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Influence of multi-walled carbon nanotubes on the effects of roxithromycin in crucian carp (*Carassius auratus*) in the presence of natural organic matter

Zhenhua Yan [a], Guanghua Lu [a, b, *], Hongwei Sun [a], Binni Ma [a]

[a] *Key Laboratory of Integrated Regulation and Resource Development on Shallow Lakes of Ministry of Education, College of Environment, Hohai University, Nanjing 210098, China*

[b] *College of Hydraulic and Civil Engineering, XiZang Agricultural and Animal Husbandry College, Linzhi 860000, China*

HIGHLIGHTS

- Multi-walled carbon nanotubes accelerated the roxithromycin accumulation in fish.
- Metabolism and oxidative stress were also enhanced by multi-walled carbon nanotubes.
- Multi-walled carbon nanotubes alter the bioavailability of roxithromycin in fish.
- Biofluid accelerate the release of roxithromycin from multi-walled carbon nanotubes.

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ABSTRACT

Carbon nanotubes are increasingly entering the aquatic environment and may interact with other co-existing contaminants, such as antibiotics. However, whether these interactions may affect their bioavailability in aquatic organisms is the subject of considerable debate. The primary objective of this study was to assess the risks arising from the coexistence of roxithromycin (ROX) and multi-walled carbon nanotubes (MWCNTs) in waters containing natural organic matter (NOM), focusing on the distribution and bioaccumulation of ROX in crucian carp (*Carassius auratus*), and the related biochemical status. There were no significant differences in ROX bioaccumulation in fish following exposure to ROX with and without NOM. However, the further addition of MWCNTs significantly facilitated the bioaccumulation of ROX in the liver (32–80%), gill (15–74%), intestine (51–113%), and bile (15–67%) in different exposure periods. Meanwhile, a 0.3-fold increase in the metabolic enzyme activity and oxidative stress in the liver were markedly accelerated by the co-exposed MWCNTs compared to ROX alone. The findings imply that the ROX adsorbed on MWCNTs may be a higher threat to fish than ROX alone. The high and fast release of ROX from MWCNTs in bile salts and serum albumin may contribute to the enhancement in bioaccumulation and bioactivity of ROX in fish with MWCNTs.

1. Introduction

The residue of antibiotics in different environments is an issue of increasing concern. The use of antibiotics in both humans and animals to treat diseases and as promoters in aquaculture and livestock husbandry have led to their continuous input into the environment and, in turn, various adverse effects on non-target organisms (Ding et al., 2016; Liu et al., 2017). The ubiquitous presence of antibiotics accelerates the development of antibiotic-resistant genes among microorganisms, even at relatively low concentrations (Dantas et al., 2008), resulting in humans and animals more susceptible to these microbes. Moreover, the specific design of antibiotics can also interact with the non-target organism via the same mode of action as that in target species, potentially impacting their growth, development and reproduction (Yan et al., 2016).

Among these antibiotics, roxithromycin (ROX) is widely used in respiratory tract and soft tissue infections and later released into

* Corresponding author. Key Laboratory of Integrated Regulation and Resource Development on Shallow Lakes of Ministry of Education, College of Environment, Hohai University, Nanjing 210098, China.
E-mail address: ghlu@hhu.edu.cn (G. Lu).

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sewage treatment plants for further elimination. However, due to the very low elimination efficiency in these plants (Göbel et al., 2007), increasing quantities of ROX are being discharged into different waters, with detected concentrations reaching as high as 3.7 μg/L in surface water and 5 μg/L in wastewater (Luo et al., 2011; Zhou et al., 2014). Recently, a great concern of the potential impact of ROX in waters has been raised due to the bioaccumulation potential in aquatic organisms and the trophic transfer in aquatic food chains (Liu et al., 2014; Ding et al., 2015). To prevent these negative effects and sufficiently remove antibiotics from waters, a serial of strong adsorption materials with extremely small size and unique physicochemical property (e.g., carbon nanotubes [CNTs]) were applied in different fields (Zou et al., 2012; Peng et al., 2014).

Certainly, as the application of CNTs rapidly increases, the potential for their release into the aquatic environment is inevitable during their use, waste disposal and recycling (Peng et al., 2014). Thus, a great deal of concerns has been dedicated to investigating their biosafety to environmental and human health (Petersen et al., 2011). Considering the concentrations of contaminants on the CNTs are always many orders of magnitude higher than those in the surrounding environment, the intake of a minor amount of CNTs by an organism could result in abundant CNT-associated contaminants in its body (Song et al., 2014; Sanchís et al., 2015). Hence, it is reasonable to suspect that CNTs will greatly modify the bioavailability and toxicity of the coexisting contaminants. Previously, several attempts have been made to understand the effects of CNTs on the bioavailability of associated contaminants in various organisms; results, however, are inconclusive and can even be contradictory. Song et al. (2014) reported an antagonistic effect *in vitro* where the addition of CNTs to a culture medium significantly reduced the cytotoxicity and bioactivity of ethinylestradiol in cultured cells. Conversely, the bioaccumulation of phenanthrene in fish was significantly facilitated by coexistent CNTs (Su et al., 2013). While the adsorption of ethinylestradiol to CNTs was irreversible, the ethinylestradiol might not be desorbed from CNTs into water, thereby decreasing the bioavailability of ethinylestradiol in cells. Conversely, the reversible adsorption of phenanthrene on CNTs leads to a high release of the adsorbed contaminants to dissolved forms, elevating their bioaccumulation in organisms. It therefore suggests that the desorption process of contaminants from CNTs may play a key role in these different changes. Additionally, Yang and Xing (2007) found that the presence of biomolecules in biofluids may significantly alter the desorption processes of contaminants from CNTs. Because of this, it is challenging to fully understand the interactive effects of contaminants and CNTs in organisms, and more attention should be paid to the desorption process of CNT-associated contaminants in biofluids.

