri* bacteria (NRRL-B-11177) were purchased from Hach Lange Ltd., Germany, while activated sludge was obtained from the Budapest Sewage Works Pte Ltd., Hungary. *Staphylococcus aureus* (ATCC 6538) was provided by the National Collection of Agricultural and Industrial Microorganisms (Szent István University, Hungary).

2.2. Irradiation

0.1 mmol dm^{-3} aqueous solutions of CIP or NOR were irradiated in a panoramic type facility with gamma-rays of a ^{60}Co source. The dose rate was 8 kGy h^{-1} and the dosimetry was performed using the ethanol-chlorobenzene dosimetry system according to ISO/ASTM 51538, 2009 standard. The unbuffered solutions were irradiated in 1 dm^3 amber glass bottles at ambient temperature. The solutions were air saturated and constantly aerated during irradiation to prevent oxygen depletion and to enhance the yield of oxygen containing radical species. It has been suggested that the $^{\bullet}OH$ is the most important reactant under these conditions (Wojnárovits and Takács, 2017). During irradiation of aqueous solutions H_2O_2 forms in self-termination reactions of $^{\bullet}OH$ and in aerated solutions also in the self-reactions of the superoxide radical anion/perhydroxyl radical pair (Illés et al., 2017). It is known that H_2O_2 interferes with some of the analytical techniques and exerts toxic effects to living organisms (Talinli and Anderson, 1992). Interferences related to H_2O_2 have been corrected in all experiments as suggested by Sági et al. (2018).

2.3. Determination of pollutant removal efficiency

The LC-MS/MS experiments were performed by Agilent 1200 LC and Agilent 6410 MS devices with gradient type elution and positive ionization mode with electrospray ionization. Chemical oxygen demand (COD) was determined according to ISO 6060:1989, 1989 standard (with the difference that 30 cm^3 sample volumes were used instead of the recommended 10 cm^3, since low COD values were expected), using a Behrotest TRS 200 COD digestion system. Test mixtures were kept at 150 °C for 2 h and the remaining potassium-dichromate oxidizing agent was determined by titration. Total organic carbon content (TOC) and total nitrogen (TN) measurements were carried out on a Shimadzu TOC-L system. Both measurements are based on catalytic combustion of the samples. CO_2 or excited NO formed following the combustion of organic carbon or nitrogen content were measured with non-dispersive infrared (TOC) or chemiluminescence (TN) detection, respectively.

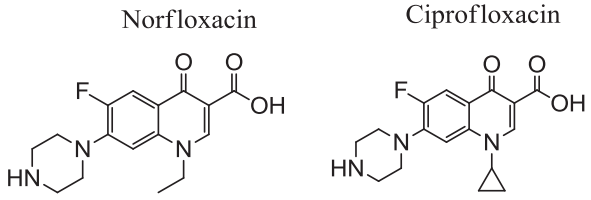

Fig. 1. The chemical structures of fluoroquinolones investigated.

2.4. Antibacterial susceptibility testing

Broth microdilution assay was performed to assess the antibacterial activity, in which the inhibited bacterial growth refers to antibacterial activity of the solutions tested. *Staphylococcus aureus* easily develops resistance to fluoroquinolones (Gade and Quazi, 2013), therefore this bacterium was chosen for susceptibility testing. Bacteria were grown in agar slant tubes (tryptone glucose (Cat. No. 1.08346.9029), yeast extract (Cat. No. 1.11926.1000) agar (TGE-Agar)). The cell density of the overnight bacterial culture was set in saline peptone water to 10^6 cells cm^{-1} by Grant bio DEN-1 suspension turbidity detector. 0.03 cm^3 of this bacteria solution was added to the microtiter plate to inoculate 0.27 cm^3 sample-trypto-casein soy broth mixture (sample-broth ratio of 1:1). The incubation time was 24 h and the temperature was set to 37 °C. The bacterial growth was measured by continuous kinetic reading mode (Multiskan Ascent optical densitometer, Thermo Electron Corporation). To determine the minimum inhibitory concentration (MIC) of CIP and NOR, measurements were also performed on the dilution series of these molecules. All the microbiological experiments were carried out under sterile conditions.

2.5. Acute toxicity testing

Acute toxicity was monitored by Microtox® test (DIN EN ISO 11348 – 3, 2007). Inhibited luminescence of *Vibrio fischeri* bacteria in the presence of test solutions indicates increasing toxicity. *V. fischeri* is a marine bacterium that requires saline environment. For this reason, 0.3 g NaCl was added to 15 cm^3 sample volumes (pH = 7–8) to reach 2% NaCl concentration recommended in the standard used. The samples were diluted by a factor of 2 to reach a well measurable range of toxicity. The liquid dried bacteria were reactivated by 12.5 cm^3 ready-to-use glucose/sodium chloride reactivation solution at 15 °C. 0.5 cm^3 of reactivated bacteria solution was exposed to 0.5 cm^3 fluoroquinolone samples at 15 ± 2 °C. The luminescence of test mixtures was determined by a LUMIStox 300 device (Hach Lange GmbH, Germany) following 30 min incubation.

2.6. Biodegradation

Biodegradability was characterized by the BOD_5/COD ratio, using 5-day biological oxygen demand (BOD_5) values determined in activated sludge. BOD_5 was measured by an OxiTop® Control BOD Respirometer System according to DIN EN 1899-1, 1998 standard measuring the pressure changes initiated by the oxygen consumption of the inoculum community. Inoculated dilution water was prepared by addition of 20 cm^3 supernatant of sedimented activated sludge to 1 dm^3 dilution water containing minerals as recommended in OECD Test No. 301, 1992. The samples (pH = 7–8) were seeded by inoculated dilution water by a dilution factor of 2. Allylthiourea solution was added to test mixtures to ensure that BOD related to the carbon content is measured solely (to inhibit nitrification). Test mixtures were incubated in airtight sealed 0.5 cm^3 amber glass bottles at 20 °C, over 5 days. However, to investigate the reactions taking place on longer time scale, the incubation period was prolonged to 21 days in some cases. Abiotic reactions have been separated by measuring unseeded samples.

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