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Effects of Waterborne Iron on Serum Iron Concentration and Iron Binding Capacity of Olive Flounder (*Paralichthys olivaceus*)

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Olive flounder (*Paralichthys olivaceus*) was exposed to waterborne iron (0.1, 0.5, 1, 5 and 10 mg/L) for 50 days. The effects of iron on blood iron status and iron binding capacity were studied. The serum iron concentration was significantly higher than in the group exposed to iron (1, 5 and 10 mg/L) in comparison to the control after 30 days of exposure to iron. A significant decrease in unsaturated iron binding capacity was found between the control and the group exposed to iron (5 and 10 mg/L, respectively) at 40 and 50 days, respectively. The total iron binding capacity of serum in the fish exposed to iron concentrations (5 and 10 mg/L) showed a significant decrease compared to that of the control at 40 days after iron exposure. Serum iron saturation values increased in the flounder exposed to iron concentration (5 and 10 mg/L) at 50 days. Our data suggest that sub-lethal exposure of waterborne iron alters the blood iron concentration and iron binding capacity, and these parameters seems to be valuable factors for screening and diagnosis of iron overload syndromes in fish.

Key words: Iron binding capacity, Iron saturation, Serum iron, *Paralichthys olivaceus*, Waterborne iron

Introduction

Iron is an important micronutrient in the aquatic environment (Cover and Wilhm, 1982). The bioavailability of aqueous iron is affected by the speciation of the metals. It appears that while the ferrous (Fe^{+2}) ion is bioavailable to organisms, the ferric (Fe^{+3}) ion is essentially unavailable for biological uptake (Albergoni and Piccinni, 1983). Although the ferric ion is generally the predominant form in aerated surface waters, it can be reduced to the ferrous state under reducing conditions (CCREM, 1992). Iron is also a component of haemoglobin (Rainbow, 1985) and thus plays an important function in the movement of oxygen in the body. Iron also plays a central role in electron transfer in the cytochromes during cellular respiration.

In man, bioavailability of iron has been extensively studied, and iron absorption is known to be influenced by the amount of iron ingested, dietary factors enhancing or inhibiting iron absorption and the iron status of the subject (Hallberg, 1981). If iron is not sufficiently present in the diet, the iron stored in the body may be depleted more rapidly than it can be absorbed, and subsequently lead to iron deficiency (Appel et al., 2001). In fish, the dietary requirement has been estimated to be 30 mg/kg for channel catfish (*Ictalurus punctatus*) (Gatlin and Wilson, 1986) and 150 mg/kg for red sea bream (*Pagus major*) (Sakamoto and Yone, 1978). Iron deficiency signs in salmonids include reduced haematocrit and depletion of hepatic iron (Andersen et al., 1996). On the other hand, high levels of iron have been suggested to be harmful to salmonids (Desjardins et al., 1987).

High levels of iron in the water also can be detrimental to aquatic organisms as it forms a precipitate on their gills and thereby reduces respiration as described by Hellawell (1986), further he concluded a situation which may lead to high levels of mortality in aquatic ecosystems. They may also affect aquatic species such as fish indirectly by killing off their benthic food sources (Hellawell, 1986).

In a previous report, we demonstrated that haematological impact in olive flounder (*P. olivaceus*)

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exposed to waterborne iron (Kang et al., 2001). High level of iron caused curvature and terminal clubbing of gill lamellae (Kang et al., 1999) and pathological changes in the ultrastructure of certain cells in the hepatopancreas in the crayfish (Roldan and Shivers, 1987).

Iron status is known to be of importance for the absorption of iron in man (Hallberg, 1981). Biochemical investigation, such as assessment of total iron (TI) level in serum or determination of serum iron binding capacity, play an essential role in the pathogenesis of anemia. The assessment of iron status in human has been reviewed and measurements include serum total iron binding capacity (TIBC) (Cook et al., 1992). Percentage iron saturation (IS) is considered to be also an auxiliary diagnostic parameter. So far nothing has been found that satisfactory knowledge about serum iron status of aquatic organisms including fish and shellfish.

This study determined serum TI, TIBC, UIBC and IS parameters in olive flounder (*P. olivaceus*) exposed to waterborne iron for 50 days.

