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Evaluation of direct and indirect photodegradation of mianserin with high-performance liquid chromatography and short-term bioassays

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ABSTRACT

The widespread use of pharmaceuticals has lead to their detection in surface and ground waters. In the last year antidepressants in particular have shown very high growth dynamics of consumption and numerous research shows that these pharmaceuticals are detected in the environment and even in drinking water. Drugs and their metabolites can be subject to two types of photoreaction, direct and indirect photodegradation. These pharmaceuticals even at low concentration can have adverse effects on aquatic life, and the resulting photoproducts can be more toxic than parents compounds.

The aim of this study was to evaluate the direct and indirect photodegradation of mianserin. The kinetics of the process and the identification of photoproducts were investigated by HPLC-PDA and HPLC–MS/MS, respectively. Ecotoxicity of mianserin before and after irradiation was assessed with a battery of assays with bacteria, protozoa and crustacea.

The results show that mianserin was not toxic to *Vibrio fischeri* (Microtox), but its toxicity to protozoan *Spirostomum ambiguum* (Spirotox) and crustacean *Thamnocephalus platyurus* (Thamnotoxkit F™) was comparable to other antidepressants. On the basis of the results of the toxicity and HPLC before and after irradiation it can be seen that the decrease toxicity of mianserin was related only to a decrease of its concentration. The photoproducts had no impact to toxicity. The direct photodegradation of mianserin was more effective in UV/vis light than vis light. However the presence of humic acid in the indirect photodegradation increases the rate of degradation without regard to the kind of used light.

1. Introduction

About 3000 different substances are used in human medicine in the European Union (Fent et al., 2006). The authors reviewed that the most commonly consumed human pharmaceuticals are non-steroidal anti-inflammatory drugs, antibiotics, lipid regulators, cardiovascular drugs, neuroactive compounds and steroids. The widespread use of pharmaceuticals has lead to their detection in surface and ground waters. Data from many countries show the magnitude of the problem of the presence of pharmaceuticals in the aquatic environment (Fent et al., 2006). For example, the amount of pharmaceuticals in Germany that is prescribed but not used and therefore disposed of totals approximately 4500 t annually (Scheytt et al., 2006). About 1500 t of pharmaceuticals were used in 2003 in Italy (Castiglioni et al., 2006). Drugs and their metabolites have been constantly introduced into the environment making them pseudopersistent pollutants, which leads to continuous exposure of aquatic organisms over their whole life cycle (Minagh et al., 2009). Pharmaceuticals even at low concentration can have an adverse effect on aquatic life (Santos et al., 2010). It is also important that pharmaceuticals do not occur individually, but as mixtures together with their metabolites, products of transformation and other pharmacologically active compounds. Chemicals dissolved in natural waters are subject many types of chemical reaction, such as hydrolysis, sorption, photolysis and oxidation (Wang and Lin, 2014). Solar radiation is one of the important abiotic factor influencing the decomposition of chemical compounds present in the environment. Drug substances and drug products, which are found in the environment, may be decomposed under exposure to light, which is confirmed by numerous studies (Calza et al., 2008; Kwon and Armbrust, 2004; Kim and Tanaka, 2009). There are two types of photoreaction, direct and indirect photodegradation (Wang and Lin, 2014). In the first case, the chemicals absorb sunlight directly and are transformed to products when unstable excited states of the molecule decompose. In the second case, photodegradation occurs when chemicals present in natural water, such as dissolved organic matters (DOMs), absorb light and form reactive oxygen species (ROS) that may oxidize a target compound. DOMs are significant photosensitizers of indirect photolysis. ROS are

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molecules and ions of oxygen that have an unpaired electron, for example triplet-state DOMs, singlet oxygen, hydroxyl and carbonate radicals, thus rendering them extremely reactive. Sunlight photolysis could increase the toxicity of pharmaceuticals (Wang and Lin, 2014). For example, research of acute toxicity on *Vibrio fischeri*, showed that during irradiation of amiloride more toxic compound(s) were created (Calza et al., 2008). Guidelines for the photodegradation of chemicals in laboratory conditions were developed by the United States Environmental Protection Agency (USEPA) and they also apply to studies of photodegradation of pharmaceuticals (USEPA 1998a, 1998b).

