Bull. Environ. Contam. Toxicol. (2006) 76:891–898

DOI: 10.1007/s00128-006-1002-2

Bulletin of Environmental Contamination and Toxicology

Effect of Cadmium on Hematological Functions in Tilapia (*Oreochromis mossambicus*)

S. M. Wu, A.-N. Deng

Department of Aquatic Biosciences, National Chiayi University, Chiayi 600, Taiwan

Received: 30 August 2005/Accepted: 8 March 2006

Cadmium is an important xenobiotic in aquatic ecosystems. In fish, Cd^{2+} has adverse effects on growth, reproduction, and the respiratory system. Waterborne cadmium can also be considered a potential source of stress, and can theoretically be detected by changes in erythrocyte parameters such as cell volume and enzyme activities (Roche and Boge, 1996).

Several researchers have reported effects of heavy metals on erythrocytes. Fish blood parameters can possibly serve as a potential tool for identification of stress caused by environmental factors (Roche and Boge, 1996), including toxicity of heavy metals. For example, Dethloff et al. (1999) reported that hemoglobin (Hb) and the hematocrit (Hct) were elevated in trout exposed to 26.9 μg/L Cu on day 3 and then returned to levels comparable to the control fish; they suggested that the changes in these hematological parameters were a nonspecific stress response. Hct, red blood cells (RBCs), and Hb were significantly higher in *Prochilodus scrfa* exposed to 29 μg/L Cu after 96 hr, and Cerqueira and Fernandes (2002) suggested a possible hemoconcentration after Cu exposure. Kotsanis et al. (2000) also investigated the hematological responses induced 6 months after treatment with Cd in rainbow trout (*Oncorhynchus mykiss*), and found a significant decrease in RBCs. All of their opinions advocated that these changes were caused by the stress of the heavy metal. However, one of the targets of Cd^{2+} toxicity is the hematological system. It was found that Cd^{2+} induces anemia and alterations in the antioxidant and metabolic status of RBCs (Kostic et al., 1993). Cu and Hg treated-fish showed an increase in RBC concentration, decreased membrane fluidity, and changed the internal viscosity of RBC. These results suggest that the possible cause of the damage of cells may be metal-protein interactions in the cells (Gwozdzinski, 1992). Erythrocyte deformability is an important indicator because it plays an essential role in the delivery of oxygen to tissues. Once Cd^{2+} changes the erythrocyte microstructure, it might affect the oxygen-carrying capacity of hemoglobin (Mojzis and Frantisek, 2000). So far, no direction evidence to demonstrate the effect of Cd on the oxygen-carrying capacity of hemoglobin in fish. Our previous study found that the levels of hemolysis were affected by Cd treatment; it appeared that the membranes of the erythrocytes had been changed by the Cd (Chang and Wu, 2003). The purpose of this study was to

Correspondence to: S. M. Wu, 300 University Road, Chiayi 600, Taiwan

use tilapia (*O. mossambicus*) as model to investigate the effect of Cd on the oxygen-carrying capacity of hemoglobin.

In this study, Hb and Hct levels were ascertained, and the indicators of the oxygen-carrying capacity of Hb, such as the oxygen partial pressure in the blood (P_{O2}), oxyhemoglobin saturation level (S_{O2}), carbon dioxide partial pressure in the blood (P_{CO2}), and Hb, were evaluated after treatment with Cd. Changes in these hematological parameters were treated as indicators of the effects of Cd on the oxygen-carrying capacity of Hb as well as on other physiological responses.