Meterials and Methods

Animals

Olive flounder (*P. olivaceus*), 170 g mean body weight, was obtained from a commercial farm in Geoje, Kyongnam, Korea. Fish were acclimated to the laboratory condition for 2 weeks at 20±1℃. The fish were held in the laboratory in 300 L aquaria with 200 L of well-aerated sea water. Fish were maintained on a 12 h light/dark cycle at all times. During this conditioning period, fish were fed extruded pellet (Jeilfeed Co., Korea; crude protein >52%, fat >7%, fibre <4%, calcium >1.2%, phosphorus >2.7% and ash <17%, according to manufacturer's specifications). Fish were fed diet twice daily (2% body weight for each meal at approximately at 08:30 and 16:00).

Test conditions

Water temperature, pH, salinity, dissolved oxygen and iron concentration of test aquaria were monitored every other days basis using standard laboratory methods as described by APHA (1985). Waterborne iron stock solution which was obtained in the form of ferrous sulfate heptahydrate ($FeSO4 \cdot 7H_2O$; 99% purity, Sigma, USA). Stock solutions of iron were mixed with sea-water to attain a nominal concentration of 0.1, 0.5, 1.0, 5.0, 10.0 mg/L. After the acclimation period, the fish were randomly distributed, 10 fish into each test chamber (glass aquaria, 120-L capacity, n=30). Fish was exposed for a period of 1, 2, 3, 4 and 5 weeks.

Blood samples

At the end of each period, fish were anesthetized in buffered 3-aminobenzoic acid ethyl ester methanesulfonate (Sigma, USA) and blood samples were taken from caudal vein. Anesthesia, measurement and blood withdrawal generally took less than 3 min/fish with minimum disturbance. The blood was allowed to clot at room temperature (22℃) for 1-2 h and then at 4℃ overnight. Serum was collected after centrifugation 3,000 g for 5 min at 4℃ (Mikro 22R, Hettich, Germany) divided into 200 μL aliquots and then stored at -80℃ until analyzed.

Biochemical studies

The total iron content (TI, μg/dL), unsaturated iron binding capacity (UIBC, μg/dL) and total iron binding capacity (TIBC, μg/dL) of the flounder serum were determined using a kit (Asan Pharm., Korea) based on the method described by Persijn et al. (1971). In this procedure, iron is released from its combination with transferrin in acid medium, reduced to its ferrous form by hydroxylamine, and reacted with ferrozine to form a violet colored complex that is measured spectrophotometrically (DR 4000, Hach, USA) at 560 nm. The percent saturation was calculated as (TI/TIBC)×100.

Statistical analysis

Statistical analyses were performed using SPSS/PC+ statistical package. Significant differences between groups were determined using one-way ANOVA and Duncan's test for multiple comparisons or the Student's t-test for two groups (Duncan, 1955). Significance level was established at $P<0.05$.

Results

Table 1 shows the monitoring result of variation of water temperature, pH, salinity, dissolved oxygen and iron concentration of test chambers for 50 days. This study showed effects of waterborne iron on olive flounder and provided information about normal serum iron status of olive flounder. Our result showed normal range of serum iron content and binding capacity in olive flounder according to control group: serum iron concentration varied from 29.6 to 54.2 μg/dL, unsaturated iron binding capacity (UIBC)

Table 1. Variation of iron concentration and water quality in each treatment group for 50 days

Test group	Temperature (℃)	pH	Salinity (PSU)	Dissolved oxygen (mg/L)	Iron concentration
Control	20.1±1.3[1]	7.8±0.5	33.7±0.8	7.3±0.8	5.02±0.9[2]
0.1 mg/L	20.3±1.2	7.5±0.4	33.5±0.5	7.6±0.6	0.12±0.04
0.5 mg/L	20.2±1.0	7.4±0.4	33.1±0.9	7.2±0.9	0.49±0.05
1 mg/L	20.3±1.2	7.2±0.3	33.4±0.9	7.3±0.5	0.97±0.08
5 mg/L	20.2±1.2	7.7±0.5	33.3±0.7	7.5±0.7	4.87±0.24
10 mg/L	20.4±1.3	7.5±0.7	33.7±0.8	7.4±0.6	9.49±0.74

[1]Values represented mean±SE of n=25. [2]ppb.

