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Selenocompounds in juvenile white sturgeon: Evaluating blood, tissue, and urine selenium concentrations after a single oral dose

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ABSTRACT

Selenium (Se) is an essential micronutrient for all vertebrates, however, at environmental relevant levels, it is a potent toxin. In the San Francisco Bay-Delta, white sturgeon, an ancient Chondrostean fish of high ecological and economic value, is at risk to Se exposure. The present study is the first to examine the uptake, distribution, and excretion of various selenocompounds in white sturgeon. A combined technique of stomach intubation, dorsal aorta cannulation, and urinary catheterization was utilized, in this study, to characterize the short-term effects of Se in the forms of sodium-selenate (Selenate), sodium-selenite (Selenite), selenocystine (SeCys), L-selenomethionine (SeMet), Se-methylseleno-L-cysteine (MSeCys), and selenoyeast (SeYeast). An ecologically relevant dose of Se (~500 μg/kg body weight) was intubated into groups of 5 juvenile white sturgeon. Blood and urine samples were repeatedly collected over the 48 h post intubation period and fish were sacrificed for Se tissue concentration and distribution at 48 h. The tissue concentration and distribution, blood concentrations, and urinary elimination of Se significantly differ ($p \leq 0.05$) among forms. In general, organic selenocompounds maintain higher blood concentrations, with SeMeCys maintaining the highest area under the curve (66.3 ± 8.7 and 9.3 ± 1.0 μg h/ml) and maximum Se concentration in blood (2.3 ± 0.2 and 0.4 ± 0.2 μg/ml) in both the protein and non-protein bound fractions, respectively. Selenate, however, did not result in significant increase of Se concentration, compared with the control, in the protein-bound blood fraction. Regardless of source, Se is preferentially distributed into metabolically active tissues, with the SeMet treated fish achieving the highest concentration in most tissues. In contrast, Selenite has very similar blood concentrations and tissue distribution profile to SeCys and SeYeast. From blood and tissue Se concentrations, Selenate is not stored in blood, but taken up rapidly by the liver and white muscle. Urinary elimination of Se is form dependent and peaks between 3 and 12 h post intubation. A basic understanding of the overall Se absorption, distribution, and elimination is provided through monitoring tissue Se concentrations, however, conclusions regarding to the dynamics and the specific processes of Se metabolism can only be inferred, in the absence of kinetic information.

1. Introduction

At low concentrations, selenium (Se) is essential for animals (NRC, 2005). It is the catalytically active component of selenoproteins, mediating numerous important biological processes ranging from antioxidant protection to thyroid hormone production (Burk et al., 2003; Papp et al., 2007). At levels found in the environment, however, Se is a potent reproductive and developmental toxin (Lemly, 2002). Its disastrous effects on fish have been well demonstrated in the incidents at Belews Lake, NC (Lemly, 1985), where a mass disappearance of fish was observed. Subsequently, Se was identified as the likely cause of other freshwater fish declines (Moyle et al., 1992; Deforest et al., 1999; Hamilton, 1999).

In the San Francisco Bay-Delta (Bay-Delta), major sources of Se include waste discharges from petrochemical and industrial manufacturing operations and, in a larger proportion, irrigation runoff from agricultural activities in the San Joaquin Valley (Luoma and Presser, 2000; Lemly, 2004). Although Se from anthropogenic sources are mostly released as inorganics, rapid production of the organic forms (i.e., selenomethionine; SeMet), by microbial biotransformations, facilitate Se bioaccumulation and

Abbreviations: Se, selenium; Selenate, sodium selenate; Selenite, sodium selenite; SeCys, selenocystine; SeMet, L-selenomethionine; MSeCys, Se-methylseleno-L-cysteine; SeYeast, selenoyeast.

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biomagnification through the trophic levels (Fan et al., 2002; Hamilton, 2004).

The dominant bivalve, in the Bay Delta, *Corbula amurensis*, has high filtering capacity (Cole et al., 1992) and can retain Se to as much as 20 μg/g dry weight (dw; Linville et al., 2002), and estimations of generic bivalve Se concentrations in the Bay-Delta range as high as 28 μg/g dw during low flow seasons (Presser and Luoma, 2006). The ability of *C. amurensis* to accumulate Se, coupled with its high abundance, has led to high concentrations of Se in benthic feeding organisms (Schlekat et al., 2000, 2002). Several bivalve predators, including white sturgeon (*Acipenser transmontanus*), a fish species of high ecological and economic value, have tissue Se concentrations exceeding toxicity thresholds (Linville et al., 2002; Stewart et al., 2004; Davis et al., 2006).

White sturgeons are indigenous to the Pacific West Coast of North America, with the largest populations residing in the Fraser, Columbia, and Sacramento Rivers; the latter region includes the population in the San Francisco Bay-Delta (Moyle, 2002). Currently, California white sturgeons are at a State S2 status (low abundance, restricted range, and potentially endangered species), as determined by the California Department of Fish and Game (CNDDB, 2009), and are considered endangered by the American Fisheries Society (Jelks et al., 2008). As high Se concentrations have been found in the liver and muscle tissues of Bay-Delta white sturgeons and at levels not seen in other carnivorous fish species or in the surrounding water (Urquhart and Regalado, 1991; Linville et al., 2002), Se toxicity is a possible explanation to the recent decline in the abundance and distribution of white sturgeon population in the Bay-Delta (Luoma and Presser, 2000).