MATERIALS AND METHODS

Male adult tilapia (*Oreochromis mossambicus*) were collected from the Mariculture Research Center of the Taiwan Fisheries Research Institute, Tailan, Taiwan and were reared in a 182-L glass aquarium with plastic chips as a substrate. Each tank was supplied with dechlorinated, circulated, and aerated local tap water (FW) at 26~28 °C under a photoperiod of 12~14 hr. Fish were fed commercial fish food pellets. Adult fish at 10~12 cm total length and 60~80 g body weight were used in the present study. The water quality was measured one time per week, and included a total hardness of 146.6 ± 5.6 ppm; Na^+ of 35.6 ± 0.3 ppm; K^+ of 3.3 ± 0.1 ppm; Ca^{2+} of 30 ± 2.3 ppm; pH of 8.2 ± 0.3~8.7 ± 0.2; and a Cd^{2+} concentration of < 1 ppb. All of these parameters and the Cd media were analyzed by atomic absorption spectrophotometry (Z-5000, Hitachi, Japan). Dissolved oxygen of 4.5 ± 0.5 ppm was measured with an oximeter (Oxi 320, WTW, Germany) before each experiment. Three adult tilapia were acclimated in an aquarium for the same treatment for 48 hr before the experiment began. Six aquariums were used for each experimental time period, and replicate aquariums were similarly prepared for each treatment. Hence, 18 adult fish were used for the three treatment groups, and every treatment was repeated one time. Three treatment groups included a Cd-injection group which was injected with 3 mg/kg body weight Cd; a control group which was injected with saline (at the same pH as the Cd-injection solution); and a blank group which was only treated by handling, i.e., being transferred from the holding to the experimental tanks. Samples were taken at 0, 6, 12, 24, and 48 hr after each treatment. The Cd was prepared using completely dried $CdCl_2$ (Sigma, USA) dissolved in 1 mL concentrated HCl; and double-deionized water was used to prepare the 10 mg/L Cd stock solution, which was then diluted with saline before being injected. The previous study has found the membrane of RBC will be impacted after injection with 3 mg/L Cd, and the treated-Cd fish were no death (Chang and Wu, 2003). Therefore, the treatment dose was still used in this study.

Fish were anesthetized with MS222, and blood was collected from the caudal vessels with a 1-mL plastic syringe and a 25-G needle; the syringe was pretreated with heparin solution (3×10^5 units/mL, Sigma, USA) to prevent blood coagulation. Blood parameters, including P_{O2}, P_{CO2}, S_{O2}, Hb, and pH, were measured using a Blood Gas System (ABL^{TM} 5, Radiometer Medical A/S,

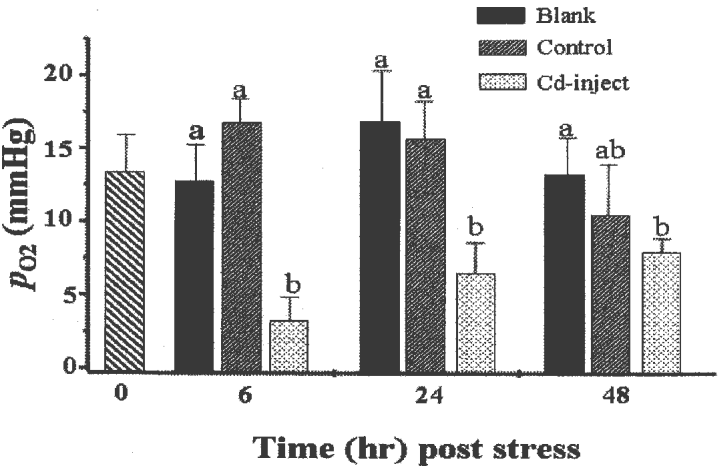

Figure 1. Changes in pO_2 levels (mmHg) of tilapia at various times after challenge with a saline injection (control group), a Cd injection (treatment group), or no injection (blank). Values are the mean ± SE (n = 10), Different letters indicate a significant difference ($p < 0.05$) between results at the same treatment time by one-way ANOVA with Tukey's comparison.

Denmark). Details of the measurements used with aquatic animals are described by Cheng et al. (2004). The blood was centrifuged at 3000 rpm for 10 min for measurement of the hematocrit (Hct). Five microliters of whole blood was diluted with 5 mL of Alserver's solution (dextrose, NaCl, sodium citrate, citric acid, and distilled water; pH 6.1 ± 0.1), RBCs were detected using a cell counter plate, and the largest sizes of the 75 RBCs were measured with a microscale using light microscopy in every group.Student's t-test was used to compare the Cd-injection and control groups in table 2. Statistical significance was accepted at a level of $p < 0.05$. One-way ANOVA with Tukey's comparison was used to compare changes with various treatments at the same time, and significance was accepted at a level of $p < 0.05$.