Table 2. Serum iron concentrations (μg/dL) of olive flounder (*Paralichthys olivaceus*) exposed to waterborne iron for 50 days

Test period	Waterborne iron concentrations					
	Control	0.1 mg/L	0.5 mg/L	1 mg/L	5 mg/L	10 mg/L
10 days	40.87±2.88[1]	45.51±2.66	47.71±3.72	47.53±3.36	49.33±3.45	48.53±2.50
20 days	45.12±1.71	44.63±2.70	48.09±3.49	46.49±3.38	50.57±3.26	53.40±5.10
30 days	43.96±2.06^{a2}	45.51±3.40^{ab}	54.22±4.32^{abc}	59.78±5.64^{bc}	57.54±5.32^{abc}	62.56±5.74^{c}
40 days	41.35±2.74^{a}	47.47±3.21^{ab}	55.15±1.41^{b}	65.77±3.51^{c}	66.52±3.80^{c}	69.41±3.74^{c}
50 days	45.68±1.69^{a}	45.25±3.71^{a}	51.48±5.80^{ab}	65.97±6.30^{bc}	78.35±5.70^{c}	73.83±7.07^{c}

[1]Values represented mean±SE of n=10.
[2]Column means within the same period having the same superscript are not significantly different (P>0.05).

from 498.1 to 634.2 μg/dL, total iron binding capacity (TIBC) from 528.8 to 679.4 μg/dL and percentage iron saturation from 5.2 to 9.3%. We have shown here that waterborne iron induces elevation of total iron levels of blood in olive flounder. The serum iron concentration was statistically significantly higher than in the iron exposed group (1, 5 and 10 mg/L) compared to the control after 30 days of iron exposure (Table 2). After 50 days of exposure, significant increment was confirmed at iron concentration of 5 and 10 mg/L, in which serum iron concentration was increased to 1.7 and 1.6 times that of the control group, respectively. In the present study, we found that the TIBC and UIBC (assessed as μg of exogenous iron that can be taken by 100 mL serum) was decreased after treatment of iron. Serum UIBC was stable of iron exposed and the control group until 30 days of exposure (Table 3), however significant decrease in UIBC were found iron in the exposed group (5 and 10 mg/L, respectively) at 40 and 50 days. Serum TIBC in the fish exposed to iron concentrations (5 and 10 mg/L) showed a significant decrease compared to the control values after 40 days iron exposure (Table 4). At 50 days, serum TIBC were increased at the iron concentrations of 5 and 10 mg/L (P<0.01) compared to the control value. Serum IS value showed insignificant change until 20 days, thereafter significant elevation was observed (Table 5). Serum IS were increased (by 90.3 and 87.1, respectively) in the groups (5 and 10 mg/L) exposed to iron at 50 days (P<0.01), but there was no significant change in the groups (0.1 and 0.5 mg/L) exposed to iron.

Discussion

Detection of iron overload is an important parameter due to its toxic effects and excess loading of iron in the liver and heart, which leads excess deposition of iron in the parenchymal tissue results in cell injury and functional disorders (Bacon and Britton, 1989). Iron overload may occur due to hereditary hemochromatosis and in homozygous thalassemic conditions, part of which is due to the transfusional iron excess in human (Brittenham, 1991). Chronic iron toxicity is not commonly observed

Table 3. Unsaturated iron binding capacity (μg/dL) of olive flounder (*Paralichthys olivaceus*) exposed to waterborne iron for 50 days

Test period	Waterborne iron concentrations					
	The control	0.1 mg/L	0.5 mg/L	1 mg/L	5 mg/L	10 mg/L
10 days	573.0±15.4[1]	569.8±25.5	569.0±10.4	570.3±13.9	567.5±19.9	557.5±12.7
20 days	572.4±14.5	554.0±15.5	561.4±14.7	557.9±16.0	557.5±19.0	560.7±26.8
30 days	592.3±16.0	588.9±10.8	568.0±21.5	564.8±17.7	542.8±28.0	557.9±20.8
40 days	575.2±13.8a[2]	582.4±17.2[a]	544.9±13.0[ab]	557.5±14.8[a]	499.7±15.4[b]	494.6±29.8[b]
50 days	570.7±14.2a	573.7±12.3[a]	539.7±12.7[a]	562.8±15.5[a]	444.8±13.6[b]	458.5±17.2[b]

[1]Values represented mean±SE of n=10.
[2]Column means within the same period having the same superscript are not significantly different (P>0.05).

