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Effects of food availability on the trade-off between growth and antioxidant responses in *Caenorhabditis elegans* exposed to sulfonamide antibiotics

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HIGHLIGHTS

- In controls, low food availability caused trade-off between growth and antioxidants.
- Sulfonamide exposure at low food availability biased trade-off toward antioxidants.
- Sulfonamide exposure with food inhibited growth more than without food.
- Sulfonamides stimulated intestinal catalase at high and low food availability.

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ABSTRACT

Adverse effects of sulfonamide antibiotics (SAs) include growth inhibition and antioxidant activation which showed trade-off effects. Yet, the influence of food availability on such effects have not been thoroughly investigated. *Caenorhabditis elegans* were exposed to four SAs at high and low food availabilities which were represented by the optical densities of bacteria at 600 nm. The nematode feeding, growth and antioxidants including superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) were determined. Results showed that the control nematodes at low food availability had less growth and greater antioxidant responses than the nematodes at high food availability. In SA exposure, the nematode growth in the presence of food (at both high and low food availability) was less than that in its absence, supporting the role of food as an exposure pathway. The nematode growth at low food availability showed significantly greater inhibition than at high food availability ($p < 0.05$). The nematode antioxidants showed stimulations, and CAT had the greatest stimulation. Moreover, the stimulation on CAT at low food availability were significantly higher than those at high food availability ($p < 0.05$). That is to say, SA exposure at low food availability further biased the trade-off effects towards more energy investment in antioxidant with less in growth. Further studies on the expression levels of CAT encoding genes demonstrated that cells in intestines were the main antioxidant response sites, which further supported the contributions of food to the observed toxicities.

1. Introduction

Sulfonamide antibiotics (SAs) are widely used in treatments of human and animal infectious diseases. After their incomplete metabolism, antibiotics reach environmental matrices (*e.g.*, soil, sediment and waterbodies) via feces/urine and through effluents of wastewater treatment plants where they were not efficiently eliminated (Binh et al., 2018). At environmental μg/L levels, they can screen and strengthen the antibiotic resistance (Szekeres et al., 2017), and result in adverse effects on various environmental organisms (Liu et al., 2018; Zhou et al., 2018). Accordingly, more and more intentions are paid to explore the hazards of SAs and the

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mechanism underlying such un-intended effects.

Among the adverse effects of antibiotics, growth inhibitions and developmental latency were widely observed in plant, photobacteria, microalgae, crustaceans and zebrafish (Liu et al., 2018). Such effects were usually accompanied with the enhancement of antioxidant responses (Liang et al., 2015; Riaz et al., 2017; Yan et al., 2018). The antioxidants, *e.g.*, superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) are essential elements of organisms to survive challenging environment (Yu et al., 2012). Notably, growth and antioxidant responses were well connected with each other, and they even showed trade-off effects in natural (Monaghan et al., 2009) and challenging environments (Yu et al., 2016).

Notably, food availability, which is one essential variable in the actual exposure conditions, can significantly influence the effects on growth and antioxidant responses. Our previous studies reported the inhibition of sulfadiazine (SD), sulfapyridine (SP), sulfamethoxazole (SMX) and sulfamethazine (SMZ) on the growth of *Caenorhabditis elegans* (one free-living nematode) (Yu et al., 2011, 2013), while Liu et al. (2013) reported that SMX at similar concentrations stimulated the nematode growth. The absence of food in our studies and the presence of food in the study of Liu et al. might be the reason for such differences. Food availability influenced effects of haloperidol and lindane on antioxidant capacity in *Daphnia magna* (Furuhagen et al., 2014), and those of copper on *C. elegans* (Yu et al., 2012). Supplementation of particular food even overcame the trade-off among indices including antioxidants (Takahashi et al., 2017).

Moreover, the contributions of food to the toxic effects of pollutants are widely debated. On the one hand, food functioned as one exposure pathway (Li et al., 2017), and its presence provoked more exposure risk and toxicities than those without food, *e.g.*, greater growth inhibition of copper on *C. elegans* (Yu et al., 2012) and more uptake of nanoparticles by intestinal cells (Lichtenstein et al., 2015) in the presence of food than those in its absence. On the other hand, food provides elements for tissue repair and growth (Mao et al., 2018), and therefore protects the organisms against the toxicities, *e.g.*, less locomotion inhibition of metals on *C. elegans* in presence of food than in its absence (Boyd et al., 2003). However, no attempt has been made so far to assess the combined effects of antibiotic exposures and food availability on growth and antioxidants and the potential trade-off effects.