Drugs acting on the central nervous system (CNS), especially antidepressants have very high growth dynamics of consumption. Antidepressant pharmaceuticals are used to treat the symptoms of depression, but can also be used for sleep and eating disorders, drug and alcohol abuse, panic, chronic pain and post-traumatic stress disorders (Santoke et al., 2012). In the global burden of disease (GBD) in 2000, depressive disorders were the third leading cause of burden after diarrhoeal diseases and lower respiratory infections (Ferrari et al., 2013). According to the latest Eurobarometer report on mental health, 6% of Polish and 7% of European citizens were treated for mental disorders with antidepressants in 2009 (Giebułtowicz and Nałęcz-Jawecki, 2014). In therapeutics, the selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine, fluvoxamine, paroxetine and sertraline are the most widely used antidepressants (Santos et al., 2010). Mianserin is atypical, tetracyclic antidepressant, the activity of which is attributed mainly to presynaptic alfa2-adrenoreceptor blocking and to serotonin receptor antagonism (Sfair et al., 2012b). Its sales volume in Poland is as high as 799 kg/a, which corresponds to a predicted environmental concentration (PEC) equal to 29 ng/l (Giebułtowicz and Nałęcz-Jawecki, 2014). This value is higher than the threshold value proposed by the European Medicines Agency (EMA, 2006), above which the ecotoxicological tests should be performed for the pharmaceutical. Due to their high sales volumes and resistance to biodegradation in wastewater treatment plants and in freshwaters, antidepressants were detected in rivers in many countries at concentrations up to several hundred ng/l. In a EU-wide monitoring survey venlafaxine, citalopram, mianserin and fluoxetine were found in 99%, 83%, 28% and 22% of municipal effluent samples, respectively (Loos et al., 2013). Due to high octanol/water partition coefficient, some antidepressants e.g. sertraline, imipramine, paroxetine, nortriptyline, fluoxetine and mianserin are expected to cause a pharmacological effect in fish (Fick et al., 2010). In the prioritization scheme for environmental risk assessment Roos et al. (2012) ranked mianserin very high due to a high PBT (persistency/bioaccumulation/ecotoxicity) index. Studies have also confirmed that SSRIs can be bioaccumulative in aquatic organisms, and the mixture of SSRIs produce toxic effects that are additive (Silva et al., 2012). Whereas, data on the lesser known antidepressants, but equally often prescribed are hardly ever found in the literature. Giebułtowicz and Nałęcz-Jawecki (2014) studied the presence of 21 antidepressant pharmaceuticals in the Vistula and Utrata rivers in Poland. They reported that the antidepressants, including citalopram, mianserin, sertraline, clomipramine, moclobemid, venlafaxin, fluoxetine, mirtazepin, and tianeptin, were detected in the Vistula river. Furthermore, the first five of these pharmaceuticals, mianserin among others to be precise, were also detected in tap water in Warsaw. These data have shown that antidepressants are present in the aqueous environment and are not indifferent to the organisms, therefore new research should be concentrated on this problem.

The purpose of this study was to evaluate the direct and indirect photodegradation of mianserin. The kinetics of the process and the identification of photoproducts were investigated by HPLC-PDA and HPLC–MS/MS, respectively. Ecotoxicity of mianserin before and after irradiation was assessed with a battery of assays with bacteria, protozoa and crustacea.

2. Materials and methods

2.1. Chemicals

Mianserin hydrochloride was extracted with methanol from MIANSEC® 30 (Jelfa SA, Jelenia Gora, Poland). Mianserin hydrochloride coated tablets were claimed to contain 30 mg of the drug and the following inactive ingredients: ludipress, magnesium stearate, colloidal anhydrous silica, ethylcellulose, macrogol 6000, titanium dioxide, indigo carmine. The extract was filtered using a 0.2 μm filter. Its purity and identify were confirmed by HPLC–MS/MS and was compared to a purchased authentic standard (Sigma-Aldrich, Poznan, Poland). The stock solution (1000 mg/l) was made up in methanol and stored at 4 °C in dark glass bottles. Working solutions of mianserin (20 mg/l) was prepared *ex tempore* by dilution of the stock solution with water or synthetic humic water. The chemical structure and relevant data for mianserin are shown in Table 1. Humic acids (sodium salt) were received from Sigma-Aldrich (Poznan, Poland). Deionized water was obtained by using Milli-Q water system (Milipore, U.S.). The high-performance liquid chromatography-grade solvent (acetonitrile) was provided by Merck (Darmstadt, Germany). Reagent grade trifluoroacetic acid was provided by J.T. Baker (Deventer, Netherlands).

2.2. Preparation of synthetic humic water

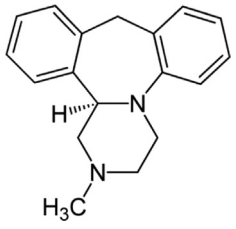
Synthetic humic water (HA) was made according to the United States Environmental Protection Agency Guidelines (USEPA, 1998b). In brief, 20 g of humic acid was extracted with 1 l of 0.1% NaOH solution by stirring for 1 h at room temperature. This mixture was decanted off and filtered through coarse filter paper. The pH was adjusted to 7.0 with dilute H_2SO_4 and the solution was filter sterilized through a 0.2 μm filter. Pre-aging was performed by exposing to direct sunlight. Before use this mixture was diluted 10-fold with 0.01 M phosphate buffer to produce a pH 7.0 solution with an absorbance of 5.00×10^{-2} at 370 nm.

2.3. Instruments

2.3.1. Liquid chromatography with photodiode array detector (HPLC-PDA)

The HPLC analyses were performed using a LC-10AT Shimadzu spectrophotometer equipped with a SPD-M10A diode-array detector and a SCL-10A system controller. The degradation products

Table 1
The chemical structure and relevant data for mianserin.

Name	Mianserin
Formula	$C_{18}H_{20}N_2$
Chemical structure	
Molar mass (g/mol)	264.37
λ_{max} (nm)	279