Characterization of dietary Ni uptake in the rainbow trout, *Oncorhynchus mykiss*

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ARTICLE INFO

ABSTRACT

We characterized dietary Ni uptake in the gastrointestinal tract of rainbow trout using both *in vivo* and *in vitro* techniques. Adult trout were fed a meal (3% of body mass) of uncontaminated commercial trout chow, labeled with an inert marker (ballotini beads). *In vivo* dietary Ni concentrations in the supernatant (fluid phase) of the gut contents averaged from 2 μmol l^{-1} to 24 μmol l^{-1}, and net overall absorption efficiency of dietary Ni was approximately 50% from the single meal, similar to that for the essential metal Cu, adding to the growing evidence of Ni essentiality. The stomach and mid-intestine emerged as important sites of Ni uptake *in vivo*, accounting for 78.5% and 18.9% of net absorption respectively, while the anterior intestine was a site of net secretion. Most of the stomach uptake occurred in the first 4 h. *In vitro* gut sac studies using radiolabeled Ni (at 30 μmol l^{-1}) demonstrated that unidirectional uptake occurred in all segments, with area-weighted rates being highest in the anterior intestine. Differences between *in vivo* and *in vitro* results likely reflect the favourable uptake conditions in the stomach, and biliary secretion of Ni in the anterior intestine *in vivo*. The concentration-dependent kinetics of unidirectional Ni uptake *in vitro* were biphasic in nature, with a saturable Michaelis–Menten relationship observed at 1–30 μmol l^{-1} Ni ($K_m = 11$ μmol l^{-1}, $J_{max} = 53$ pmol cm^{-2} h^{-1} in the stomach and $K_m = 42$ μmol l^{-1}, $J_{max} = 215$ pmol cm^{-2} h^{-1} in the mid-intestine), suggesting mediation by a channel or carrier process. A linear uptake relationship was seen at higher concentrations, indicative of simple diffusion. Ni uptake (at 30 μmol l^{-1}) into the blood compartment was significantly reduced in the stomach by high Mg (50 mmol l^{-1}), and in the mid-intestine by both Mg (50 mmol l^{-1}) and Ca (50 mmol l^{-1}). In both regions, kinetic analysis demonstrated reductions in J_{max} with unchanged K_m, suggesting non-competitive interactions. Therefore the Mg and Ca content of the food will be an important consideration affecting the availability of Ni.

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1. Introduction

Ni is found in bodies of water impacted by both natural processes through the erosion and weathering of rocks such as silicates (Bencko, 1983) and industrial processes such as mining, electroplating and smelting (Eisler, 1998; Ptashynski and Klaverkamp, 2002; Brix et al., 2004). Ni is a transition metal which is considered to be essential micronutrient in most plant species, bacteria, invertebrates and perhaps mammals (Bencko, 1983; Nielsen et al., 1993). Ni essentiality has been well established in terrestrial organisms (see review by Phipps et al., 2002). In microorganisms and plants, Ni-containing enzymes are involved in nitrogen fixation, hydrogen metabolism and carbon cycling (Ragsdale, 2005). In fish, its essentiality status remains uncertain (Muyssen et al., 2004), but recent evidence does indicate that it is homeostatically regulated in at least one freshwater fish species, the rainbow trout (*Oncorhynchus*

mykiss) (Chowdhury et al., 2008). However, Ni may also be toxic. For example, in mammals Ni has been shown to cause apoptotic damage to cells (Park et al., 2007), allergies (Bocca et al., 2007) and renal disorders (Denkhaus and Salnikow, 2002).

Relative to other metals, waterborne Ni levels causing toxicity to aquatic organisms are relatively high (i.e. toxicity is low), and rates of Ni entry into the organism across the gills are also high (Meyer et al., 1999; Pane et al., 2003a,b, 2004a; Brix et al., 2004; Deleebeeck et al., 2007). Waterborne Ni appears to be primarily a respiratory toxicant with marked inhibitory effects on branchial gas exchange during acute high level exposures (Pane et al., 2003a,b), and more subtle pathological effects during chronic low level exposures (Pane et al., 2004b). The acute effects are due to external Ni, not internal Ni (Pane et al., 2004a). The exact mechanism of these effects is not known, but perhaps may reflect inflammatory swelling of the branchial epithelium associated with allergic reactions.