In the present study, *C. elegans* were exposed to SAs including SD, SP, SMX and SMZ at high and low food availability. Growth and antioxidants including SOD, CAT and GSH were determined in both control and SA exposed nematodes. Further studies were performed on the expressions of CAT encoding genes searching for the antioxidant response sites. Results demonstrated that SA exposure and low food availability provided a platform to show the trade-off effects between growth and antioxidant responses. Such trade-off effects indicated the adaptation strategy of nematodes in challenging environments and partially explained the multigenerational toxicities of SAs. Further studies are needed to better evaluate the effects of SAs on organisms in the actual environments.

2. Materials and methods

2.1. Chemicals

Sulfadiazine (SD, CAS RN: 68-35-9, $C_{10}H_{10}N_4O_2S$), sulfapyridine (SP, 144-83-2, $C_{11}H_{11}N_3O_2S$), sulfamethoxazole (SMX, 723-46-6, $C_{10}H_{11}N_3O_3S$) and sulfamethazine (SMZ, 57-68-1, $C_{12}H_{14}N_4O_2S$) were purchased from Sigma-Aldrich (USA). The stock solutions of SAs were prepared with sterile K-medium containing 1% dimethyl sulfoxide (DMSO, Amresco, USA), and they were maintained at 4 °C in the dark (Yu et al., 2011, 2013). The SA concentrations in the stock solutions were 200.0 mg/L, and two lower concentrations (*i.e.*, 2.0 and 0.02 mg/L) were diluted with K-medium containing 1% DMSO. The concentration of 0.02 mg/L was chosen to represent environmental levels of SAs (Ji et al., 2012). The concentrations of SAs were determined on high performance liquid chromatography (Agilent 1200, USA, with Agilent TC-C18 column) using 80% H_2O (0.1% formic acid) and 20% acetonitrile (v/v) as the mobile phase (1.0 mL/min) (Yu et al., 2013). The actual exposure concentrations were half of the aforementioned values due to the experimental settings described below.

2.2. Preparation of the nematodes

Wild-type *C. elegans* (N2) and its food, *E. coli* OP50, were provided by Department of Biochemistry and Molecular Biology, Southeast University Medical School, Nanjing, China. Nematode growth medium (NGM), and lysogeny broth medium (LBM) were used to culture nematodes and bacteria, respectively (Brenner, 1974; Yu et al., 2013). After an incubation in LBM at 120 rpm overnight at 37 °C, the bacterial suspensions (150 μL) were spread across each NGM plate followed by a further incubation overnight at 37 °C to form a bacterial lawn. Then, the NGM plates were used to culture the nematodes at 20 °C.

Gravid nematodes were bleached for 5–10 min at room temperature in fresh clorox solutions containing 0.5 M NaOH and 1% NaOCl (diluted from sodium hypochlorite solution, Analytical reagent, Aladdin Industrial Co., China) to obtain age-synchronized eggs. Then, the eggs were washed in sterile K-medium and transferred onto NGM plates with a bacterial lawn. The nematodes were incubated for 36 h at 20 °C to reach L3 stage (Emmons et al., 1979; Van Gilst et al., 2005). The L3 nematodes were harvested and fasted in K-medium for 2 h to digest and evacuate the food in the guts, and then they were used in chemical exposure (Reinke et al., 2010; Yu et al., 2013). Copper chloride was used as a reference toxicant to verify that the nematodes were healthy and their responses to toxicants remained statistically constant throughout our study (Yu et al., 2017).

2.3. Exposure

The toxicity experiments were performed according to previous studies with some modifications (Yu et al., 2017). Preliminary experiments showed no significant differences between nematodes in the solvent control (1% DMSO in K-medium) and the absolute control (K-medium). In the formal experiments, three SA concentrations (200, 2.0 and 0.02 mg/L) and one solvent control (1% DMSO in K-medium) were arranged in the 24-well culture plates with six wells for each concentration or control. Each well typically contained 500 μL SMX solutions or solvent control, 300 μL K-medium containing approximately 100 L3 nematodes and 200 μL bacterial suspensions in K-medium.

The bacterial suspensions were obtained according to previous report (Yu et al., 2012). Briefly, *E. coli* OP50 were incubated in LBM at 120 rpm overnight at 37 °C, and then were collected into 15 mL sterile centrifuge tubes which were centrifuged at 4000 rpm for 5 min at 20 °C. After the supernatants were discarded, the pellets were resuspended with sterile K-medium. Next, the centrifuge tubes were stored at −26 °C overnight and thawed at 4 °C for 8 h. The freeze-thaw cycle was repeated another four times to kill the bacteria. The validation of such killing method was confirmed by a further incubation with the treated bacteria in LBM at 120 rpm overnight at 37 °C where the optical densities at 600 nm (OD_{600}) showed no significant changes between the beginning and the end of the incubation. Then, the bacterial suspensions were mixed with