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Internal bioavailability of waterborne and dietary zinc in rainbow trout, *Oncorhynchus mykiss*: Preferential detoxification of dietary zinc

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ABSTRACT

Internal bioavailability of zinc (Zn) in the liver and intestine of juvenile rainbow trout (*Oncorhynchus mykiss*) was investigated following exposure to 150 or 600 μg l^{-1} waterborne Zn, 45 or 135 μg dietary Zn g^{-1} fish day^{-1}, and a combination of 150 μg l^{-1} waterborne and 45 μg dietary Zn g^{-1} fish day^{-1} for 40 days. At the organ/tissue level the concentrations of Zn in the intestine were 15–25 times those in the liver, and a transient partially additive accumulation was observed in the intestine. At the subcellular level Zn distribution was ubiquitous with the accumulation pattern in the liver being heat stable proteins (HSP) > mitochondria > nuclei-cell debris > heat denaturable proteins (HDP) > microsomes-lysosomes (M-L) = NaOH resistant fraction, while in the intestine it was nuclei-cell debris > HSP > NaOH resistant fraction > mitochondria > M-L = HDP. The majority of cellular Zn was biologically available in both tissues with the estimated putative metabolically active pools (MAP) being 65–78% in the liver and 59–75% in the intestine. We show, for the first time, preferential streaming of dietary Zn into the metabolically detoxified pool (MDP) and that of waterborne Zn to the MAP. Specifically, in the liver the cellular Zn load shifted to MAP in the waterborne Zn and combined exposures, and to the MDP in the dietary Zn exposures. In the intestine the proportion of detoxified Zn increased in the dietary Zn-exposed fish but was unchanged in the waterborne and combined exposures despite elevated concentrations. Under the experimental conditions used in the present study, uptake from the food drove the accumulation of Zn in the intestine while uptake from both sources was important in the liver, consistent with its central location. Further, additive accumulation in the MDP (hepatic and intestinal), intestinal HSP, and hepatic HDP was revealed. Overall these data suggest that fish are better insulated from dietary than waterborne Zn toxicity.

1. Introduction

Zinc is an essential trace metal required in more than 1000 structural, regulatory and catalytic proteins necessary for normal physiology, growth, and development in all animals (Vallee and Falchuk, 1993; Eide, 2006; Maret and Krężel, 2007). For this micronutrient, the dose–response relationship is stereotypically U-shaped denoting high incidence of adverse effects at extremes of the dose axis due to deficiency (low dose) and toxicity (high dose). Therefore a requirement for safe Zn utilization by animals is homeostatic regulation of its cellular concentrations. In fish the maintenance of Zn homeostasis under varying ambient concentrations involves the modulation of uptake, accumulation, and excretion (Bury et al., 2003). However, because uptake of metals occurs via two main pathways (branchial and gastrointestinal) in fish, understanding Zn homeostasis and toxicity presents unique challenges. It has been observed that following Zn uptake from water via the gills and from food via the gastrointestinal tract (Hogstrand and Wood, 1996; Clearwater et al., 2002) the ensuing distribution is tissue-specific and uneven. Thus delineating the source (water or food) may reveal the fate and effects of accumulated Zn. A second feature that confounds our understanding of Zn (and other metals) homeostasis and toxicity is the existence of a variety of physiological handling strategies to deal with accumulated metals. These handling strategies render some cellular pools of metals unavailable for physiological use or to cause toxicity. Indeed it is now widely acknowledged that the bioavailability of metals for biological functions or toxicity depends on their speciation and intracellular localization (Campbell, 1995; Vijver et al., 2004; Campbell et al., 2005).

Speciation is the partitioning of a metal among specific chemical forms (species), distinguished by isotopic composition, oxidation or electronic state, and complex or molecular structure (Templeton et al., 2000). It is dictated by the chemical composition of the medium in which the metal is found. For example, in aqueous media metal ions generally bond with extant organic and inorganic com-

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plexing ligands leading to decreased bioavailability and toxicity (Campbell, 1995). This is the basis of the free-ion activity model (FIAM, Campbell, 1995) which contends that the biological effects of metals are best predicted by the activity of the free metal ion or labile metal complexes. Typically, measurement of metal speciation and bioavailability in aqueous external environment is achieved by a variety of approaches including equilibrium calculations using thermodynamic modeling programs such as MINEQL+ (Schecher and McAvoy, 1994), WHAM (Tipping, 1994), and MINTEQA2 (Brown and Allison, 1987). The importance of these modeling approaches in aquatic toxicology is portrayed in the role they played in the development of the biotic ligand model (BLM), a novel environmental monitoring and regulatory tool which was recently approved for copper and is under development for other metals. Based in part on this "success story" of speciation analysis in the external environment, a popular opinion in the aquatic toxicology community is that knowledge of speciation of accumulated metals would greatly improve the link between exposure, accumulation and effects. To achieve this goal, the critical tissue residue concept (CTR; McCarty and Mackay, 1993) has been proposed. For metals however, CTR does not appear to be a good predictor of toxicity in part because the metal handling strategies exhibited by various organisms affect bioavailability of accumulated metals but are not considered (Rainbow, 2002; Ahearn et al., 2004; Vijver et al., 2004). Thus procedures for measuring bioavailability that consider the physiological fate of accumulated metals are necessary for effective application of CTR to metals. To this end differential centrifugation (Wallace et al., 2003) has been employed to measure bioavailability of metals in the internal environment. This approach is analogous to sequential extraction of metals in sediments (Campbell et al., 2005) and permits the quantification and detection of changes in sizes of cellular pools of metals leading not only to a better understanding of the fate of internalized metals but also a more precise linkage of accumulation and effects.