However there is a growing awareness that metal uptake and toxicity from the diet may be more important in many field situations (Dallinger and Kautzky, 1985; Meyer et al., 2005), and this may well be the case with Ni (reviewed by Ptashynski et al., 2001). Histo-pathological lesions in livers, kidney, and intestine have been recorded in response to high concentrations of Ni in the

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diet (Ptashynski et al., 2001, 2002). Fish readily take up Ni from spiked food (Ptashynski et al., 2001; Ptashynski and Klaverkamp, 2002), and when Ni is infused into the stomach (Chowdhury et al., 2008). In isolated intestinal sac preparations from trout, uptake rates of Ni were greater than those of five other metals (Ojo and Wood, 2007). However, at present, nothing is known about the mechanism(s) of gastro-intestinal Ni uptake in fish, apart from the fact that it can be homeostatically down-regulated after chronic exposure to elevated waterborne Ni levels (Chowdhury et al., 2008).

Cellular mechanisms of Ni transport have not been completely elucidated. However, some studies suggest that Ni may use Ca and/or Mg channels (see Eisler, 1998 for a review), or the proton-coupled divalent metal transporter variously known as DCT1, DMT1, or Nramp2 (Gunshin et al., 1997). Ni interacts antagonistically with both Ca and Mg in a number of different systems. Ni is an effective blocker of several different types of Ca channels (McFarlane and Gilly, 1998; Todorovic and Lingle, 1998; Lee et al., 1999). Costa (1991) outlined the competitive binding behaviour between Ni and Mg in mammalian studies, and there is similar evidence at many other phylogenetic levels including bacteria (Kaltwasser and Frings, 1980; Smith et al., 1995), mold (Adiga et al., 1962), yeast (Ross, 1995), invertebrates (Pane et al., 2003a,b), and amphibians (Brommundt and Kavaler, 1987).

In fish, there is now molecular and physiological evidence for the occurrence of DMT1 (Dorschner and Phillips, 1999; Donovan et al., 2002; Bury et al., 2001, 2003; Cooper et al., 2006; Nadella et al., 2007), though the possible involvement of this promiscuous divalent metal carrier in Ni transport (Gunshin et al., 1997) has not been investigated. Both Ca and Mg protect against waterborne Ni toxicity (Meyer et al., 1999; Deleebeeck et al., 2007). There are also some indications for Ca and Mg interactions on Ni transport in fish, with greater evidence for the latter. Pane et al. (2003a,b, 2005) reported that plasma [Mg], but not plasma [Ca], was marginally depressed during acute exposure of trout to high levels of waterborne Ni. This effect appeared to be associated with an inhibition of Mg reabsorption by the kidney (Pane et al., 2005). In *in vitro* tests, Ni uptake into renal brush border membrane vesicles was inhibited by Mg at a 100:1 Mg to Ni molar ratio, and by both Mg and Ca at a 1000:1 molar ratio, and these properties were altered by chronic sublethal Ni acclimation (Pane et al., 2006a,b). In marine toadfish (*Opsanus beta*), Ni infusion lowered Mg concentrations in intestinal fluids (Pane et al., 2006c).

With this background in mind, the aim of the present study was therefore to determine the mechanistic nature of Ni transport in the gut of rainbow trout. We hypothesized that Ni uptake would be carrier-mediated and would be significantly reduced by either or both Mg and Ca. Our first objective was to determine the normal levels of Ni in the fluid phase of the chyme, and the relative importance of the different segments (stomach, anterior, mid, and posterior intestine) in net Ni uptake *in vivo*, as a single meal (commercial trout chow) of normal Ni content passed through the digestive tract. A recently validated technique (Bucking and Wood, 2006a,b) which uses X-radiography of glass ballotini beads as an inert marker (McCarthy et al., 1992) was employed to provide a point of reference (a non-absorbed, non-secreted label) against which net Ni movements into and out of the chyme could be quantified. The approach has been used successfully to quantify the uptake of other divalent metals (Cu: Nadella et al., 2006a; Ca, Mg: Bucking and Wood, 2007).