Although numerous studies have examined the toxicological effects of Se or Se tissue burden in fish, few had looked at the responses of initial Se exposure, which could provide a better understanding of the absorption, distribution, and elimination processes. Furthermore, data pertaining to white sturgeon, an evolutionary ancient Chondrostean fish with a morphology and physiology different from those of modern teleosts, are relatively scarce. Recently, Tashjian and Hung (2006) demonstrated the effectiveness of a newly developed combined technique of stomach intubation, dorsal aortic cannulation, and urinary catheterization, to examine changes in tissue Se concentrations in 48 h after a single oral intubation of graded levels of L-selenomethionine (SeMet) in white sturgeon. However, the study did not provide information on the effects of Se forms and the Se tissue distribution was insufficiently described as only two tissues were measured.

In this study, we provided a more comprehensive and comparative evaluation of the initial exposure to Se of white sturgeon, a benthic fish that is evolutionary distinct from modern teleosts and at a high risk from Se exposure, by using different Se forms. We hypothesize that the Se form has an effect on total Se blood concentration, tissue distribution, and urinary excretion in white sturgeon over a 48 h exposure period. Furthermore, the Se dose and form used in the current study are ecological relevant.

2. Materials and methods

2.1. Animal maintenance and experimental setup

White sturgeons, obtained from Sterling Caviar (Elverta, CA, USA), were maintained at the Center for Aquatic Biology and Aquaculture (University of California, Davis, CA, USA) for the duration of the experiment. Thirty-five juveniles (1.12 kg ± 0.03; mean ± standard error of mean (SEM)) were kept in outdoor 400 L circular fiberglass tanks supplied with aerated well water (18–19 °C) at a flow rate of 15 L/min. Fish were fed a commercial trout feed with Se at 0.6 μg Se/g dw. Fish were fasted for 24 h and then were fitted with an aorta cannula, a stomach tube, and a pair of urinary catheters, as described by Deng et al. (2000). Postoperative animals were transferred into indoor round tanks (400 L) with continuous water flow, restrained in triangular Plexiglas® chambers (21 cm sides and 90 cm in length), and allowed 48 h to recover. Animal operation and tissue sampling procedures complied with protocols approved by the Campus Animal Care and Use Committee.

2.2. Treatment and sampling

As a benthic predator, white sturgeon is potentially impacted by Se exposure (Luoma and Presser, 2000) and, therefore, is an appropriate model for the study. The Se dose used was calculated from the tissue concentration of *C. amurensis*, the major prey item of the Bay Delta white sturgeon, at a Se concentration of 20 μg Se/g (Linville et al., 2002), and at a dietary consumption rate of 2% body weight (BW)/day (Cui and Hung, 1995).

Groups of five sturgeons were orally intubated with a single dose of either 0 (control) or a target dose of 500 μg Se/kg BW (Sigma-Aldrich, St. Louis, MO, USA). Selenium was delivered as one of the two inorganic forms: sodium selenate (Selenate; 502 ± 20 μg Se/kg BW) or sodium selenite (Selenite; 494 ± 32 μg Se/kg BW), or as one of the four organic forms: selenocystine (SeCys; 486 ± 27 μg Se/kg BW), L-selenomethionine (SeMet; 496 ± 26 μg Se/kg BW), Se-methylseleno-L-cysteine (MSeCys; 514 ± 46 μg Se/kg BW), or selenoyeast (SeYeast; 507 ± 29 μg Se/kg BW). Starch gel, made from dissolvable potato starch (Sigma-Aldrich), was used as a carrier and the control.

Whole blood (1 ml) was taken at 0, 1.5, 3, 6, 12, 24, and 48 h post intubation from the same animal through the dorsal aortic cannula and replaced with an equal amount of fish heparin saline (Gisbert et al., 2003). The 0 h samples were taken immediately prior to Se intubation for baseline values. Urine was collected continuously from the paired urinary catheters, and samples were taken at the end of 6 time periods (0–1.5, 1.5–3, 3–6, 6–12, 12–24, and 24–48 h post intubation). Fish were killed at 48 h post intubation with an overdose of MS-222 (0.5 g/L, Argent Chemical Laboratories, Redmount, WA, USA). Gills, heart, spleen, liver, gastro-intestinal tract (GIT), kidneys, and a cubical section (~2 cm) of white muscle at the midpoint of the body were removed from each fish. The GIT was rinsed in saline solution. All samples were immediately frozen in liquid nitrogen and stored at −80 °C pending Se analysis. The remaining whole bodies (RWB) were weighed and stored at −20 °C pending Se analysis.

2.3. Selenium analysis

A tracer study by Krishnamurti et al. (1989), performed in ewes, reported two kinetically heterogeneous pools of blood ^{75}Se, in which the Se in the trichloroacetic acid (TCA) precipitate disappeared at a much slower rate than it did in the supernatant portion, which is protein free. Based on this information and the assumption that protein bound Se is highly available, in the current study, the sturgeon blood was separated into the protein-bound blood fraction (PB) and the non-protein bound (NPB) fractions by TCA extraction. PB was precipitated from 100 μL of whole blood through two washings with 100 μL 10% TCA dissolved in 0.1 N trace metal grade hydrochloric acid. Blood Se was analyzed separately from the PB and NPB fractions. Organs and RWB were freeze-dried and pulverized before analyses. Selenium concentrations were determined as described by Fan et al. (1998), with modifications. In brief, samples were microdigested in trace metal grade nitric acid at room temperature, derivatized with 2,3-diaminonaphthalene (Dojindo Laboratories, Kumamoto, Japan), and Se intensity measured with a florescence spectrophotometer (Perkin-Elmer, Buckinghamshire,