The objective of this study therefore was to quantify the biologically available Zn in rainbow trout liver and intestinal tissues following chronic waterborne and dietary Zn exposures to better understand the interactions between the two primary routes of metals uptake. We predicted that (i), subcellular partitioning and physiological fate of Zn in a centrally located organ such as the liver would reflect uptake from both water and food, while in the intestine it would reveal the intracellular fluxes and ligand interactions important in the regulation of Zn uptake and excretion, and (ii), upon internalization Zn would be handled similarly regardless of source. Other than our recent publication (Sappal et al., 2009), the handling, interactions, and relative contribution of waterborne and dietary Zn have not been studied at the subcellular level in fish. Lastly, this study sought to identify a common subcellular target that accumulates Zn irrespective of the tissue and route of uptake. Identification of such a target would facilitate exposure characterization during risk assessment of metals.

2. Materials and methods

2.1. Fish

Juvenile rainbow trout (mean initial weight 14 g) were obtained from Ocean Trout Farm, Brookvale, PE, and acclimated to laboratory conditions for 1 month at the Atlantic Veterinary College (AVC) Aquatic Research Facility. Laboratory conditions consisted of a single 250-l tank supplied with aerated flow-through well water containing: Na 47.1, Cl 137.3, Ca 58.8, Mg 27.6, hardness 260 (as $CaCO_3$) and dissolved organic carbon 1.5, all in mg l^{-1}. The water pH and temperature were 7.5–8.0 and 11.5–12 °C, respectively. During the acclimation period, fish were fed 2% bw daily ration of commercial granulated 3.0 grade trout chow (Corey Feed Mills Ltd., Fredericton, NB) containing crude protein 46% (minimum), crude fat 26% (minimum), crude fibre 1.7% (maximum), Ca 1.3% (actual), phosphorous 1.0% (actual), Na 0.6% (actual), vitamin A 4400 i.u. kg^{-1} (minimum), vitamin D3 3200 i.u. kg^{-1} (minimum), and vitamin E 2000 i.u. kg^{-1} (minimum).

2.2. Experimental diets

Experimental diets were made in-house by supplementing unpelleted trout chow with the required amount of Zn calculated to deliver nominal 45 and 135 μg Zn g^{-1} fish day^{-1} in the low and high dietary Zn exposure groups, respectively. This was achieved by making diets containing nominal Zn concentrations of 1500 and 4500 mg kg^{-1}, which were kept at −20 °C until use. The actual Zn concentrations of the diets (means ± SEM, $n = 5$) determined by flame atomic absorption spectroscopy (FAAS) were 228 ± 6, 1441 ± 76 and 4820 ± 200 mg kg^{-1} for the control, low and high Zn diets, respectively.

2.3. Exposure regime

Experimental fish were exposed to 150 or 600 μg l^{-1} waterborne Zn, 45 or 135 μg g^{-1} fish day^{-1} dietary Zn, and a combination of 150 μg l^{-1} waterborne and 45 μg g^{-1} fish day^{-1} dietary Zn for 40 days. A control group with no Zn added to the water and food was maintained. The elevated Zn concentrations were approximately 5 and 20 times the control levels in both the water and food. All the Zn exposure groups and the control were in triplicates and the experiment was carried out in a battery of eighteen 10-l tanks in a completely randomized block.

Briefly following the 1-month laboratory acclimatization period, the fish were equally ($n = 16$–17) distributed into the experimental tanks without bias using a random number set. Exposure to 150 and 600 μg l^{-1} waterborne Zn was achieved via a constant drip (3.0 ml min^{-1}) of stock solutions containing, respectively, 50 and 200 mg l^{-1} Zn ($ZnSO_4 \cdot 7H_2O$; Sigma–Aldrich, Oakville, ON) from Mariotte bottles into head tanks receiving 1000 ml min^{-1} of AVC well water. Uniform distribution of Zn in the head tanks was achieved by constant vigorous aeration. There were three head tanks designated low, high, and combined, and each of them supplied three experimental tanks at a flow rate of 333 ml min^{-1}. Actual concentrations of Zn in the low, high waterborne and combined exposure experimental tanks were 179.90 ± 9.43 ($n = 49$), 649.50 ± 14.80 ($n = 53$) and 189.60 ± 10.60 ($n = 49$), μg l^{-1}, respectively. Note that the US Environmental Protection Agency's water hardness corrected numerical limit of total allowable waterborne Zn for this water is 710 μg l^{-1}. Measured in-tank Zn concentrations in the control, low dietary and high dietary experimental tanks were 34.71 ± 14.13 ($n = 32$), 31.23 ± 9.37 ($n = 32$) and 36.27 ± 12.35 μg l^{-1} ($n = 30$), respectively. Dissolved oxygen and ammonia were 9.95 ± 0.06 ($n = 42$) and 0.44 ± 0.04 ($n = 30$) and mg l^{-1}, respectively, while the pH was 7.89 ± 0.08 ($n = 30$), throughout the experimental period.

During the experiment, fish were fed the designated diets at a ration of 1.5% wet bw twice a day, once in the morning (09:00–10:00 h) and again in the evening (21:00–22:00 h). The fish were allowed to feed for 1 h, after which fecal material was siphoned off. Visual examination during the feeding revealed that the fish readily ingested the diets. Water samples collected after 1 h of feeding were analyzed for Zn concentrations in all the treatments and no significant leaching of Zn from food into the water was observed.