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Kinetic of Copper Accumulation and Elimination in Rockfish (*Sebastes schlegeli*) Tissues Exposed to Dietary Copper

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Experiments were carried out to investigate the accumulation and elimination changes in the tissue of juvenile rockfish (*Sebastes schlegeli*) after sub-chronic dietary Cu (0, 50, 125, 250 and 500 mg/kg) exposure for 60 days and depuration for 30 days. The profile of Cu accumulation in the tissue of rockfish was dependent on the exposure periods and Cu concentration. Liver of rockfish is a more important storage tissue than other tissues, and the order of Cu accumulation in tissues was liver > intestine > kidney > gill > muscle. The accumulation factors were increased with the exposure period in gill, intestine, liver, kidney and muscle. An inverse relationship was observed between the accumulation factor and the exposure concentrations in the gill, kidney and muscle. Cu elimination in tissues of rockfish were decreased with periods for the 30 days of depuration except kidney and muscle. The order of Cu elimination in organs during depuration was intestine > liver > gill.

Key words: *Sebastes schlegeli*, Dietary copper, Accumulation, Elimination

Introduction

As copper (Cu) is an essential metal for all organisms including fish, its function plays an important role in metabolism, and its concentration is well regulated (Cousins, 1985). However, Cu is one of the most toxic metals to fish and affects various blood parameters, growth parameters, enzyme activity, and reproduction (Horning and Nieheisel, 1979; Sorenson, 1991).

Fish accumulate Cu from polluted environment resulting in accumulation in their tissues and then redistribute. Cu accumulation between tissues varies depending on the source of uptake, food or waterborne (Sorensen, 1991). Although the sub-chronic toxic effects of metal on fishes are well documented, that is mostly in fishes exposed to waterborne metal, but few studied have been conducted on the effects of dietary metal (Handy, 1996). The realization that dietary uptake of metal is a major cause of long-term contamination in wild fish (Dallinger et al., 1987; Farag et al., 1995) has renewed interest in the nutritional and toxicological effects of metal in the food of fishes (Handy, 1996). Bioaccumulation patterns of metals in fish tissues can be utilized as effective indicators of environmental metal contamination. Moreover, tissues specific accumulation of metal has been proposed as a key indicator of chronic exposure (Larsson et al., 1985). So, the kinetics of Cu accumulation in fish tissues are obviously of great importance.

Several factors influence the elimination of metals from the tissues of fish. These include time, temperature, interacting agents, age of fish, metabolic activity of fish and biological half life of the metal (Larson et al., 1985; Heath, 1995; Nielsen and Andersen, 1996). Metal elimination studies are important in view of health protection, allowing the determination of the self-cleansing ability of contaminated organisms and assessment of biological half-lives for different metallic contaminants.

The rockfish (*Sebastes schlegeli*) is an economically important food fish in Korea that is commonly cultured in marine based cages (Jung, et al., 2001). Despite its importance, relatively little information is known on the effect of Cu, particularly through dietary exposure. The aims of present study is to investigate the Cu accumulation and elimination in tissue of the juvenile rockfish (*S. schlegeli*) after

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sub-chronic dietary Cu exposure.

Material and Methods

Diet preparation

Diets were supplemented with 0 (control), 50, 125, 250 and 500 mg/kg diet, using $CuSO_4 \cdot 5H_2O$ (Aldrich, USA). Copper sulfate pentahydrate was dissolved in 1000 mL acidified water and mixed well with other feed ingredients prior to pelleting. All ingredients were mixed and pelleted by a laboratory pellet machine without heating using a 2 mm diameter module (Baokyong Commercial Co., Korea). After processing, all the diets were packed into small bags and stored at -20℃ until they were fed to the fish use. Proximate analyses of the diets indicated a crude protein of 48.0%, crude lipid 5.0%, carbohydrate 4.0%, ash 15%, calcium 1.0% and phosphrous 2.7%.

Experimental fish and treatment

The juvenile rockfish (*S. schlegeli*) were obtained from rockfish nursery in Koge island, Korea. The rockfish were acclimated in 1000 L aerated running seawater tank for 1 month to the laboratory conditions (Table 1). Each tank received a flow of 7 L/min and was supplied with continuous aeration. Fish were fed Cu-free diet daily at a rate of 2% body weight (as twice 1% meals per day). After 1 month in acclimating tanks, fish were randomly transferred to 150 L tank (flow = 1.2 L/min), which were running water test with continuous aeration. After transferred to exposure tanks, the rockfishs were acclimated to experimental conditions. Fish were selected of mean body length 11.83±0.03 cm (mean±SE, n=600), body weight 26.02±0.23 g for the experiment of dietary Cu exposure. Each of the four experimental diets fed to rockfish for 60 days and then Cu-free diet fed to for another 30 days.

