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Photocatalytic degradation of selected anticancer drugs and identification of their transformation products in water by liquid chromatography–high resolution mass spectrometry

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ABSTRACT

A study on the fate of two antineoplastic drugs, methotrexate and doxorubicin, in the aquatic environment is presented. The investigation involved a study of their decomposition under dark experiments, homogeneous photolysis and heterogeneous photocatalysis using titanium dioxide, the identification of intermediate compounds, as well as the assessment of acute toxicity over time. The analysis were carried out using LC (ESI positive mode) coupled with LTQ-Orbitrap analyser; accurate mass-to-charge ratios of parent ions were reported with inaccuracy below 10 mmu, which guarantee the correct assignment of their molecular formula in all cases, while their MS^2 and MS^3 spectra showed several structural-diagnostic ions that allowed to characterize the different transformation products and to discriminate the isobaric species. Fourteen and eight main species were identified subsequently to doxorubicin or methotrexate transformation. The major transformation processes for doxorubicin involved (poli)hydroxylation and/or oxidation of the molecule, or the detachment of the sugar moiety. Methotrexate transformation involved decarboxylation or the molecule cleavage. Acute toxicity measurements showed that not only the two drugs exhibit high toxicity, but also their initial transformation products are highly toxic.

1. Introduction

Pharmaceuticals have become a major group of emerging contaminants as widespread pollutants of surface water, groundwater and drinking water [1–4]. Among them, anticancer drugs are of great importance, owing to their cytotoxicity, mutagenesis and genotoxicity. Therefore, it is crucial to monitor their presence in the environment and to extend the investigation to their transformation products (TPs), as they could contribute to the biotoxic and mutagenic potential [5].

The number of studies investigating cytostatic pharmaceuticals in water samples is limited, probably due to their presence at traces level (ng/L and below) and to their further transformation into other products [5]. Little information is likewise available on the transformation products of these drugs, although some human metabolite and biodegradation products have been already monitored [6].

Liquid chromatography is the primary separation technique for the analysis of antineoplastic drugs [7–11]. Trace level analysis requires high sensibility, specificity and accuracy, achievable by using LC/MS. Mass spectrometry is the most selective technique to identify unknown compounds and, using ESI-MS, it is possible to obtain structurally significant fragmentation ions [12–17].

With this in mind, in a previous work we have investigated the transformation of cyclofosfamide and mitomycin C [18], while in this work we focus on doxorubicin and methotrexate, whose presence was already documented in hospital effluents [17,19] or surface waters [20]. Though, only few studies investigate their biotic or abiotic transformation [21–23].

We aimed to monitor the fate of the selected drugs through a combined evaluation of their photostability, identification of TPs and assessment of their toxicity. Photo-induced reactions are certainly known to play a key role among the abiotic transformation; for such, the disappearance of the two substances and the formation of TPs have been evaluated under UV-A light in ultrapure water or after addition of TiO_2. Heterogeneous photocatalysis is indeed a widely used method not only to achieve the decontamination of aquatic systems [24–27], but also to simulate the transformation processes occurring in the environment. This approach was successfully used in previous studies and permitted to identify several TPs, alongside the parent compounds, in water samples [28–30].

Degradation products were characterized by multiple stage mass spectrometry, using a high resolution ion trap (LTQ-Orbitrap)

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in order to confirm the elemental composition, together with high resolution MS^n detection, which provides diagnostic identification and characterization of unknown transformation products.

The future application of the present work could be the more complete analysis of pollution of water (wastewater, surface or groundwater samples) with the extension of the analytes scenario to the products of degradation of anticancer drugs.

2. Experimental

2.1. Material and reagents

Experiments were carried out using TiO_2 Degussa P25 as the photocatalyst. TiO_2 powder was irradiated and washed with distilled water until no signal due to chloride, sulphate or sodium ions could be detected by ion chromatography, in order to avoid possible interference from ions adsorbed on the photocatalyst.

Doxorubicin and methotrexate were purchased from Aldrich and used as received. HPLC grade water was from MilliQ System Academic (Waters, Millipore). HPLC grade methanol (BDH) and acetonitrile (Aldrich) were filtered through a 0.45 μm filter before use. Reagent grade formic acid was from Fluka Chemie (Sigma).

2.2. Irradiation procedures

The irradiation experiments were carried out in Pyrex glass cells, filled with 5 ml of drug solution (15 mg/L) or of a suspension containing the drug (15 mg/L) and TiO_2 (200 mg/L). Samples were subjected to different irradiation times (ranging from 2 min to 4 h), using a Philips TLK/05 lamp 40 Watt with maximum emission at 360 nm. The temperature reached during irradiation was 26 °C. After illumination, the entire content of each cell was filtered through a 0.45 μm filter and then analyzed.

