Aquatic Toxicology 147 (2014) 92–104

Contents lists available at ScienceDirect

Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aquatox

Effects of the antibiotic enrofloxacin on the ecology of tropical eutrophic freshwater microcosms

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ARTICLE INFO

Article history:
Received 11 October 2013
Received in revised form
12 November 2013
Accepted 8 December 2013

Keywords:
Antibiotics
Enrofloxacin
Microcosms
Ecological risk assessment
Tropics

ABSTRACT

The main objective of the present study was to assess the ecological impacts of the fluoroquinolone antibiotic enrofloxacin on the structure and functioning of tropical freshwater ecosystems. Enrofloxacin was applied at a concentration of 1, 10, 100 and 1000 μg/L for 7 consecutive days in 600-L outdoor microcosms in Thailand. The ecosystem-level effects of enrofloxacin were monitored on five structural (macroinvertebrates, zooplankton, phytoplankton, periphyton and bacteria) and two functional (organic matter decomposition and nitrogen cycling) endpoint groups for 4 weeks after the last antibiotic application. Enrofloxacin was found to dissipate relatively fast from the water column (half-dissipation time: 11.7 h), and about 11% of the applied dose was transformed into its main by-product ciprofloxacin after 24 h. Consistent treatment-related effects on the invertebrate and primary producer communities and on organic matter decomposition could not be demonstrated. Enrofloxacin significantly affected the structure of leaf-associated bacterial communities at the highest treatment level, and reduced the abundance of ammonia-oxidizing bacteria and ammonia-oxidizing archaea in the sediments, with calculated NOECs of 10 and <1 μg/L, respectively. The ammonia concentration in the microcosm water significantly increased in the highest treatment level, and nitrate production was decreased, indicating a potential impairment of the nitrification function at concentrations above 100 μg/L. The results of this study suggest that environmentally relevant concentrations of enrofloxacin are not likely to result in direct or indirect toxic effects on the invertebrate and primary producer communities, nor on important microbially mediated functions such as nitrification.

1. Introduction

Antibiotics used in human and veterinary medicine can enter aquatic ecosystems directly, through the discharge of waste water treatment plant effluents or aquaculture residues, or indirectly, by leaching and runoff of agricultural soils amended with manure from livestock facilities (Ternes et al., 2004; Sarmah et al., 2006; Rico et al., submitted for publication). Over the last few years, a considerable amount of work has been done on assessing the occurrence and environmental fate of antibiotics in the aquatic environment, indicating that measured water concentrations are, in most cases, relatively low (i.e. from 0.001 μg/L to about 10 μg/L) (Kümerer, 2009). Acute and chronic laboratory studies suggest that antibiotics are not expected to result in direct toxic effects on fish and aquatic invertebrates at environmentally relevant concentrations (Robinson et al., 2005; Park and Choi, 2008). However, several experiments indicated that cyanobacteria and non-phototrophic microbial communities could be affected by antibiotic pollution at concentrations that are orders of magnitude lower than the threshold concentrations derived from toxicity data for standard test species (Maul et al., 2006; Ebert et al., 2011; Yergeau et al., 2012; Wunder et al., 2013). Possibly, effects of antibiotics on cyanobacteria could affect the community structure of primary producers, which might propagate to primary and secondary consumers (Rico et al., submitted for publication). Furthermore, the disruption of important ecosystem processes such as organic matter mineralization (Maul et al., 2006), nitrification (Klaver and Mattews, 1999), and/or degradation of organic pollutants (Näslund et al., 2008) could result in changes in water quality and might induce additional stress to aquatic organisms. To date, our knowledge on the

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http://dx.doi.org/10.1016/j.aquatox.2013.12.008

effects of antibiotics on ecological interactions is still very limited and, therefore, further research needs to be undertaken to assess the potential side effects of antibiotics on ecological functions and on the structure of aquatic communities in multitrophic systems.

Model ecosystem studies (i.e., microcosms and mesocosms) have been used in the risk assessment of pesticides and veterinary medicines since they provide more ecological realism than laboratory bioassays and allow the identification of potential interactions between aquatic communities and ecosystem functions (Van den Brink et al., 2005). The number of studies evaluating the fate and effects of antibiotics on aquatic model ecosystems is very limited, and all of them have been performed under temperate climatic conditions (e.g. Wilson et al., 2004; Knapp et al., 2005; Maul et al., 2006). Recent monitoring studies have detected antibiotic residues in several rivers impacted by urban and intensive animal production in (sub-)tropical regions of Asia (Yang et al., 2010; Shimizu et al., 2013; Rico et al., submitted for publication), suggesting that the study of the potential ecotoxicological effects of antibiotics in the tropical zone requires further attention.