Table 1. Chemical components of seawater used in the dietary Cu exposure experiment (mean±SE).

Parameter	Value
Temperature (℃)	18.00 ± 0.20
pH	8.10 ± 0.20
salinity (‰)	32.70 ± 0.40
NH_4-N (μg/L)	12.66 ± 1.25
NO_2-N (μg/L)	1.37 ± 0.28
NO_3-N (μg/L)	9.62 ± 1.01
PO_4-P (μg/L)	5.05 ± 0.96
SS (mg/L)	5.62 ± 0.20
dissolved oxygen (mg/L)	6.74 ± 0.84
COD (mg/L)	1.52 ± 0.08
Cu (μg/L)	2.32 ± 0.12

Cu analysis

In experiment, fish were starved for 24 h prior to sampling to allow all feed to be excreted. The gill, intestine, kidney, liver and muscle were sampled every 10 days for analysis of metal concentration. Ten fish were removed each test concentration and the control. Tissue samples were dried at 65℃ and kept in a desiccators until digestion. Dry tissue was digested with 1:1 HNO_3 (Suprapur grade, Merck) and samples were fumed to near dryness on a hot plate at 120℃ for overnight. After digestion, the residue was dissolved in 20 mL of 0.2 N HNO_3 and kept in a refrigerator until analysis for trace metal. Cu concentrations of tissues were measured using a flame atomic absorption spectrophotometer (AAS, Perkin-Elmer 3300). Cu concentration in the tissues of rockfish were expressed as μg/g dry wt. Accumulation factor (AF) was used to compare the body burden of an organism with the degree of contamination in the water. The following definition is used here:

$$\text{Accumulation Factor (AF)} = \frac{[\text{Me}]_{\text{exp}} - [\text{Me}]_{\text{control}}}{[\text{Me}]_{\text{diet}}}$$

where $[Me]_{exp}$, $[Me]_{control}$, $[Me]_{diet}$ are the metal concentration in the experimental group, the control group and diet, respectively, in μg/g (Holwerda, 1991). Elimination rate (%) is used as a percentage decrease of initial value (60 days).

Statistical analysis

Data are expressed as means±standard error (SE). Statistics were using one-way analysis of variance (ANOVA) followed by Duncan's multiple comparisons test of mean values if significant differences were found. The probability limit $P<0.05$ was considered significant.

Results

Cu accumulation

Cu accumulation in gill was significantly increased with exposure period and concentration for the 60 days (Fig. 1a). During the first 10 days, Cu accumulation did not vary significantly. After 60 days of exposure, Cu accumulation values were approximately 2-fold higher than in the control at 250 mg/kg and 500 mg/kg Cu dietary exposure.

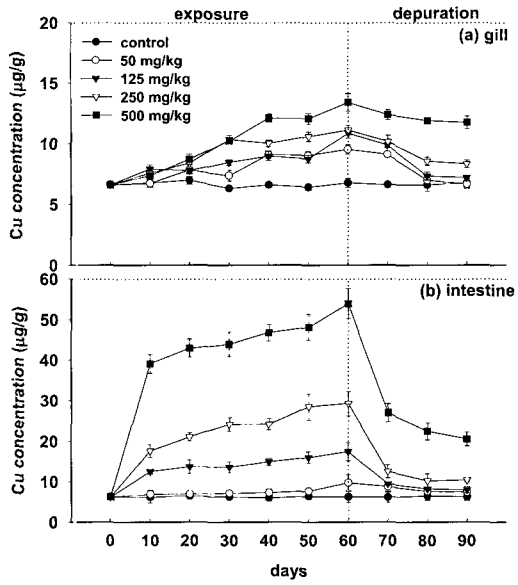

Fig. 1. Cu concentration over time in gill and intestine of *Sebastes schlegeli* exposed to dietary Cu for 60 days, followed by a depuration period of 30 days (mean ± SE).

