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Effects of Dietary Cadmium on the Respiratory Burst of Phagocytes and the Antioxidant Defense in Cultured Red Seabream (*Pagrus major*)

Chun Soo Kim and Ki Hong Kim*

Department of Aquatic Life Medicine, College of Fisheries Sciences, Pukyong National University, Pusan 608-737, Korea

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To examine effects of cadmium on the respiratory burst of kidney phagocytes and antioxidant defense in liver, juvenile red seabream *Pagrus major* were fed a cadmium-incorporated diet (1 g $CdCl_2$/kg diet). The respiratory burst activity measured by chemiluminescence (CL) was significantly reduced by oral intake of cadmium. Lipid peroxidation in liver expressed as thiobarbituric acid reactive substances (TBARS) was significantly higher in the fish fed a cadmium-incorporated diet than that of the fish fed a control diet both on Day 3 and Day 9. Liver Glutathione S-transferase (GST) activitiy was significantly increased both on Day 3 and Day 9 by feeding a cadmium-incorporated diet, when compared with the controls. From the present results, it can be concluded that oral intake of cadmium in red seabream is associated with marked reduction of respiratory burst capacity of kidney phagocytes which can elevate susceptibility of fish against infecting pathogens. Cadmium administration also elicits significant increment of lipid peroxidation in liver, and fish try to detoxify cadmium by increasing GST activity.

Key words: Cadmium, Red seabream (*Pagrus major*), Respiratory burst, Lipid peroxidation, TBARS, Glutathione S-transferase

Introduction

The association between chemical contamination of various coastal environments and diseases in aquatic organisms has not been well researched, although immunosuppression has been suggested to be a factor linking disease in fishes to highly contaminated areas (Anderson, 1993). Since many fish and shellfish species are found or cultured in shallow coastal waters, where human activities are concentrated and toxicant concentrations are often elevated, investigations of contaminant effects on the immunological and antioxidative responses in fish are very important.

Cadmium is a widespread environmental pollutant that is highly toxic and affects biological systems in various ways. Since cadmium is a common contaminant in the aquatic environment, the biological response of aquatic organisms to cadmium intoxication is of significant interest.

Several studies have documented the toxic effects of cadmium on the immune system in mammals. Cadmium has been shown to enhance humoral immune responses at low levels of exposure, whereas high doses are immunosuppressive (Koller et al., 1976; Fujimaki, 1985; Krzystyniak et al., 1987). In fish, both suppressive and stimulatory effects of cadmium on humoral and cellular immune response have been demonstrated (Thuvander, 1989; Albergoni and Viola, 1995; Lemaire-Gony et al., 1995). Hutchinson and Manning (1996) reported that dab (*Limanda limanda*) exposed to various concentrations of cadmium showed significantly reduced respiratory burst of kidney phagocytes. Fish, like other vertebrates, respond to infectious pathogens in specific and non-specific ways. However, the non-specific defences are the first a pathogen

*Corresponding author: khkim@pknu.ac.kr

encounters, and it has been suggested that they are very important in the resistance of fish to infectious agents (Blazer, 1991). Granulocytes and macrophages possess a phagocytic activity which is the initial step in the immune response in fish, and is the major line of defence for all foreign material, including pathogenic agents (Olivier et al., 1986). During phagocytosis, fish macrophages increase their oxygen consumption as well as the production of reactive oxygen intermediates (ROIs) (Chung and Secombes, 1988) such as the superoxide (O_2^-), hydrogen peroxide (H_2O_2), and the hydroxyl radical (OH^-). These ROIs play an important role in the antimicrobial activity of phagocytic cells (Allen et al., 1972; Babior, 1984). This final stage is termed the respiratory burst (Secombes and Fletcher, 1992).