The main objectives of the present study were (i) to get a better understanding on the potential direct and indirect toxic effects of antibiotic pollution on tropical aquatic ecosystems, (ii) to identify sensitive structural and functional endpoints for the risk assessment of antibiotics, and (iii) to assess whether the use of threshold concentrations derived from laboratory toxicity data would result in a sufficient level of protection for tropical aquatic ecosystems. For this, we assessed the effects of the fluoroquinolone antibiotic enrofloxacin on five structural (macroinvertebrates, zooplankton, phytoplankton, periphyton and bacteria) and two functional (organic matter decomposition and nitrogen cycling) endpoint groups in outdoor freshwater microcosms in tropical Thailand. Enrofloxacin was chosen as test compound because of its broad use in livestock and aquaculture production in tropical countries (e.g. Lampang et al., 2007; Rico et al., 2013), and because of the availability of data on its environmental fate and aquatic toxicity (Knapp et al., 2005; Robinson et al., 2005; Park and Choi, 2008; Ebert et al., 2011; Rico et al., submitted for publication). In our study, enrofloxacin was applied in daily pulses for a period of 7 days to eutrophic microcosms, simulating exposure patterns in tropical ecosystems receiving aquaculture effluents that contain enrofloxacin residues (Rico and Van den Brink, 2014). Enrofloxacin shows antibacterial activity against a broad spectrum of (Gram-positive and Gram-negative) bacteria and is believed to act by inhibiting bacterial DNA gyrase or topoisomerase IV, thus preventing bacterial DNA synthesis and reproduction (Hooper, 1999). Under environmental conditions, enrofloxacin is rapidly deethylated to form ciprofloxacin (Knapp et al., 2005), which is an antibiotic that has been listed as critically important for its use in human medicine (WHO, 2011). The occurrence of antibiotics such as enrofloxacin and ciprofloxacin in the environment has raised concerns about their selective pressure on clinically relevant bacteria and the development of antibiotic resistance (Suzuki and Hoa, 2012), and therefore the assessment of their degradation and transformation under tropical conditions adds crucial information to perform refined exposure assessments.

2. Materials and methods

2.1. Experimental design

The present experiment was performed in ten outdoor microcosms at the Faculty of Fisheries of Kasetsart University (KU, Bangkok, Thailand). Each microcosm consisted of a PVC tank (top diameter: 122 cm; bottom diameter: 101 cm; total depth: 80 cm; water depth: 63 cm; water volume: 600 L) initially filled with approximately 3 cm of silica-based fine gravel (1–2 mm diameter) extracted from natural rivers in the north of Thailand, and tap water pre-stored for 1 week to allow dissipation of possible chlorine residues. An aeration system was installed in each microcosm in order to provide mixing of the water during the experimental period. The experiment was performed during March and April 2012 (dry season). The weather conditions during the experimental period were: air temperature 32 (24–40) °C (mean, minimum–maximum), relative humidity 63 (50–75)%, and daily precipitation 1.7 (0–37) mm (rained on 19% of days) (Don Muang Weather Station, Bangkok, Thailand). The microcosms were stocked with plankton and macroinvertebrates collected from freshwater outdoor tanks located at the Ornamental Fish Facilities of KU, from a water reservoir at KU, from the water canal located at the Asian Institute of Technology (AIT, Bangkok, Thailand) described in Daam and Van den Brink (2011), and from outdoor freshwater tanks located at the hatchery of the AIT. These sampling sites were selected because they were uncontaminated sources that showed a relatively high biodiversity of phytoplankton and invertebrates native to Thailand. The stock of the macroinvertebrates was made up by distributing the same number of animals into each microcosm, and the stock of plankton by introducing equal volumes of concentrated plankton sample into each microcosm. The planktonic and macroinvertebrate communities were allowed to establish themselves for a period of 4 weeks prior to the application of the test substance. During this period, water was exchanged between microcosms biweekly in order to homogenize the structure of the communities between the systems. Nitrogen (1.4 mg/L as urea) and phosphorus (0.18 mg/L as triple super phosphate) were added biweekly to the systems according to the recommendations provided by Daam and Van den Brink (2011) during the entire experimental period. The resulting experimental systems were plankton dominated and showed a high eutrophication level, mimicking uncontaminated aquatic systems receiving nutrient-rich effluents from aquaculture or livestock production areas which may be contaminated by antibiotic residues.

2.2. Application of the test substance

Enrofloxacin was applied to the microcosms in daily pulses (at around 4 pm) at a nominal concentration of 1, 10, 100 and 1000 μg a.i./L during a period of seven days (starting on April 3, 2012). The selected dosing scheme tried to simulate exposure regimes in aquatic ecosystems resulting from antibiotic treatments used in aquaculture or livestock production. The enrofloxacin application was performed in eight microcosms in duplicate replicated treatments, while the remaining two microcosms were used as controls. Enrofloxacin stock solutions (667 mg/L) were prepared daily with enrofloxacin powder purchased from Sigma–Aldrich (purity ≥98%, Lot Number: 0001369030). In order to dissolve the enrofloxacin crystals, the weighted amount of the compound was introduced with distilled water in a volumetric flask and sonicated for 30 min at 45 °C. Subsequently, 200 μL of ammonia solution (25%, v/v ammonia) were introduced in the volumetric flasks. The solutions were shaken gently by hand and then sonicated for another 15–30 min under the same temperature conditions until the compound was completely dissolved. Dosing solutions of 0.60, 6.03, 60.3, and 603 mg/L were created by diluting aliquots of the stock solutions in 1 L of distilled water. Finally, the prepared dose solutions were poured over the water surface of the microcosms and mixed by stirring with a wooden stick.

2.3. Sampling and analytical verification

The concentration of enrofloxacin and ciprofloxacin (main by-product of enrofloxacin) were determined in water samples