Our further objectives were pursued using a now well-characterized *in vitro* gut sac preparation which allows metal uptake to be measured in a relatively short period (typically 2–4 h) during which transport rates are stable, and to be partitioned into mucus-bound, mucosal epithelium, and blood space components (Nadella et al., 2006a, 2007; Ojo and Wood, 2007). Our second goal was to compare Ni uptake rates and their partitioning in the different gut segments, and our third goal was to determine

the concentration-dependence of Ni uptake ('kinetics'). Saturation kinetics would suggest transporter involvement, whereas linear kinetics would suggest simple diffusion. The range of luminal Ni concentrations was selected based on the *in vivo* sampling experiment. A final objective was to assess the possible antagonistic effects of Ca and Mg on Ni uptake, using realistic levels of these cations known to occur in chyme (Bucking and Wood, 2007), and to investigate the nature of any interactions observed. To this end, possibly confounding effects of alterations in transepithelial potential (TEP; Nadella et al., 2007) were also assessed.

2. Methods

2.1. Experimental organisms

Adult rainbow trout (200–300 g) of both sexes were obtained from Humber Springs Trout Hatchery, Orangeville, Ontario, Canada. Rainbow trout were acclimated to their 500 L tanks for two weeks prior to the experiments in aerated, flowing, dechlorinated Hamilton (Ontario, Canada) tap water with a water composition of (in mmol l^{-1}) Na$^+$ = 0.5, Cl$^-$ = 0.7, Ca = 1.0, hardness $\sim$140 ppm as CaCO$_3$, pH $\sim$ 8; $12 \pm 2\,°C$. The background Ni concentration in the Hamilton tap water was 20–30 nmol l^{-1}. Fish were fed a 2% of body weight ration daily with Martin's commercial dried pellet feed (5 point; Martin Mills Inc., Elmira, ON, Canada, containing 41.0% crude protein, 11.0% crude fat, 3.5% crude fiber, 1% Ca, 0.85% total P, 0.45% Na). The measured concentration of Ni in the food was 431 ± 14 nmol g^{-1}.

2.2. In vivo determination of Ni uptake

Stored samples from the *in vivo* experiments conducted by Bucking and Wood (2006a,b, 2007) were re-analyzed for Ni content in the present study. The experimental procedures are briefly described below.

2.2.1. Diet preparation

Martin's commercial dried pellet feed (5 point) was minced using a Braun PowerMAX Jug Blender (Gillette Company; Massachusetts, USA). The fine powder was transferred to an automatic pasta maker (Ronco Inventions; California, USA) and 8.5 grade lead–glass ballotini beads (0.40–0.45 mm in diameter; Jencons Scientific, PA, USA Inc.) were added at a density of 4% dry food mass. The beads and food powder were mixed for 30 min, NANO pure-II water was then added (ratio 2:1) and the wet mixture was mixed for an additional 30 min to ensure even distribution of the beads throughout the wet food mixture. The mixture was then extruded and hand-shaped to resemble the 5 point trout chow the fish previously consumed. The pellets were air-dried and refrigerated at $-20\,°C$ until further use. Tests by Gregory and Wood (1998) showed that the ballotini beads did not affect the palatability of the food, which was readily consumed to the same extent as the normal food.

2.2.2. Feeding and sampling procedures

Rainbow trout were starved for one week prior to experiment to allow for gut clearance and then fed the pelleted food described in Section 2.2.1 until satiation. The mean meal size amounted to 3.06 ± 0.02% of body mass (Bucking and Wood, 2006b, 2007). Six fish were sacrificed with a cephalic blow at each of the seven time intervals (2, 4, 8, 24, 48, 72 h) and weighed, as chemical anesthesia induced vomiting in earlier trials. An incision was made above the pelvic fin at the esophagus to just above the anal fin at the rectum. The gastrointestinal tract was tied off using silk ligature at each boundary between the stomach and anterior (including the caeca), mid and posterior intestine to contain all the gut contents in the appropriate segment. The GI tract was then dissected out and