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Acute toxicity evaluation for quinolone antibiotics and their chlorination disinfection processes

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ABSTRACT

Acute toxicity of 21 quinolone antibiotics was monitored using photobacterium *Vibrio fischeri* assay. The minimum IC_{20} (inhibitory concentration for 20% luminescence elimination) was obtained at the least 18.86 μmol/L for the tested quinolones. A quantitative structure–activity relationship model was established to investigate the possible mechanism for the acute toxicity. The critical physicochemical descriptors, describing σ and π atom electronegativity, implied that the electron transfer might occur between the quinolones and photobacterium *V. fischeri*. Although the quinolones exhibited limited acute toxicity to photobacterium, toxicity elevation was detected after their chlorination. Hence, chlorination disinfection treatment of quinolone-containing water should be of concerns.

Introduction

Antibiotics are one of the most widely used groups of pharmaceuticals due to their potency against diseases in human, veterinary and industrial farming (Cruz Moreno-Bondi et al., 2009; Kummerer, 2009; Pan et al., 2011). Quinolones are among the five classes of antibiotics (β-lactam, macrolides, quinolones, sulfonamides, and tetracyclines) used to treat a broad variety of Gram (+) and Gram (–) bacterial infections. They kill target bacteria by inhibiting the activity of bacterial DNA gyrases, which are required for replication and transcription in prokaryotes (Bryan et al., 1989; Hooper, 2001). Owing to the advantages of broad-spectrum antibacterial activity, high potency, non-cross resistance and low price, quinolones have been extensively used in recently years. However, due to the incomplete assimilation and metabolism in organism, a considerable fraction of those drugs has been discharged into the environment. The removal of quinolones in municipal sewage treatment plants plays a crucial rule in their pollution control (Jia et al., 2012), while low removal efficiency and a high discharge from secondary effluents lead to a serious pollution to the aquatic environment. Consequently, quinolones have been frequently detected in various environmental matrices. For example, eight quinolones were the prominent contaminants in sediments and aquatic plants of the Baiyangdian Lake, China, with the concentrations of 65.5–1166 and 8.37–6532 μg/kg, respectively (Li et al., 2012). Ciprofloxacin, one of the most commercial quinolones, was found in Switzerland hospital effluents as high as 89 μg/L (Hartmann et al., 1998). It was reported that ciprofloxacin and enrofloxacin were not readily biodegradable by sewage sludge organism (Ebert et al., 2011). The hazard quotients (HQs) for the aquatic environment of ciprofloxacin and ofloxacin were 3.5 and 1.5 to algae in Baiyangdian Lake, indicating that those two compounds were harmful to algae in the lake water (HQ > 1 means that the harmful ecological impact is significant for the selected antibiotic) (Hernando et al., 2006; Li et al., 2012). Hu et al. (2007) investigated genotoxicity potential of 20 quinolones by umuC bioassay; the result indicated that all the tested compounds showed high toxicity with 10% of the maximum response concentration (EC_{10}) ranged from 0.61 to 2917 nmol/L. In

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addition, it was reported that genotoxicity in the wastewater of the hospital was mainly caused by quinolone antibiotics (Hartmann et al., 1998). The removal of those compounds in sewage treatment plants is a complex issue, and their toxicity patterns are flexible under various treatment processes, such as ozonation, UV photolysis and chlorination disinfection. Report regarding acute toxicity elevation has been disclosed in levofloxacin chlorination process (El Najjar et al., 2013). Therefore, it is necessary to consider the toxic effects for not only quinolone precursors but also their transformation products. Biological test, which can reflect the toxicity formation during the treatment process as a whole, would be an efficient way to evaluate those toxicity risks to the environment.

Limited research has been conducted on the aquatic ecotoxicity of quinolone antibiotics. Therefore, the main aim of this study is to provide basic toxicity data for quinolone antibiotics towards the photobacterium *Vibrio fischeri*, which is a standard aquatic toxicity model species representing decomposer trophic level. The 21 quinolones examined in this study were chosen on the basis of their wide usage in human and veterinary, as well as their detection levels in the environment. As all the compounds share the same quinolone skeleton, the quantitative structure–activity relationship (QSAR) method was used to investigate the relationship between toxicity and molecular structure. Finally, the acute toxicities of those quinolones after chlorination were measured to evaluate their toxicity changes during chlorination disinfection treatment. This study could provide useful information on evaluating the potential risks of quinolones towards the aquatic environment.