RESULTS AND DISCUSSION

A comparison of the P_{O2} of fish among the blank, control, and treatment groups indicated that there was a significant decrease 6~24 hr after the Cd^{2+} injection. However, there was no significant difference between the treatment and control groups after 48 hr. There were also no obvious differences in the level of P_{O2} from 0 to 48 hr in the blank group (Fig. 1). No significant differences in erythrocyte (RBC) counts appeared between 0 ([30.1 ± 5.6] × 10^4/mm^3) and 6 hr ([29.5 ± 8.3] × 10^4/mm^3) after transfer, but it was significantly (about 2-fold) higher 6 hr after treatment compared to the control and blank groups. However, counts were restored to the control level after 12~24 hr, and no significant differences among

the groups were observed (Table 1). We suggest that the hematological changes were a response to Cd-induced stress, as in rainbow trout (*Oncorhynchus mykiss*) with higher erythrocyte numbers in the blood and a lower spleen somatic index following 6 hr of confinement, but which was restored to the control level after 24 hr after the confinement (Runane et al., 2000). They suggested that the contraction of the spleen resulted in the release of blood cells into the circulation and may have accounted for the increase in erythrocytes seen after 6 hr of confinement. Hb, Hct, and erythrocyte size were compared 6 hr after fish were treated with a Cd^{2+} injection or a saline injection, in order to observe if the stress

Table 1. Effects of treatment with either a Cd^{2+} or saline injection (the control), or a blank after 6, 12, and 24 hr on the erythrocyte number ($10^4/mm^3$) of tilapia

Treatment	Time (hr) post-treatment		
	6	12	24
Blank	29.5±8.3[a]	32.1±3.8[a]	31.3±5.0[a]
Control	39.7±8.6[a]	39.6±4.6[a]	27.9±1.3[a]
Cd injection	73.4±17.8[b]	48.6±26.2[a]	20.4±5.4[a]

Data are presented as the mean ± SD (n = 15). Different superscript letters in a column indicate a significant difference ($p < 0.05$) between different tests at the same time by one-way ANOVA with Tukey's comparison.

of Cd toxicity induced the erythrocyte number to rise. Neither Hb nor Hct showed a significant difference, but the RBC size appeared smaller in Cd^{2+}-injected fish than in saline-injected fish (Table 2). We suggest that these smaller RBCs might have been released from a storage organ of blood cells, but it was useless for enhancing the P_{O2} until 24 hr in the treated tilapia group. Therefore, we determined the curve of S_{O2} of Hb in order to check that if the P_{O2} decreased following the impact of the affinity of Hb with O_2 after 24 hr of Cd exposure. In fish injected with Cd^{2+} (treatment) or saline (control), and those with no injection (blank) after 24 hr, the level of saturation of hemoglobin gradually increased following the rise in P_{O2} until complete saturation appeared to have been reached. A comparison of the curve of saturation of hemoglobin among the blank, control, and treatment groups revealed a downward shift with treatment. But it was interesting to determine why the curve of the blank group appeared to have shifted to the right compared to the control group (Fig. 2). The level of P_{CO2} was 13 mmHg at 0 hr, and showed no obvious difference with that at 6 hr in the blank group. However, it was significantly lower when compared with the control and blank groups at 24 hr after treatment. The pH levels showed no obvious changes among the groups (Fig. 3).

This study reveals that the oxygen-carrying capacity of hemoglobin was affected

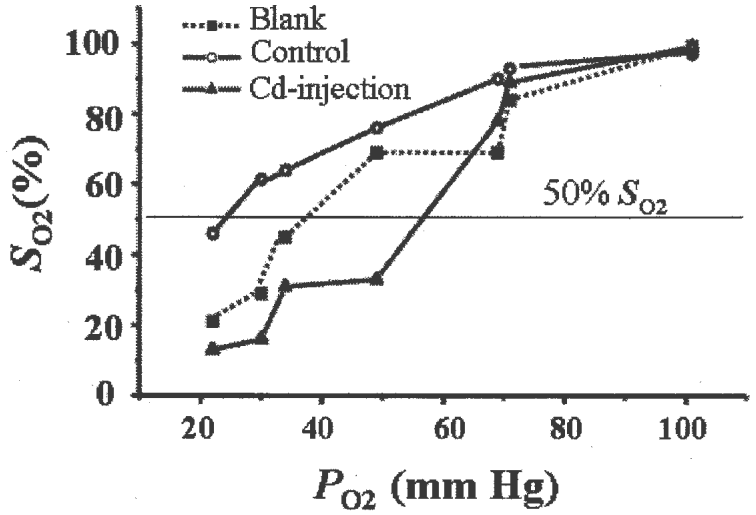

Figure 2. Effect of injection with 3mg/kg Cd (treatment group), saline (control group), or no injection (blank group) after 24 hr on the percent saturation of hemoglobin (S_{O2}) in tilapia.

