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Inhibitory effect of naringin on microcystin-LR uptake in the freshwater snail *Sinotaia histrica*

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ABSTRACT

Gastropods are an important food source for aquatic animals, and have been demonstrated to transfer microcystin (MC) to higher trophic levels through the food web. In this study, we performed an oral administration experiment to evaluate whether naringin can inhibit MC-LR uptake in the freshwater snail *Sinotaia histrica*. We also observed the effect of MC-LR on the organizational pathology of the hepatopancreas in *S. histrica*. Following exposure to cells of *Microcystis ichthyoblabe*, *S. histrica* showed vacuolization and separation of the basal lamina from cells in the hepatopancreas. Initial treatment with 1 mM naringin resulted in the prevention of MC-LR uptake rate by approximately 60% over 8 days, whereas initial treatment with 10 mM naringin suppressed microcystin uptake in 2 days, despite an increase in MC-LR levels in the snail from days 5 to 8. With continuous treatment of 10 mM naringin, the uptake prevention rate was 100%. Overall, we observed a strong inhibitory effect against MC-LR with naringin treatment. This study provides a potential mechanism to prevent the uptake of microcystin in the aquatic food web, thereby limiting its toxicity in cyanobacterial bloom-polluted areas where the environment can be controlled and may have further applications in the aquaculture of gastropods.

1 Introduction

Cyanobacterial blooms can create significant water quality and human health problems because some species of cyanobacteria are capable of producing toxins. Microcystins (MCs) are the most commonly reported cyanotoxin and occur in numerous variants, many of which are potent hepatotoxins (Carmichael, 1997). MCs are usually associated with freshwater environments, and their bioaccumulation by aquatic animals, including mussels, snails, zooplankton, shrimps, frogs and fish has been reported in several studies (Amorim and Vasconcelos, 1999; Ozawa et al., 2003; Ferrão-Filho et al., 2002; Chen et al., 2004; Gkelis et al., 2006; Xie et al., 2004, 2005, 2007).

Gastropods are an important food source not only for fish but also for waterfowl, crayfish and amphibians. Hence,

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serious ecological implications exist for the transfer of MCs to higher trophic levels through the food web (Ozawa et al., 2003). Recently, the decline of a gastropod community was demonstrated over a 10-year study in a lake due to recurrent MC-producing cyanobacteria proliferations (Gérard et al., 2008, 2009). In addition, Lance et al. (2010a) demonstrated that both abundance and MC tissue concentrations of gastropods varied greatly according to the occurrence of MC-producing cyanobacteria.

MCs are strong hepatotoxins that cause sickness and death in both humans and animals (Carmichael, 1997). MCs have been shown to be taken up into hepatocytes via OATPs (organic anion transporting polypeptides), which are expressed on the hepatocyte membrane (Fischer et al., 2005). In isolated rat hepatocytes, algal toxins have been shown to induce a disruption of various cytoskeletal elements (Eriksson et al., 1989a; Falconer and Yeung, 1992; Ohta et al., 1992; Blankson et al., 1995) as well as to elicit apoptotic cell death (Bùe et al., 1991). Since algal toxins are protein phosphatase inhibitors, which cause an over-phosphorylation of cellular proteins (Bialojan and Takai, 1988; Haystead et al., 1989), some of their biochemical effects can be counteracted using protein kinase inhibitors. For example, naringin, a grapefruit flavonoid, has been found to protect rat hepatocytes against toxin-induced disruption of the cytoskeleton and cell death in a recent study (Blankson et al., 2000). Moreover, two weeks oral administration of naringin (80 mg/kg) significantly inhibited 3-nitropheniolic acid induced neuronal apoptosis in rats via inhibition of Bax-Bcl-2 pathway (Gopinath et al., 2011). Naringin (4′,5,7-trihydroxy flavanone 7-rhamnoglucoside), isolated from the grape and citrus fruit species, has immense therapeutic potential (Adil et al., 2014). Naringin was reported to possess anti-inflammatory, anti-oxidant, anti-ulcer, anticancer, antiatherogenic, hepatoprotective, and neuroprotective activities (Adil et al., 2014; Benavente-Garcia and Castillo, 2008). It also was defined as a major and selective clinical inhibitor of organic anion-transporting polypeptide 1A2 (OATP1A2) (Bailey et al., 2007). Naringin has been used commercially in certain food products and been given safely to humans in several studies (Bailey et al., 1993a,b; Ameer et al., 1996). However, limited information is available about naringin in the aquatic environment. It is poorly known whether naringin is capable of protecting aquatic animals in MC-polluted areas.