Chemiluminescence (CL) measures the respiratory burst activity of phagocytic cells in which oxygen is converted into reactive oxygen intermediates. Respiratory burst assays have been employed in order to monitor the activity of phagocytes sampled from several estuarine and marine fish species exposed to chemical contaminants (Elsasser et al., 1986; Wishovsky et al., 1989; Roszell and Anderson, 1993). Cadmium has been found to stimulate lipid peroxidation in fish causing serious tissue damage (Thomas and Wofford, 1993), the liver being the major target organ in which a marked fibrosis occurs (Lemaire-Gony and Lemaire, 1992). The glutathione S-transferases (GSTs), however, are generally involved in the detoxication of activated, electrophilic xenobiotics. Thus, the investigation of the capacity of cadmium to alter the activation and detoxication balance has great importance.

In the present study, the effects of dietary cadmium on the CL response of kidney phagocytes and the lipid peroxidation and GST activities in liver of red seabream (*Pagrus major*) were investigated.

Materials and Methods

Diet preparation

The experimental diets were prepared by mixing the commercial powder feed (Woosung feed Co., Korea) and $CdCl_2$ (Sigma) at the rate of 0 and 1 g $CdCl_2$/kg diet. The $CdCl_2$-free diet was used as a control diet. After pelleting the powder by using the meat grinder, the experimental diets were stored at −20℃ until needed. Prior to use as feed, small quantities were stored at 4℃.

Experimental procedure

Net-pen reared juvenile red seabream, *Pagrus major,* averaging body weight 50 g, were obtained from a local red seabream farm in Tongyoung, Korea. A total of 20 fish were stocked into two tanks containing 100 L seawater at 20±1℃ with aeration. Before the start of the experiment, the fish were acclimated for one month and fed on a control diet. After acclimation, fish were fed each of the diets (control and cadmium-incorporated diet) once daily to satiation during the experimental period. At 3rd and 9th day of feeding of each experimental diet, 5 fish were randomly selected from each tank for analysis.

Chemiluminescence (CL) assay

Fish were anaesthetized with 200 mg/L tricaine methanesulfonate (MS-222, Sigma), and blood was withdrawn by caudal vein venipuncture. Head kidney was removed aseptically and was passed through a 100 μm nylon mesh using Hank's balanced salt solution (HBSS, Sigma) containing heparin (10 units/mL, Sigma), penicillin (100 μg/mL, Sigma) and streptomycin (100 U/mL, Sigma). The resulting cell suspension was placed on a 34/51% Percoll density gradient and centrifuged at 400×g for 30 min at 4℃. The interphase was collected and the cells were washed twice at 400×g for 5 min in HBSS containing heparin and antibiotics. The cell viability was examined with trypan blue exclusion and was evaluated to be greater than 95%. The leucocytes, including neutrophils and macrophages, were adjusted to 1×10^6 cells/mL HBSS.

Zymosan (Sigma) was mixed with the serum of an adult of red seabream and incubated at 30℃ for 30 min. Zymosan was separated from the serum by centrifugation, washed three times and suspended in HBSS.

The ROIs (reactive oxygen intermediates) produced by stimulated phagocytes was quantified using an automatic photoluminometer (Bio-Orbit 1251, Finland). Each test cuvette (4 mL) contained 0.7 mL luminol (Sigma) made according to the method of Scott and Klesius (1981), 0.5 mL cell

suspension, and 0.3 mL opsonized zymosan, which was added just prior to measurement. Blank cuvette contained luminol and cell suspension, but opsonized zymosan was replaced by HBSS. The measurements were made for 100 minutes and the light emission was recorded as mV.

Thiobarbituric acid reactive substances (TBARS)

TBARS in liver homogenates were determined by mixing a 100 μL aliquot of the homogenate with 200 μL of sodium dodecyl sulfate (8.1%, Sigma) and 1.5 mL of 20% acetic acid (pH 3.5). Then, 1.5 mL of 0.8% (w/v) thiobarbituric acid (TBA, Sigma) in water containg 0.025% 2,6-di-tert-butyl -p-cresol (BHT, Sigma) was added. The mixture was incubated in a boiling water bath for 60 min, centrifuged at 5000×g for 5 min, and its absorbance was read at 535 nm.