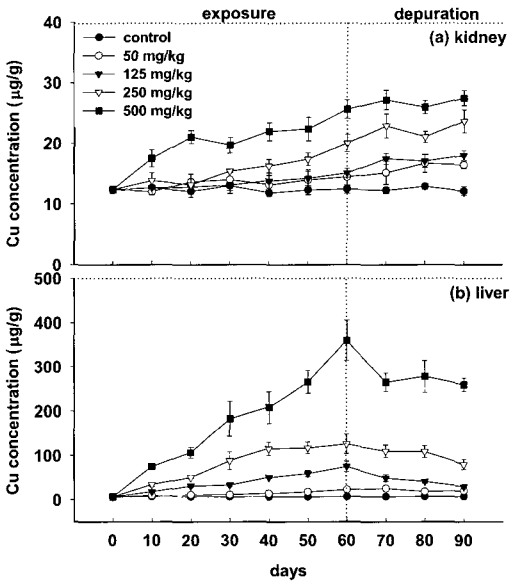

Fig. 2. Cu concentration over time in kidney and liver of *Sebastes schlegeli* exposed to dietary Cu for 60 days, followed by a depuration period of 30 days (mean ± SE).

For intestine, Cu accumulation profile depended upon the dietary Cu concentration (Fig. 1b). This profile showed two steps in the accumulation process. During first 10 days, Cu accumulation increased sharply reaching 2-6 fold (125, 500 mg/kg) against the control group. After that, Cu accumulation increased slowly reaching 3-9 fold value after end of Cu dietary exposure.

Cu accumulation in kidney were about an order of magnitude lower than those of the intestine and liver, and increased significantly after 10, 30 day at 500 mg/kg, 250 mg/kg, respectively (Fig. 2a). Unlike the intestine, Cu accumulation profile in the kidney showed a gradual increase at the 250 mg/kg and 500 mg/kg diet group.

Cu accumulation in liver was significantly in- creased with dietary exposure period and concen- tration for 60 days (Fig. 2b). During first 10 days, Cu concentration increased sharply reaching a value 10-fold increase compare to the control at 500 mg/kg Cu diet group. Cu accumulation was significantly increased after first 10 days at 125, 250 and 500 mg/kg Cu diet group. Finally, after 60 days of Cu dietary exposure, the Cu concentration in the liver was approximately 11-fold, 18-fold and 51-fold higher than in the control diet group at 125, 250 and 500 mg/kg Cu diet group, respectively. On the other hand, Cu accumulation in liver did not vary significant at 50 mg/kg Cu dietary exposure during the first 10 days. After 60 days of exposure, Cu concentration values were 22.80±0.28 μg/g.

Low Cu accumulation was observed in muscle and did not vary significant after 30 days with dietary exposure concentration (Fig. 3). After 40 days of exposure, Cu accumulation significantly increased at 500 mg/kg Cu diet group compared to the other diet group. After 60 days of Cu exposure, the order of Cu accumulation in tissues were liver > intestine > kidney > gill > muscle.

The accumulation factors are presented for gill, intestine, kidney, liver and muscle at 50, 125, 250 and 500 mg/kg exposure in Fig. 4. The accumulation factors were increased with the exposure period in gill, intestine, liver, kidney and muscle. An inverse relationship was observed between the accumulation factor and the exposure concentrations in the gill, kidney and muscle. Although the accumulation factor in the intestine and liver increased with exposure periods, it did not increase with exposure concentrations.

Cu elimination

Cu elimination in gill, intestine, kidney, liver and muscle of *S. schlegeli*, as a function of exposure time and exposure concentration are shown in Figs. 1-3. Cu elimination in tissues of the rockfish were

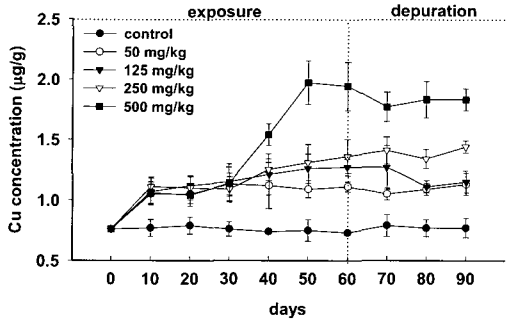

Fig. 3. Cu concentration over time in muscle of *Sebastes schlegeli* exposed to dietary Cu for 60 days, followed by a depuration period of 30 days (mean ± SE).

