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Effects of lomefloxacin on survival, growth and reproduction of *Daphnia magna* under simulated sunlight radiation

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ABSTRACT

Lomefloxacin, an antibacterial agent with known photo-induced toxicity in clinical studies, is frequently detected in aquatic environments. Investigating the photo-induced toxicity of lomefloxacin in aquatic organisms is therefore of importance for assessing its ecological risks. In this study, the effects of lomefloxacin on survival, growth and reproduction of *Daphnia magna* under simulated sunlight radiation (SSR) were investigated, and the mechanism of action was revealed. Results indicated that SSR containing UV radiation increased the acute toxicity of lomefloxacin to *Daphnia magna* relative to white fluorescent light irradiation. Under SSR, 100 μM lomefloxacin significantly enhanced reactive oxygen species (ROS) generation and lipid peroxidation, and decreased activities of superoxide dismutase and catalase. The biochemical observations and apparent effects on the organism indicate that oxidative stress plays a central role in the acute photo-induced toxicity. Chronic toxicity results showed that SSR significantly affected growth and reproduction of *Daphnia magna*, whereas lomefloxacin reduced the damage of UV radiation in SSR through light shielding. This study provides insight into the mechanism of photo-induced toxicity and can support the risk assessment of chemicals in the aquatic environment by including the impacts of sunlight irradiation on toxicity.

1. Introduction

The majority of aquatic and terrestrial species are frequently exposed to chemicals in combination with natural stressors like sunlight radiation (Gergs et al., 2013; Holmstrup et al., 2010; Roberts et al., 2017; Schipper, 2005). Sunlight radiation, an important component of ecosystems, can play a role as an ecological stressor. Exposure to specific wavelengths of sunlight, such as ultraviolet radiation, can cause oxidative stress and damage to biological macromolecules. Besides, sunlight can interact with chemicals in a phenomenon known as photo-induced toxicity (Larson and Berenbaum, 1988; Ribeiro et al., 2011; Roberts et al., 2017). Photo-induced toxicity as a concern in aquatic systems was first suggested by Jodlbauer and Tappeiner (1905) who demonstrated that anthracene was phototoxic to *Paramecium caudatum*. Subsequently, an increasing number of chemicals including polycyclic aromatic hydrocarbons, anthraquinone dyes, pesticides, metals, ultraviolet sunscreens and engineered nanoparticles like fullerenes, carbon nanotubes and metal oxide nanoparticles have been shown to cause photo-induced toxicity to aquatic organisms (Kawakami and Gaspar, 2015; Kim et al., 2009, 2015; Li et al., 2014, 2012; Roberts et al., 2017; Verma et al., 2008; Wang et al., 2009). Investigating photo-induced toxicity of chemicals to aquatic organisms is significant for evaluating their ecological risks.

Photo-induced toxicity as a side effect of pharmaceuticals such as fluoroquinolones, sulfonamides and tetracyclines has been recognized clinically (Kim et al., 2009, 2015; Man et al., 1999). Lomefloxacin, for which the molecular structure, physicochemical properties, environmental levels and ecotoxicity data are summarized in the Supplementary Data (Table S1), is a broad spectrum antibiotic used for the treatment of human or animal infections (US Environmental Protection

Abbreviations: ROS, Reactive oxygen species; SOD, Superoxide dismutase; CAT, Catalase; GSH-Px, Glutathione peroxidase; GSH, Reduced glutathione; GSSG, Oxidized glutathione; DCFH-DA, 2, 7-dichlorodihydrofluorescein diacetate; DMSO, Dimethyl sulfoxide; WFLR, White fluorescent light radiation; SSR, Simulated sunlight radiation; DCF, 2′,7′-dichlorofluorescein; TBA, Thiobarbituric acid; MDA, Malondialdehyde

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Agency (USEPA), 2012; Gothwal and Shashidhar, 2017; Chang et al., 2010; Yiruhan et al., 2010; Ma et al., 2015; Dodder et al., 2014; Robinson et al., 2005; Backhaus et al., 2000). The phototoxic potential of lomefloxacin has been examined in clinical trials (Alshibani, 2017; Man et al., 1999; Oliveira et al., 2000). It is reported that sunlight irradiation can induce adverse reactions (i.e., eczematous lesions, acute oedematous sunburn-like lesions, ectropion and thick hyperkeratotic scaling) on the skin of humans who take lomefloxacin (Koker et al., 2010; Man et al., 1999; Oliveira et al., 2000). Photogenotoxicity and photocarcinogenesis of lomefloxacin have been confirmed in mice and rats (Chignell et al., 2003; Itoh et al., 2005; Reus et al., 2012). Although the photo-induced toxicity of lomefloxacin has been known in mammals (mice, rats and human), photo-induced toxicity of lomefloxacin to aquatic organisms has rarely been reported.

Conventional toxicological studies on aquatic organisms are typically conducted in laboratory settings with white fluorescent lights to simulate the irradiation of sunlight. Lack of realistic irradiation conditions (i.e., UV irradiation) could significantly underestimate the toxicity of chemicals in the aquatic environment (Jung et al., 2008; Kim et al., 2009; Roberts et al., 2017). Existing aquatic toxicity studies on lomefloxacin ignored the photo-induced toxicity potential and were limited to acute toxicity (Robinson et al., 2005; Backhaus et al., 2000). Therefore, it is essential to investigate the acute and chronic toxicity of lomefloxacin to aquatic organisms under simulated sunlight radiation. Furthermore, the mechanisms of toxic action underlying the photo-induced toxicity of lomefloxacin to aquatic organisms need to be revealed.