In waters, the widespread presence of natural organic matter (NOM) could also alter the behaviour of the CNT-associated contaminant in organisms by affecting the transformation and fate of CNTs in natural systems (Hyung and Kim, 2008; Wang et al., 2011a; Sun et al., 2013). Because NOM has many binding ligands and can interact with the surface of nanomaterials, the competition between NOM and contaminants for the same binding ligands in CNTs could elevate the levels of dissolved contaminants in waters (Kim et al., 2009; Linard et al., 2015). However, an increase in binding sites in CNTs may also appear because of better dispersion of CNTs in the presence of NOM, accelerating the sorption process of contaminants from waters to CNTs. Though researchers have noticed the effects of NOM in the behaviour of contaminants on CNTs, relatively little attention has been given to the influence of CNTs on the biological effects of contaminants in waters containing NOM.

Therefore, the primary objective of this study was to investigate the potential of multi-walled CNTs (MWCNTs) to alter the distribution, bioaccumulation and toxicity of ROX in crucian carp (*Carassius auratus*) in the presence of NOM. As a particular category of CNTs, MWCNTs consist of multiple rolled layers of graphene with a higher thermal, chemical, electrical and mechanical stability. Moreover, MWCNTs also exhibit advantages over other CNTs, such as a simple preparation process, low price and large production scale leading to wider application (Yu et al., 2016). MWCNTs were thereby selected as the target CNTs. The concentrations of ROX in different tissues were quantified, and the biochemical status of fish, including metabolic and oxidative status, were measured to evaluate toxicity. Moreover, the desorption process of ROX from MWCNTs was simultaneously conducted in different simulated biofluids to understand the interaction in fish preliminarily.

2. Materials and methods

2.1. Materials and animals

Standard roxithromycin (CAS 80214-83-1) with a purity of 99% was purchased from Labor Dr. Ehrenstorfer (Augsburg, Germany). MWCNTs synthesized via chemical vapour deposition technique (>99.9% purity) were obtained from XFNANO Materials (Nanjing, China). According to the manufacturer's instructions, the average inner and outer diameter of MWCNT was 8 nm and 3 nm, respectively, with a length 0.5–2 μm. These characteristics were further verified by dynamic light scattering and transmission electron microscope. Bile salts were obtained from J&K Chemical (Shanghai, China), with a mixture of sodium cholate and sodium deoxycholate (1:1). Bovine serum albumin (BSA) and humic acids (used as NOM) were purchased from Sigma Chemical (St. Louis, USA). Methanol (HPLC grade) was obtained from Merck Serono (Darmstadt, Germany). Ultrapure water was purified with a Millipore Milli-Q integral water purification system (Milford, USA).

The stock solution of NOM was prepared in ultrapure water, with the final pH value of 7.4 adjusted by sodium hydroxide. The working solutions of NOM were further diluted with test water to a final concentration of 1 mg/L, because the normal concentration of NOM typically ranges from 0.1 mg/L to 20 mg/L in surface water (Rodrigues et al., 2009). Moreover, the concentration of NOM in each treatment was further verified using dissolved organic carbon analysis (OI Analytical, USA). The stock solution of ROX was prepared in methanol and stored in the dark at −20 °C, and was further diluted to the working concentrations in NOM solution. To investigate the effect of NOM on the bioavailability, ROX was also prepared in test water without NOM. The stock solution of MWCNTs were prepared in NOM solution by sonication for 24 h at 25 °C with an output of 100 W and 40 kHz, as described by Alpatova et al. (2010). The working solution was further diluted with NOM solution to achieve final concentrations by sonication. In these solutions, MWCNTs were well suspended with a little aggregate. All working solutions were allowed to come to equilibrium for 24 h before use.

Juvenile carp (14 ± 1.5 g, 8 ± 1.2 cm) were obtained from the Nanjing Institute of Fishery Science (Nanjing, China) and later acclimatized for at least two weeks prior to the exposure in dechlorinated tap water at steady state (temperature at 20 ± 1 °C; pH at 7.4; dissolved oxygen > 95%). All fish were fed once a day at the rate of 6% of body weight. Faeces and uneaten food were removed daily.

2.2. Exposure treatments

Fish were randomly exposed to the following different treatments: ROX alone (ROX, 4 and 20 μg/L), ROX with NOM (ROX + NOM, 4 and 20 μg/L), MWCNTs with NOM (CNT, 100 μg/L), and ROX plus MWCNTs with NOM (ROX + NOM + CNT, 4 + 100 μg/L