Glutathione S-transferase (GST)

GST activity was determined by the method of Habig et al. (1974). Briefly, the reaction mixture consisted of 1.65 mL sodium phosphate buffer (0.1 M, pH 6.5), 0.1 mL reduced glutathione (1 mM, Sigma), 0.05 mL 1-chloro-2,4-dinitrobenzene (CDNB, 1 mM, Sigma) and 0.2 mL fish liver homogenate (10%) in a total volume of 2 mL. The change in absorbance was recorded at 340 nm and the enzyme activity was calculated as nmol CDNB conjugate formed/min/mg protein.

Statistical analysis

The mean and standard deviation of the mean, as well as standard error was calculated for each treatment. Students *t*-test was employed to evaluate the level of significance and the difference was considered significant when $P<0.05$.

Results

Chemiluminescence (CL) assay

The fish fed a cadmium-incorporated diet showed significantly ($P<0.05$) reduced CL responses at Day 3 than the fish fed a control diet (Fig. 1). Although there was no statistical significance in CL responses between the two experimental groups at Day 9, the mean value of the group fed cadmium was considerably lower than the control group.

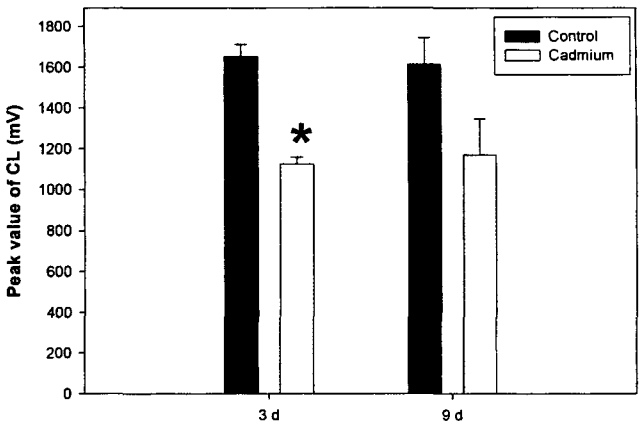

Fig. 1. The peak value of chemiluminescent (CL) response of kidney phagocytes of cultured red seabream, *Pagrus major*, fed a cadmium-incorporated diet or control diet. Data represent the mean ± S.E. *denotes statistically significant differences ($P<0.05$).

Thiobarbituric acid reactive substances (TBARS)

The TBARS values of the fish fed a cadmium-incorporated diet were significantly higher than those of the fish fed a control diet both on Day 3 and Day 9 (Fig. 2).

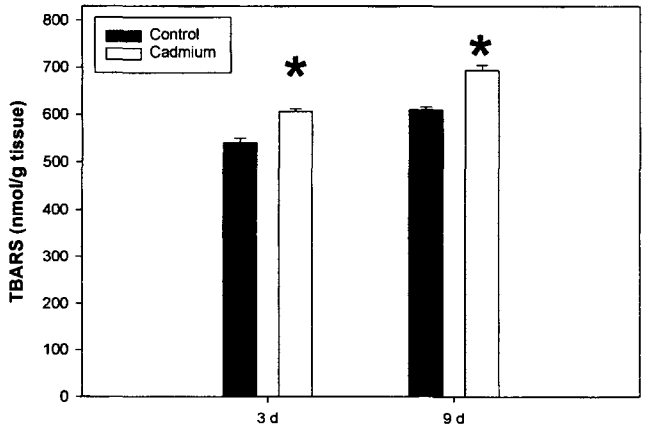

Fig. 2. The value of thiobarbituric acid reactive substances (TBARS) of liver of cultured red seabream, *Pagrus major*, fed a cadmium-incorporated diet or control diet. Data represent the mean ± S.E. *denotes statistically significant differences ($P<0.05$).

Glutathione S-transferase (GST)

Liver GST activitiy was significantly increased both on Day 3 and Day 9 by feeding a cadmium-incorporated diet, when compared with the controls (Fig. 3).