1. Materials and methods

1.1. Reagents and chemicals

Based on their usage in human and veterinary as well as their availability from the manufactory, 21 quinolone antibiotics were chosen in this study. Cinoxacin (CIN), ciprofloxacin (CIP), danofloxacin (DAN), difloxacin hydrochloride (DIF), enoxacin (ENO), enrofloxacin (ENR), fleroxacin (FLE), levofloxacin (LEV), lomefloxacin hydrochloride (LOM), moxifloxacin hydrochloride (MOX), norfloxacin (NOR), ofloxacin (OFL), pazufloxacin (PAZ), pipemidic acid (PIP), sarafloxacin hydrochloride (SAR) and sparfloxacin (SPA) were purchased from Sigma-Aldrich as HPLC or analytical reagent (>98% purity, MO, USA). Balofloxacin (BAL), gatifloxacin (GAT), nadifloxacin (NAD) and pefloxacin (PEF) were obtained from the National Institutes for Food and Drug Control of China (at least 97% purity, Beijing, China). Rufloxacin hydrochloride (RUF) with 99% purity was obtained from International Laboratory (USA). The NaClO (8%) aqueous solution was obtained from Wako Co. (Tokyo, Japan). All reagents were used directly without further purification. The stock solutions of all studied compounds were prepared in ultrapure water produced by a Milli-Q ultrapure water system (Millipore MA, USA).

1.2. Chlorination disinfection

The chlorination experiments were performed in borosilicate glass bottles. Water bath and magnetic stirring apparatus were used to maintain the reaction temperature at 25 °C. In order to investigate the formation characteristics of disinfection byproducts in chlorination disinfection treatment of quinolones, the conception and significance of disinfection byproducts formation potential recommended by APHA (1998) were referenced. In addition, it was reported that OFL, CIP and NOR were labile during chlorination treatment within pH 6.0–8.0 (Li and Zhang, 2012). Therefore, in this study, the chlorination treatment on the target compound was performed with 10 molar equivalents of free available chlorine at pH 7. The 0.02 mol/L phosphate buffer solution was used to maintain the pH at 7 during the reaction period. After 60 min, sodium sulfite solution (1.5 equivalents to free available chlorine) was added to quench reaction. The quenched reaction solution was freeze-dried; 10 mL of mixed solvent methanol/acetone (1/1, *V*/*V*) was added to extract organic components. Supernatants were collected and dried with gentle N_2 flow, dissolved with dimethyl sulfoxide for toxicity test. The chlorination experiment for each quinolone compound was conducted in triplicate. A blank control without adding free available chlorine was set as well.

1.3. Toxicity test

The photobacterium acute toxicity test quantifies the effects of pollutants by measuring the decrease of luminescence intensity of the test bacteria. The test bacteria strain (*V. fischeri*, freeze-dried powder) was provided by the Institute of Soil Science, Chinese Academy of Sciences (Nanjing, China). The quinolone samples (both before and after chlorination) were diluted into a series of exposure solutions, and 20% inhibition concentration (IC_{20}) was calculated after the 15 min exposure. For each test, a dose–response curve of Hg^{2+} ($HgCl_2$) as positive control was conducted as well.

1.4. QSAR method

The ADRIANA.Code program (Ver 2.2.4) was applied to calculate physicochemical parameters of the target molecules. In total, 8 shape descriptors, 29 global molecular descriptors and 88 2D property-weighted autocorrelation (or topological) descriptors were calculated. All calculated descriptors were selected as independent variables and pIC_{20} ($-\log IC_{20}$) values were selected as dependent variables. Stepwise multiple linear regression method was used to establish QSAR model.

2. Results and discussion

2.1. Acute toxicities of 21 quinolones

The toxicity values for 21 quinolone compounds are listed in Table 1. The IC_{20} values for all the tested compounds ranged from 18.86 to >700 μmol/L. Among those compounds, DIF and MOX exhibited a notable higher toxic effect than the others, with IC_{20} values of 18.86 and 22.85 μmol/L, respectively. It is interesting to note that DIF and MOX are the third generation of quinolones with wider antimicrobial activity and stronger potency than those of the first and second generation ones. However, CIN, one of the first generation quinolones with limited antimicrobial activity, is the second toxic compound (with IC_{20} value of 35.02 μmol/L) among all the tested ones. This may attribute to more O atoms contained in CIN structure (a substituent of 1,3-dioxolane at 6- and 7-positions), and this will be explained later (Section 2.2). Hu et al. (2007) have found that the genotoxic potential of the earliest quinolones