In this study, we performed an oral administration experiment to evaluate whether naringin can inhibit MC-LR uptake in the freshwater snail *Sinotaia histrica*. We also examined the effect of MC-LR on the organizational pathology of the hepatopancreas in *S. histrica*.

2 Materials and methods

2.1. Sampling site

Lake Suwa (36°3′N, 138° 5′E), a hypereutrophic shallow lake, is located in Nagano Prefecture, central Honshu, Japan, at an altitude of approximately 760 m. The surface area of the lake is 13.3 km^2, with a maximum depth of 6.8 m and an average depth of approximately 5 m. Due to its shallow depth, vertical mixing by wind occurs even during the summer. A dense bloom of *Microcystis* containing MC-RR and MC-LR has occurred during the summer since the 1970s (Park et al., 1998).

2.2. Experiment design

Two experiments were conducted, one was an organizational pathology experiment, and the other one was a naringin inhibition experiment. Naringin (>95% pure, C27H32O14, MW 580.53) was purchased from Sigma–Aldrich (Oakville, Ontario, Canada). Seven glass culture vessels (each 10 L in volume) were used in experiments, and the vessels were aerated with an air pump. Treatments assigned to each vessel are described below. Snails (*S. histrica*) (10 in each vessel, mean weight of 3 ± 1 g, the snail density is similar to the density that was found in Lake Suwa, 2010) were collected from Lake Suwa in October 2010. Prior to the experiment, the snails were fed with commercial carp food and acclimated in the vessels (room temperature: 23 ± 1 °C, water temperature: 20 ± 1 °C, dechlorinated tap water) for one week. The cyanobacterial species used as food for *S. histrica* was *Microcystis ichthyoblabe*. A unialgal culture of *M. ichthyoblabe* strain TAC95 (Tsukuba Algal Collection, National Museum of Nature and Science, Tokyo, Japan) was grown in 10 L of MA medium (*Microcystis aeruginosa* medium) at 23 ± 1 °C under illumination at ca. 16 μmol m^{-2} s^{-1} and a 12:12 h light:dark cycle. *Microcystis* cultures were harvested during the late exponential growth phase or the early stationary growth phase. The strains were cultured according to Watanabe (1996) for MC-LR production. *M. ichthyoblabe* strain TAC95 produces only microcystin-LR (Yokoyama and Park, 2003). The *Microcystis* cells were lyophilized, and the MC-LR was extracted using methods described by Park et al. (1998). To quantify the number of cells, the samples were agitated by gentle ultrasonication to disrupt the colonies into single cells. *Microcystis* cells were quantified microscopically using an improved Fuchs–Rosenthal hemacytometer (KAYAGAKI marks grid volume: 1/16 mm^2, depth 1/5 mm).

In the organizational pathology experiment, there were three treatments, including one control and two types of exposures (no replicates for control and 3 replicates for each exposure treatment). Control l contained no *M. ichthyoblabe* and no nagrigin exposure; Exposure 1 (E1) contained no *M. ichthyoblabe* and 10 mM naringin (5.81 g/L), which was placed into the vessel each day throughout the experiment (8 days); Exposure 2 (E2) contained *M. ichthyoblabe* at cell densities of approximately 1.0×10^7 cells/mL and no narigin exposure. After 8 days, three snails (out of ten) were removed from each treatment for histological investigation. The snail tissues were removed from the shell, and hepatopancreas samples were fixed in 10% formalin. Hepatopancreas samples were then sliced into serial 3- to 5-μm-thick longitudinal sections and stained with hematoxylin and eosin (H&E) (Martoja and Martoja-Pierson, 1967). The hepatopancreas sections were imaged via an optic microscope using 100× to 400× magnification. 25 sections from each of the three snails per exposure were then evaluated to qualitatively and quantitatively assess the MC-induced pathology.

In the naringin inhibition experiment, there were four treatments, including one positive control (control 2) and three exposures (A, B, C) (3 replicates for positive control and each