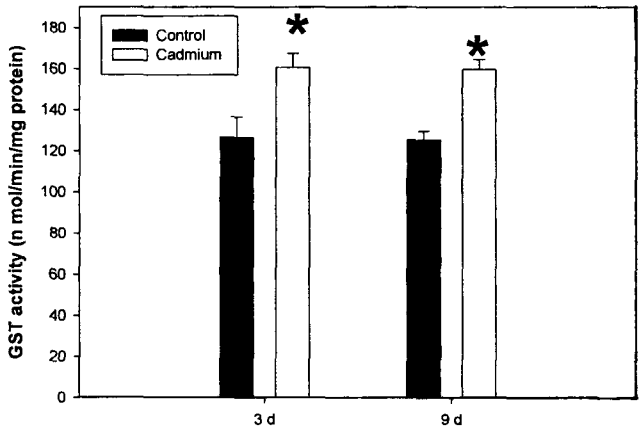

Fig. 3. Glutathione S-transferase (GST) activity of liver of cultured red seabream, *Pagrus major*, fed a cadmium-incorporated diet or control diet. Data represent the mean ± S.E.
*denotes statistically significant differences ($P<0.05$).

Discussion

The present results clearly showed that oral intake of cadmium inhibited the respiratory burst activity of head kidney phagocytes in cultured red seabream *Pagrus major*. The *in vivo* inhibitory effect of cadmium on the respiratory burst of head kidney phagocytes have been reported in marine fish species such as sea bass *Dicentrarchus labrax* (Bennani et al., 1996) and dab *Limanda limanda* (Hutchinson and Manning, 1996). Although the exact mechanism of cadmium-induced immunotoxicity in fish is not yet elucidated, it is known that in other vertebrates cadmium is concentrated in the kidney and may act as a potent nephrotoxin (Goyer, 1986). Romeo et al. (2000) and Cattani et al. (1996), also, found that kidney was the main organ for storage of cadmium in fish. Therefore, it is probable that toxic levels of cadmium may have accumulated in the kidney tissue of fish, primarily damaging the renal tissue but in turn having an impact upon the adjacent lymphoid tissue. One possible outcome of this may be the decreased respiratory burst capacity of the kidney phagocytes according to the severity of the toxic insult. Tort et al. (1996) reported that the serum cortisol levels of rainbow trout were significantly increased by intraperitoneal injection of cadmium. The mechanism of stress-mediated suppression of phagocytic activity in fish is not fully understood, but appears to be mediated by the endocrine system (Bayne and Levy, 1991). Angelidis et al. (1987) assumed that the decrease in the CL response in stressed fish might be based on the corticosteroid effects. Therefore, in the present results, cadmium-associated stress might be a cause of respiratory burst reduction.

Metal toxicity may also be exerted through lipid peroxidation considered as a first step of cellular membrane damage by xenobiotics (Viarengo, 1989). The significantly increased thiobarbituric acid reactive substances (TBARS) both on Day 3 and Day 9 in the liver by feeding a cadmium-incorporated diet in the present study indicate that cadmium may alter the structure of cell membranes by stimulating the lipid peroxidation process.

According to the present results, the activity of glutathione S-transferase (GST), which catalyzes the conjugation of glutathione to heavy metals and detoxicates lipid peroxides (Nakagawa, 1991), was increased in the liver of cadmium administered fish. Increased activity of GST in liver by treatment with cadmium has been reported only in rats (Planas-Bohne and Elizalde, 1992) and in guinea-pigs (Iscan et al., 1994). The present results indicate that fish, at least for red seabream, also respond to cadmium by increasing liver GST activity. This increase might be a physiological response for coping with the toxicity of cadmium.

From the present results, it can be concluded that oral intake of cadmium in red seabream is associated with marked reduction of respiratory burst capacity of kidney phagocytes which can elevate susceptibility of fish against infecting pathogens. Cadmium administration also elicits significant increment of lipid peroxidation in liver, and fish try to detoxify cadmium by increasing GST activity.

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