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Bioaccumulation, tissue distribution and joint toxicity of erythromycin and cadmium in Chinese mitten crab (*Eriocheir sinensis*)

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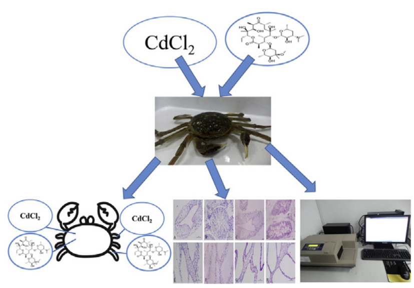
HIGHLIGHTS

- Determined bioaccumulation and tissue distribution of erythromycin and cadmium in *Eriocheir sinensis*.
- Cadmium and erythromycin bioaccumulation decreased as gill > hepatopancreas > muscle > ovary.
- The highest cadmium bioaccumulation reached 1.15 mg/g dry weight in gill.
- Cadmium increased erythromycin bioaccumulation in crab tissues.
- Significantly positive correlation between acetylcholinesterase and erythromycin residues in hepatopancreas.

GRAPHICAL ABSTRACT



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ABSTRACT

The bioaccumulation of erythromycin (ETM) and cadmium (Cd) in Chinese mitten crab (*Eriocheir sinensis*) and subsequent toxicity on pathological changes and enzymatic activities were investigated during 21-day exposure to ETM, Cd, and Cd + ETM mixture. The bioaccumulation of Cd and ETM residues in crab tissues decreased as gill > hepatopancreas > muscle > ovary, with higher Cd bioaccumulation than ETM. The highest Cd bioaccumulation in crab reached 1.15 mg/g dry weight in gill and 461.29 μg/g in hepatopancreas, on the 14th day of Cd treatment. Cd exposure promoted the bioaccumulation of ETM in four tissues. ETM exposure caused tubular vacuolization in epithelial and edema and degeneration of hepatic ducts in hepatopancreas, and disconnected gill epithelial layer and indistinctly cellular structure in gill. During Cd exposure, mitochondria acted as a main biomarker to identify the damage, including reduced and swollen mitochondria, and broken mitochondrial structure. Moreover, Chinese mitten crab showed defence capability against ETM and Cd exposure by physiological adjustment of metabolic enzymes glutathione S-transferase activity.

1. Introduction

In recent years, pharmaceuticals and personal care products (PPCPs) have received growing attention as emerging organic pollutants for their possible threats to aquatic environment and

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human health (Bu et al., 2013; Chen et al., 2017). As an important group of PPCPs, antibiotics have been found to be ubiquitous in the aquatic environment throughout the world (Ahmed et al., 2015). Sulfonamides, chloramphenicols, fluoroquinolones, tetracyclines, and macrolides e.g. sulfadiazine and erythromycin (ETM), have been detected in wastewater, surface water and groundwater in Europe, North America and China (Barnes et al., 2008; García-Galán et al., 2010; Hu et al., 2010; Chen and Zhou, 2014). The most commonly reported antibiotics included triclosan, sulfamethoxazole, lincomycin and ETM (Lapworth et al., 2012). ETM was listed in a new contaminant candidate list (CCL-3) by the US Environmental Protection Agency (Richardson and Kimura, 2007). The concentrations of ETM in Chinese surface water were similar to the global levels at < 1 μg/L, except for Haihe River in Tianjin and Shijing River in Guangzhou at up to 3.7 μg/L (Gao et al., 2012; Bu et al., 2013). ETM was one of the most frequently detected pharmaceuticals in the effluent of sewage treatment plants and in river water (Zhou et al., 2012; Chen and Zhou, 2014).

The modernization of industry and the presence of intensive human activities in urban areas, such as vehicle emissions and industrial discharges, have exacerbated heavy metal contamination in urban soils (Sun et al., 2010). As the most persistent pollutants in the ecosystem, the long-term input of heavy metals could result in surface water and groundwater contamination because of their resistance to decomposition in natural condition (Belhaj et al., 2016; Sara et al., 2017). In addition to river water pollution, some coastal zones are highly polluted, e.g. the Bohai Sea (China) where the concentrations of heavy metals e.g. As, Cd, Cr and Hg are high especially in estuarine water, sediment and organisms (Gao et al., 2014). In the Pearl River Estuary, which is the largest estuary in Southern China, heavy metal pollution by Cr, Ni, Pb and Zn was widely detected in sediments (Yu et al., 2010). In the sediments of Yangtze River Estuary, the contamination of heavy metals followed a declining order: As > Cr > Cd > Ni > Mn > Pb > Zn > Cu, with the pollution magnitude decreasing from rivers to adjacent sea (Wang et al., 2014).