2.3. Analytical procedures

All samples were analyzed by HPLC/HRMS. The chromatographic separations, monitored using an MS analyzer, were run on a Phenomenex Luna 150 mm × 2.1 mm, using an Ultimate 3000 HPLC instrument (Dionex, Milan, Italy). Injection volume was 20 μL and flow rate 200 μL/min. Gradient mobile phase composition was adopted: 5/95–100/0 in 40 min methanol/formic acid 0.05% in water when run on ESI positive mode or acetonitrile/ammonium acetate 0.1 mM in the negative mode.

A LTQ Orbitrap mass spectrometer (Thermo Scientific, Bremen, Germany) equipped with an atmospheric pressure interface and an ESI ion source was used. The LC column effluent was delivered into the ion source using nitrogen as both sheath and auxiliary gas. The tuning parameters adopted for the ESI source were: capillary voltage 37.00 V, tube lens 65 V. The source voltage was set to 3.5 kV. The heated capillary temperature was maintained at 275 °C. The acquisition method used was optimized beforehand in the tuning sections for the parent compound (capillary, magnetic lenses and collimating octapole voltages) to achieve maximum sensitivity. Mass accuracy of recorded ions (vs calculated) was ±10 millimass units (mmu) (without internal calibration).

Analyses were run using full MS (50–1000 m/z range), MS^2 and MS^3 acquisition in the positive ion mode, with a resolution of 30,000 in FTMS mode. The ions submitted to MS^n acquisition were chosen on the base of full MS spectra abundance without using automatic dependent scan. Collision energy was set to 35 (arbitrary units) for all of the MS^n acquisition methods. MS^n acquisition range was between the values of ion trap cut-off and m/z of the fragmented ion. Xcalibur (Thermo Scientific, Bremen, Germany) software was used both for acquisition and for elaboration.

2.4. Toxicity measurements

The toxicity of samples collected at different irradiation times was evaluated with a Microtox Model 500 Toxicity Analyzer (Milan, Italy). Acute toxicity was evaluated with a bioluminescence inhibition assay using the marine bacterium *Vibrio fischeri* by monitoring changes in the natural emission of the luminescent bacteria when challenged with toxic compounds. Freeze-dried bacteria, reconstitution solution, diluent (2% NaCl) and an adjustment solution (non-toxic 22% sodium chloride) were obtained from Azur (Milan, Italy). Samples were tested in a medium containing 2% sodium chloride, in five dilutions, and luminescence was recorded after 5, 15 and 30 min of incubation at 15 °C; no substantial differences were found between the three contact times, for which we report the results related to 5 min of contact. Inhibition of luminescence, compared with a toxic-free control to give the percentage inhibition, was calculated following the established protocol using the Microtox calculation program.

3. Results and discussion

3.1. Drugs degradation under homogeneous and heterogeneous photocatalysis

Experiments were run in ESI positive mode, which appeared to be more sensitive and suitable both for the parent compound and for most of the photogenerated products. Fig. 1 shows the disappearance of doxorubicin (top) and methotrexate (bottom) as a function of irradiation time under direct photolysis or in the presence of TiO_2. The process of photolysis in ultrapure water promoted a partial drugs degradation as within 1 h of irradiation, almost 60% of methotrexate and 10% of doxorubicin disappeared. TiO_2 addition endorsed a fast degradation and both drugs were efficiently degraded ($t_{1/2}$ 3 and 2 min for doxorubicin and methotrexate,

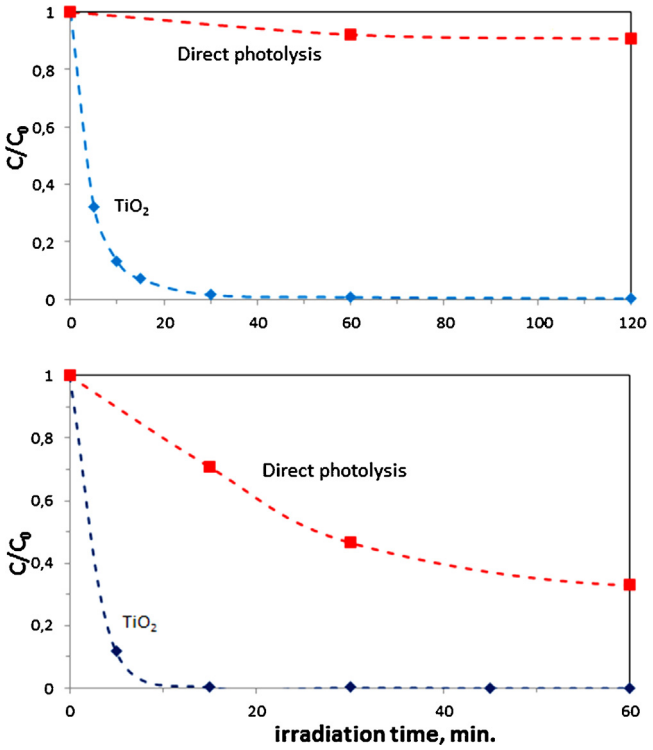

Fig. 1. Degradation of doxorubicin 15 mg/L (top) and methotrexate (bottom) as a function of irradiation time in ultrapure water or ultrapure water spiked with TiO_2 (200 mg/L).

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