The potential mechanisms of photo-induced toxicity can be generally classified as photomodification or photosensitization. For aquatic systems, photosensitization leads to the formation of reactive oxygen species (ROS, e.g., $^1O_2$, $O_2^{\bullet-}$ or $^{\bullet}OH$), which is thought to be the most important mechanism of photo-induced toxicity (Arfsten et al., 1996; Diamond, 2003; Roberts et al., 2017). Under UV irradiation, lomefloxacin can generate $^1O_2$ and $O_2^{\bullet-}$ in aqueous media (Ferguson, 1995; Kawada et al., 1999; Martinez et al., 1998). In our previous studies, $^1O_2$ photogenerated by lomefloxacin in *Daphnia magna* was also verified (Luo et al., 2017). On the one hand, excess formation of ROS can harm cellular components such as membrane lipids, proteins and nucleic acids, eventually leading to impairment of vital cellular functions and cell damage or death. Lomefloxacin has been found to induce photosensitized lipid peroxidation in vitro (Kawada et al., 1999). On the other hand, antioxidant enzyme systems including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and others in biota can counteract and regulate the overall ROS levels (Finkel and Holbrook, 2000; Li et al., 2018; Vale et al., 2016). Similar to ROS, lipid peroxidation and changes of antioxidant enzymes activity can also be measured to probe the mechanism of photo-induced toxicity of lomefloxacin to aquatic organisms.

Daphnia magna, a typical invertebrate, acts as a key species linking primary producers and organisms at higher tropic levels. Due to its transparent body, as well as important role in aquatic systems, *Daphnia magna* has often been chosen as a model organism to investigate photo-induced toxicity (Lee et al., 2017a; Mansfield et al., 2015; Wang et al., 2009).

In the present study, acute and chronic toxicity experiments were conducted in which *Daphnia magna* was exposed to lomefloxacin under simulated sunlight radiation. To understand the underlying mechanisms, ROS generation, lipid peroxidation, and antioxidant enzymes activities in *Daphnia magna* during and after exposure were measured. Results of this study are helpful for assessing the ecological hazards of lomefloxacin and other fluoroquinolone antibiotics with photo-induced toxicity.

2. Materials and methods

2.1. Chemicals and reagents

Lomefloxacin hydrochloride (CAS: 98079-52-8, 98% purity) was purchased from J&K Chemicals. 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA, 97%) and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich. A DCFH-DA stock solution was prepared in DMSO and stored at − 20 °C. Other reagents (purity > 99.0%) were purchased from Kermel Chemical Reagent Co., Ltd. Ultrapure water was obtained from an OKP ultrapure water system (Shanghai Lakecore Instrument Co.).

2.2. Maintenance of Daphnia magna

The test organisms used in this study were offsprings of *Daphnia magna* cultured in the laboratory since 2011. The culture medium was prepared from dechlorinated tap water that was aerated three days and saturated with dissolved oxygen. Three times a week the culture medium was renewed. All adult daphnids were cultured in glass beakers and fed with a suspension of *Chlorella pyrenoidesa* once a day. Both the daphnids and green algae shared the same conditions of 20 ± 1 °C with a 16:8 h light: dark cycle in an artificial climate incubator (MGC-350HP, Shanghai Yiheng technology Co. Ltd.).

2.3. Exposure lighting system

An artificial climate incubator with internally installed trihedral white fluorescent light tubes was employed in the experiment. The light irradiance of the white fluorescent light tubes is shown in Fig. 1. It can be seen that almost no UV radiation was present in this white fluorescent light radiation (WFLR). Considering the realistic irradiation in the field environment, an incubator was additionally equipped with an array of UV and visible light tubes (two UV-A tubes, one UV-B tube and three fluorescent tubes, 8 W, Philips Co., Ltd.) in the upper ceiling, as shown in Fig. 1. This combination of light sources (Fig. 1) was defined as simulated sunlight radiation (SSR) in this study. The emission spectrum of the SSR was measured using a spectroradiometer (TriOS-RAMSES, Germany).

Many previous studies employed fluorescent light tubes to simulate sunlight irradiation and investigate photo-induced toxicity (Overmans et al., 2018; Wormington et al., 2017; Wang et al., 2009). Although the emission spectrum of xenon lamps is close to the spectrum of sunlight (Zhang et al., 2014), xenon lamps can hardly be employed in breeding *Daphnia magna*, as xenon lamps can release heat that quicken the evaporation of water from the culture media.

The samples were positioned approximately 0.60 m beneath the light tubes array. The intensities of the incident UV-A (320–400 nm) and UV-B (290–320 nm) radiation inside the glass beakers were measured to be 47.6 ± 0.8 μW/cm^2 and 9.3 ± 0.4 μW/cm^2, respectively. The UV-blocking of glass beakers is negligible as the radiation direction of the UV light is vertical. Dias and von Sperling (2017) reported that the UV-A intensity of solar radiation at 10 cm below pond waters was in the range of 2–100 μW/cm^2. During the incubation, the samples were exposed to WFLR/SSR on a 16:8 light to dark cycle (Fig. S1), and the added light array was turned on at 10:00 a.m. and shut down at 6:00 p.m.

2.4. Acute toxicity assays

The 48 h acute toxicity test using *Daphnia magna* (< 24 h) were performed according to OECD 202 guideline (Organisation for economic co-operation and development, 2004). All the tests were carried out in reconstituted water (Table S2) that was aerated for three days. Standard reference toxicity tests with potassium dichromate ($K_2Cr_2O_7$) were run to assure comparable sensitivities of cultured daphnids over