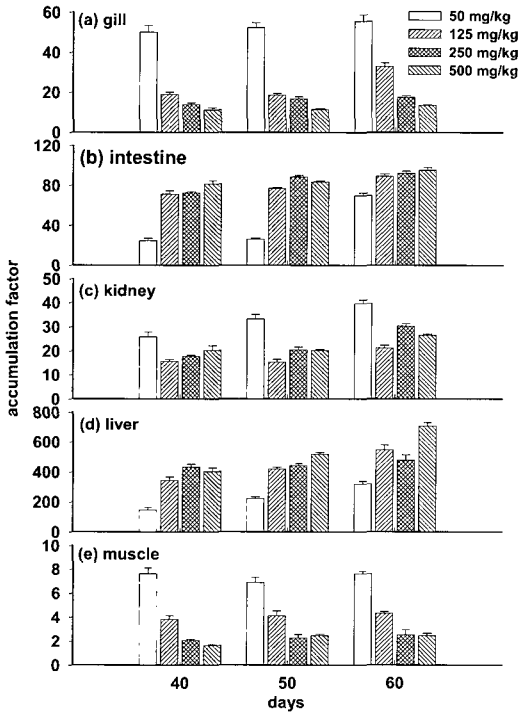

Fig. 4. Accumulation factor (AF) over time in gill, intestine, kidney, liver and muscle of rockfish, *Sebastes schlegeli* (mean ± SE), exposed to dietary Cu.

decreased with periods for the 30 days of depuration except kidney and muscle.

After exposed to dietary Cu, Cu concentration in the gill decreased slowly following the end of the exposure period. The elimination rates at the end of depuration periods were 25.12% for 250 mg/kg Cu exposure and 12.32% for 500 mg/kg Cu exposure (Fig. 1a).

Intestine showed fastest elimination rates of Cu at all concentration (Fig. 1b). During first 10 days of depuration, Cu concentration decreased sharply reaching a value 9.45±0.28, 12.54±1.62 and 26.95± 2.22 μg/g at 125 mg/kg, 250 mg/kg and 500 mg/kg Cu diet group, respectively. At the end of the depuration period, the Cu elimination rate were 53.94%, 64.41% and 61.89%, respectively.

Cu elimination in liver was about an order of magnitude lower than that of intestine (Fig. 2b). After 30 days of depuration, the elimination rate were 37.03% in the fish exposed to 250 mg/kg, and 27.93% in the fish exposed to 500 mg/kg.

The Cu concentration in the kidney continued to increased after the end of dietary Cu exposure (Fig. 2a). In muscle, Cu concentration slowly increased or remained constant (Fig. 3), and did not vary significantly ($P<0.05$). The order of Cu elimination in organs during depuration period was intestine > liver > gill.

Discussion

Metal accumulation in tissues of fish depends on exposure dose and time as well as other factors such as temperature, age of fish, interaction with other metals, water chemistry and metabolic activity of the fish (Pagenkopf, 1983; Heath, 1995). The profile of Cu accumulation among tissue in rockfish is dependent on the exposure periods and Cu concentration. Similar patterns of Cu accumulation were also shown in other aquatic animals (Miller et al., 1993; Berntssen et al., 1999; Kamunde et al., 2002).

In this study, Cu accumulation in the liver of rockfish was approximately 3-7 fold higher than in the intestine, and the order of Cu accumulation in tissues were liver > intestine > kidney > gill > muscle. These results indicated that Cu accumulation in liver of rockfish was more effective than that in the intestine exposed dietary Cu. Miller et al. (1993) reported that in rainbow trout elevated Cu concentrations in the water of more than 11 times increase from normal levels already induced an accumulation of liver Cu. These authors concluded that diet appeared to be dominant source of Cu in liver compared to waterborne Cu. Kamunde et al. (2002) found that the Cu level in the liver of rainbow trout (*Oncorhynchus mykiss*) was 33-fold higher than control at 282 mg/kg dietary exposure and he suggested that the role of the liver is central in fish Cu metabolism. The same results were observed that rainbow trout (Lanno et al., 1985; Wong et al., 1999), Atlantic salmon (Lorentzen et al., 1998) and channel catfish (Gatlin

and Winson, 1986) accumulated Cu highly in the liver. However, Lundebye et al. (1999) reported that 50% of the whole body burden in Atlantic salmon exposed to 500 and 700 mg/kg Cu diet was present in the intestine and these results indicate that the intestine plays an important role in regulating the uptake of dietary Cu. This difference might be explained by the fish species (trout vs. salmon) because effectively tissue Cu regulation in fish varies strongly with fish species. In rockfish, Cu exposure resulted in increased Cu accumulation of liver, because it plays a major role in detoxification and excretion of metals through the induction of metal-binding proteins such as metallothioneins (MTs; Roesijadi, 1992). Therefore, it can be concluded that liver of rockfish is a more important storage tissue than other tissues, and Cu accumulation clearly reflected the level of dietary exposure.