Table 4. Total iron binding capacity (μg/dL) of olive flounder (*Paralichthys olivaceus*) exposed to waterborne iron for 50 days

Test period	Waterborne iron concentrations					
	The control	0.1 mg/L	0.5 mg/L	1 mg/L	5 mg/L	10 mg/L
10 days	613.8±17.8[1]	615.3±23.5	616.8±9.3	617.8±12.1	616.9±18.9	606.1±11.4
20 days	617.5±13.4	598.6±14.3	609.5±15.0	604.3±16.7	608.0±16.5	614.1±26.0
30 days	636.2±16.3	634.4±9.9	622.2±18.3	624.6±16.4	600.4±24.5	620.4±19.4
40 days	616.5±12.1a[2]	629.9±17.1[a]	600.0±11.8[ab]	623.3±17.7[a]	566.2±14.3[b]	564.0±29.3[b]
50 days	616.4±14.1a	619.0±14.9[a]	591.2±11.9[a]	628.8±17.5[a]	518.2±15.5[b]	532.3±17.2[b]

[1]Values represented mean±SE of n=10.
[2]Column means within the same period having the same superscript are not significantly different (P>0.05).

Table 5. Serum iron saturation (%) of olive flounder (*Paralichthys olivaceus*) exposed to waterborne iron for 50 days

Test period	Waterborne iron concentrations					
	The control	0.1 mg/L	0.5 mg/L	1 mg/L	5 mg/L	10 mg/L
10 days	6.6±0.33[1]	7.5±0.71	7.8±0.64	7.7±0.62	8.1±0.66	8.0±0.49
20 days	7.3±0.41	7.5±0.56	7.9±0.56	7.7±0.53	8.4±0.70	8.8±0.99
30 days	6.9±0.33[a2]	7.2±0.57[ab]	8.8±0.91[abc]	9.6±0.99[abc]	9.8±1.21b[c]	10.1±0.97[c]
40 days	6.7±0.54[a]	7.6±0.55[ab]	9.2±0.39[bc]	10.5±0.34[cd]	11.8±0.76[de]	12.5±1.04[e]
50 days	7.4±0.33[a]	7.3±0.47[a]	8.7±0.97[ab]	10.5±0.94[b]	14.1±0.98[c]	13.9±1.35[c]

[1]Values represented mean±SE of n=10.
[2]Column means within the same period having the same superscript are not significantly different (P>0.05).

amongst fish since iron dose not usually reach sufficiently high levels in the aquatic environment. However, toxic levels of iron in the aquatic environment can arise from mining activities as a result of water percolating through exposed rocks and leaching the metals (Gonzalez et al., 1990).

A reduced level of serum iron and an elevated TIBC are required for a diagnosis of iron deficiency, whereas an increased proportion of saturation is suggestive of iron overload in human (Finch and Huebers, 1982). TIBC is the maximum amount of iron that plasma protein (mainly transferrin) can bind and is often decreased in iron overload (Fairbanks and Klee, 1999). In human, if the serum iron levels

are very high and the transferrin saturation is greater than 60%, additional clinical study such as iron binding capacity are required. In guinea pigs fed iron-containing diet, the serum iron concentration and transferrin saturation became significantly increased (Schwartz et al., 1993). Even though index related with iron status seems very important for evaluating the human health, little has been done to determine the physiological system of fish. Thus we test serum iron status in olive flounder (*P. olivaceus*) which is one of most important for aquaculture species in Asia including Korea and Japan in particular.

To our knowledge, this is the first report of waterborne iron exposure on iron binding capacity of olive flounder. We found that the TIBC and UIBC in vivo decreased after treatment of iron. A significant increase was observed in serum iron concentration of the flounder intoxicated with waterborne iron. The difference became statistically significant in the iron concentration of 1, 5 and 10 mg/L. The UIBC and TIBC tended to decrease with increasing waterborne iron concentrations after 30 days of exposure. Decreased serum TIBC may be due to associated with decline serum UIBC exposed to waterborne iron in olive flounder.

The stores regulator can influence the amount of iron uptake by about 2 to 3 factors in iron-deficient conditions (Finch, 1994). In the present study 50 days exposure of waterborne iron at the concentration of 5 and 10 mg/L, increased the IS levels by 90.3% and 87.1% ($P<0.01$), respectively, in comparison to the control group. It is likely that iron absorption is indirectly influenced by the saturation of plasma transferrin with iron. However, the exact molecular details of the activity of the stores regulator are presently not known. In human, to confirm true iron overload, those with a high screening iron saturation should determine serum ferritin levels. In the absence of a toxic or inflammatory cause, a high level of serum iron saturation (IS) is highly suggestive of iron overload.

Therefore, we assume that changes in serum iron concentration, iron binding capacity and serum iron saturation reflect the sensitive tool of waterborne iron overload, and these parameters seems to be valuable factors for screening and diagnosis of iron overload syndromes in fish.

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