Crabs are typical benthic organisms, and are considered as good indicators reflecting the contamination levels in water and even in surface sediment, because crabs are known to reside in the surficial sediment and feed on benthic prey living among contaminated sediments (Zhao et al., 2012; Cheng et al., 2017). However, there have been few studies, up to now, on the bioaccumulation and potential toxicity of antibiotics in benthic organisms (Garcia-Galan et al., 2017). Recently Cheng et al. (2017) studied the bioaccumulation of sulfadiazine in Chinese mitten crab (*Eriocheir sinensis*) which is one of the most important cultured crustacean species, and is a native freshwater crab species distributed in the Yangtze Estuary, China. Moreover, antibiotics are widely used for the treatment of bacterial diseases in aquaculture production causing systematic adverse effects (Limbu et al., 2018), and administered concentrations in feeds are as high as 500 mg/kg-body weight (Liu et al., 2017). ETM has been widely used as antibacterial agents in aquaculture (Kümmerer, 2009). On the other hand, Cd is a heavy metal with significant bioaccumulation in the food chain, reaching 33.7 ± 4.5 μg/g in guano samples from the Xisha archipelago, South China Sea (Liu et al., 2012). A survey by Sun et al. (2010) found the maximum concentrations of Cd reached 2.08 mg/kg and 69.3 mg/kg in soils from Shenyang (China) and Ibadan (Nigeria), respectively.

Due to the highly complex aquatic environment, aquatic organisms are frequently exposed to multiple contaminants. Thus, the aims of this study were to determine the bioaccumulation and tissue distribution of ETM and Cd (singly and jointly) in Chinese mitten crab's hepatopancreas, gill muscle and ovary, providing valuable data on the correlation between benthic organisms and the surrounding environment of aquaculture areas. Secondly, histopathology and ultrastructure of exposed tissues were examined to qualitatively reveal the potential toxicities of ETM and Cd on Chinese mitten crab. In addition, the activities of four metabolic enzymes i.e. acetylcholinesterase (ACHE), glutathione S-transferase (GST), metallothionein (MT) and superoxide dismutase (SOD), were quantified after contaminant exposure to investigate the changes in metabolic activities.

2. Materials and methods

2.1. Materials

Organic solvents (acetonitrile, methanol, acetone, formic acid) of high performance liquid chromatograph (HPLC) grade, and concentrated perchloric acid and nitric acid (65%) of suprapur grade were purchased from Merck (Darmstadt, Germany). The ultrapure water used in the sample pretreatment and instrumental analysis was prepared from a Milli-Q Gradient system (Millipore, Billerica, MA, USA). The standards of the target compound ETM and its internal standard roxithromycin (RTM)−d_7 were of the highest purity (>99%) commercially available and both were supplied by Dr. Ehrenstrofer GmbH (Augsburg, Germany). The stock solutions of ETM and RTM-d_7 standards were prepared in methanol at 1 g/L and then diluted with methanol to 10 mg/L. The stock solutions of $CdCl_2$ (1 g/L) were diluted with ultrapure water before using. Standard solutions of ETM and $CdCl_2$ were stored at −20 °C and 4 °C, respectively prior to use. The GF/B glass microfiber filters (47 mm diameter, pore size 1 μm) from Whatman (Maidstone, UK) were soaked in methanol for 1 h, and washed twice with methanol to remove interferences before usage. All glassware used in the experiments was washed with tap water and deionized water, and then heated at 500 °C for 4 h prior to use.

2.2. Experimental animals and study conditions

Healthy female adult crabs (n = 120, 100 ± 10 g wet weight) were collected from Tongchuan aquatic product market in Shanghai, China, and acclimated for 1 week at 18 ± 2 °C in filtered and constantly aerated freshwater. Crabs were fed once daily, a maintenance diet of a commercial crab feed (Xiangsheng Aquatic Products Corporation, Shanghai) without any antibiotics.

All experiments were conducted under the conditions described for acclimation. The exposure concentration was designed as 1 mg/L based on the early investigations of administered concentrations of ETM in feeds and the concentrations of Cd in soils. Prior to exposing, each aquarium (67 cm length, 45 cm width, 35.5 cm height) was filled with 15 L aerated water and 1.5 mL of standard solution (10 g/L) before stirring. The dissolved oxygen concentration in the aquarium was 6.5 ± 0.2 mg/L with constant aeration. There were three experimental groups: ETM group (1 mg/L), Cd group (1 mg/L), Cd + ETM group (1 mg/L each), and a control group (no contaminant), all with 2 replicate aquaria containing 12 crabs in each aquarium. After exposing for 4, 7, 14 and 21 days, two crabs were taken from each aquarium, respectively. Tissues (hepatopancreas, gill, muscle and ovary) were harvested, quickly frozen in liquid nitrogen, and stored at −80 °C prior to freeze drying. The hepatopancreas of Chinese mitten crab is the major digestive gland, not only involved in the synthesis and secretion of digestive enzymes related with food digestion and absorption, but also in lipid and carbohydrate metabolism and immunity function. Gill is particularly vulnerable to external contaminants and the damage has been linked to the mortality correlating with the physiological responses of crustaceans. Additionally, muscle, ovary and hepatopancreas tissues are the major edible portions of Chinese mitten