Table 2. Effects 6 hr after an injection with Cd^{2+} or saline on hemoglobin (g/dL), hematocrit (%), and red blood cell size (μm) of tilapia

Injection	Blood parameter		
	Hemoglobin ($n = 10$)	Hematocrit ($n = 10$)	Red cell size ($n = 75$)
Cadmium	7.3±0.6	31.5±2.5	10.5±0.2 *
Saline	7.0±0.2	35.0±3.7	13.9±0.2

Data are presented as the mean ± S.D. * $p < 0.05$. The cell size was significantly lower in Cd^{2+}-injected than saline-injected fish (Student's *t*-test) at the same time.

By Cd^{2+} toxicity, and the fish compensated by increasing the number of erythrocytes, but the P_{O2} after treatment was still lower than that of the blank group until the end of the experiment. A similar recovery appeared 48 hr after treatment in comparison with the control group. The result was that Cd^{2+}-induced toxicity lowered the oxygen-carrying capacity of hemoglobin, and then induced P_{O2} depression 24 hr after treatment. However, the P_{O2} had gradually recovered by 48 hr after treatment. The affinity of oxygen binding by hemoglobin increases linearly with a rise in the hematocrit (Wells et al., 2003). The higher

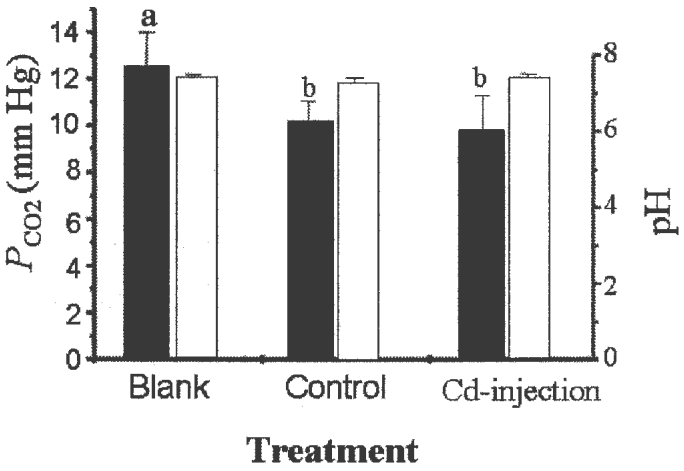

Figure. 3. Effect 24 hr after an injection of either 3 mg/kg Cd (treatment group) or saline (control group), or no injection (blank) on pH (empty columns) and p_{CO2} (filled columns). Values are the mean ± SE (n = 10). Different letters indicate a significant difference ($p < 0.05$) between treatments by one-way ANOVA with Tukey's comparison. pH levels showed no significant differences between tests

concentrations of hemoglobin and the hematocrit were an indirect response to the apparent enhancement of the oxygen-carrying capacity. Hemoglobin saturation curves represent the proportion of oxyhemoglobin produced by a given P_{O2}. Generally, an upward shift in the curve shows that oxygen is binding with hemoglobin more efficiently, while a downward shift in the curve indicates that oxygen is more easily lost. The basic functions of the curve respond to changes in temperature, pH, and P_{CO2}. Therefore, changes in these parameters might explain the oxygen-binding capacity with hemoglobin. This study showed that P_{O2} was significantly lower 6 hr after the Cd^{2+} injection compared to the other groups, and the condition was prolonged until 48 hr (Fig. 1). Although the number of circulating erythrocytes increased rapidly in response, their level returned to the normal state 12 hr after the Cd^{2+} injection (Table 1), and was comparable to the concentrations of hemoglobin and hematocrit values after 6 hr in the Cd-injection and control groups. No significant difference between the groups was seen, and the size of the RBCs was significantly smaller in the Cd-injection than in the control group ($p < 0.05$) (Table 2). We suggest that many smaller erythrocytes might have been newly created for compensation since Cd^{2+} affected the oxygen-binding capacity of hemoglobin. This evidence is also supported by the group of curves, because the lowest values of the percent saturation of hemoglobin were shown in Cd^{2+}-injected fish. This result demonstrates that Cd^{2+} caused the affinity of oxyhemoglobin to drop But we could not determine the effect of Cd^{2+} on iron absorption or the structure of hemoglobin (Huebers et al.,