Cu accumulation in the gill was significantly elevated at dietary concentration and periods. Miller et al. (1993) reported Cu level in gill increased with increasing dietary Cu concentration, and he suggested that Cu uptake from the diet is well regulated in fish. However. Lundebye et al. (1999) observed that gill Cu concentration was significant increase not among treatments, but over time. Although little literature has been explained this difference, may be the re-distribution through transport from the liver and blood.

Cu accumulation in muscle was not affected by exposure concentration except on 40 days in higher exposure concentration (500 mg/kg). Thus, Cu accumulation in muscle was homeostatically regulated (Kamunde et al., 2001) and related to exposure periods.

The calculated accumulation factor has two major purposes: first, to measure how much Cu is accumulated with respect to aqueous exposure concentration; second, to find the finite limit in the ability of fish to accumulate metals (Sorensen, 1991). The accumulation factor of rockfish increased with exposure period. Similar patterns of accumulation factor were also shown carp (Cinier et al., 1999) and eel (Yang and Chen, 1999). Thus, Cu accumulation in rockfish strongly influenced dietary exposure periods, and the ability of fish to accumulates Cu agreed with tissue accumulation order.

Cu elimination route may be including urinary, branchial and also biliary and fecal excretion, the principle Cu excretion routes in mammals (Gregus and Klaassen, 1986). Muramoto (1983) observed that the sequence of increasing elimination rate in the organ of carp (*Cyprinus carpio*) is intestine > gill, after 90 days of depuration periods. Viarengo et al. (1985) reported that Cu was rapidly eliminated from the mussel tissue, and Cu concentration in the gill and digestive gland were of the same magnitude as the control at 24 days of depuration periods. Geffard et al. (2002) found that Cu elimination rate in the digestive gland of oyster was higher than that of gill, and he suggested that fast elimination of Cu in the digestive gland could result from the physiological role of this organ in essential element homeostasis and in protein metabolism. Moreover, intestine showed faster elimination rate, and higher amount of elimination than gill and liver in this study. This result indicated that metal elimination in intestine was more effective than other organ. Harrison and Klaverkamp (1989) suggested that enteric excretion with faces is a major excretion route for metal in fish. Moreover, fish exposed to waterborne metal do not excrete significant amount of metal in the urine (Giles, 1988), and marine teleosts was fewer glomeruli so must function exclusively as a secretory kidney than freshwater teleosts and probably would have little ability to excrete metals via the routes (Heath, 1995). Therefore, it can be concluded that the capability for elimination of metal in the urine may insufficient compared to intestine, and intestine of fish is a more important elimination route than hepatic-biliary excretory route.

During the 30 days of depuration periods, the Cu concentration in kidney increased and that of muscle remained constant. Wicklund et al. (1988) showed that Cd continued to be accumulated in liver and kidney of zebrafish (*Brachydanio rerio*) throughout the depuration period. Kuroshima (1987) also reported that Cd level in kidney of girella remained constant after the end of exposure, and he suggested that Cd once taken up in a body is hardly excreted but is redistributed among tissues. This phenomena is seems to the redistribution of Cu among tissue before its elimination. During the depuration period, the accumulation Cu may be transferred from liver and gill to kidney and muscle for redistribution.

In conclusion, Cu accumulation clearly reflected the level of dietary exposure. The liver of the rockfish is a more important storage tissue than other tissues, and the order of Cu accumulation in tissues were liver > intestine > kidney > gill > muscle. Cu elimina-

tion in organ of rockfish was time-dependent until the end of the depuration periods except kidney and muscle. The order of Cu elimination rate in organ of rockfish during depuration periods was intestine > liver > gill, and intestine of fish is a more important elimination route than other excretory route. The application of laboratory accumulation and elimination data to natural situation must be careful due to complex factors such as metal sepciation, species, uptake route, chemical and biological factors which may affect the accumulation and elimination of metal (Cinier et al., 1999). Therefore, further research on more toxicological consideration is necessary to set concentration of feed guideline in for protect fish health and human safety.

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