1987; Mojzis and Nistiar, 2000). In addition, we noted that the curve appeared higher in the control group than in the blank group. We suggest that the shift to the left in the control group was due to injection of acid-saline into tilapia causing P_{CO2} depression, and the pH was well conserved due to the excellent buffering system of the fish (Fig. 3).

The present study is the first report of the affinity of oxyhemoglobin being affected by cadmium exposure. This data showed that these effects were brief under our experimental conditions and that P_{O2} values had gradually recovered by 48 hr post Cd exposure.

Acknowledgments. The National Science Council of Taiwan financially supported this study (grant no. NSC91-2815-C-415-003-B) and some work described in this paper was supported by Chiayi University, Chiayi, Taiwan. Thanks are extended to Dr. P. P. Hwang for his assistance during this study.

REFERENCES

Cerqueira CCC, Fernandes MF (2002) Blood cell responses of the tropical fish *Prochilodus scrofa* to acute copper exposure and subsequent recovery. In: Kennedy C, Kolok A, Kinlay DM (ed) V. International Congress on the Biology of Fish, 21-26 July, Vancouver, Canada. Aquatic Toxicology: Mechanisms and Consequences, 99-108

Chang ZM, Wu SM (2003) Effects of cadmium on erythrocyte and lymphocyte of tilapia (*Oreochromis mossambicus*). Bioformosa 38:71-78 (in Chinese)

Cheng W, Liu CH, Cheng SY, Chen JC (2004) Effect of dissolved oxygen on the acid-base balance and ion concentration of Taiwan abalone Haliotis diversicolor supertexta. Aquaculture 231: 573-586

Dethloff GM, Jonathan STH, Howard CB (1999) Alterations in physiological parameters of rainbow trout (*Oncorhynchus mykiss*) with exposure to copper and copper/zinc mixtures. Ecotoxol Environ Safe 42:253-264

Gwozdzinski K (1992) Structural changes of proteins in fish red blood cells after copper and mercury treatment. Arch. Environ. Contam Toxicol 23: 426-430.

Huebers HA, Huebers E, Csiba E, Rummel W, Finch CA (1987) The cadmium effect on iron absorption. American J Clin Nutr 45:1007-1012

Kostic MM, Ogjanovic B, Dimitrijevic S, Zikic RV, Stajn A, Rosic GL, Zivkovic RV (1993) Cadmium-induced changes of antioxidant and metabolic status in red blood cells of rates: in vivo effects. European J Haematol 51:86-93

Kotsanis N, Iliopoulou-Georgudaki J, Kapata-Zoumbos K (2000) Changes in selected haematological parameters at early stages of the rainbow trout, *Oncorhynchus mykiss*, subjected to metal toxicants: arsenic, cadmium and mercury. J App Ichthyol 16: 276-278

Mojzis J, Nistiar F (2000) Cadmium-induced changes in cation-osmotic haemolysis in rats. Envir Toxicol Pharm 8:79-81

Roche H, Boge G (1996) Fish blood parameters as a potential tool for identification of stress caused by environmental factors and chemical

intoxication. Mar Environ Res 41:27-43
Ruane NM, Nolan DT, Rotliant J, Costelloe J, Wendelaar Bonga SE (2000) Experimental exposure of rainbow trout *Oncorhynchus mykiss* (Walbaum) to the infective stages of the sea louse *Lepeophtheirus salmonis* (Kroyer) influences the physiological response to an acute stressor. Fish Shellfish Immuno 10: 451-463
Wells RMG, Baldwin J, Seymour RS, Baudinette RV, Christian K, Bennett MB (2003) Oxygen transport capacity in the air-breathing fish, *Megalops* cyprinoids: compensations for strenuous exercise. Comp Biochem Physiol 134A: 45-53