Haematological changes in *Bufo maculatus* treated with sublethal concentrations of Cadmium

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Abstract

Adult *Bufo maculatus* was exposed to sublethal cadmium concentrations of 0.25, 0.50, 1.00 and 2.00 mg/L. The toxicant from which the cadmium concentrations were prepared was cadmium chloride ($\text{CdCl}_2 \cdot \text{H}_2\text{O}$). There were three replicate tanks per treatment and three individuals per tank including control groups. The hematologic alterations based on the examination of blood indices during the 28 days of exposure showed that total erythrocyte count (TEC), hematocrit (Hct) and hemoglobin (Hb) concentration decreased ($P < 0.05$) relative to controls. The decline was concentration-dependent as concentration of cadmium increased. The decline in hemoglobin and hematocrit in the experimental organism could be due to a decrease in the synthesis or release of erythrocytes into the circulation or an increase in the rate of erythrocyte destruction inflicted by cadmium toxicity. There was significant ($P < 0.05$) elevation in total leukocyte count (TLC) with increase in the concentration of cadmium. The increase in total leukocyte count observed in this study could be attributed to a stimulation of the immune system in response to tissue damage caused by cadmium toxicity. The study has shown that the exposure of the *Bufo maculatus* toad to cadmium can inflict alterations in the hematologic indices, which could induce unfavorable physiological changes in the amphibian, which may lead to death. There is, therefore, the need to protect amphibians in order to sustain the biodiversity in the Nigerian Niger Delta ecological zone.

Introduction

In the past few decades, scientists have reported global declines in amphibian populations worldwide.¹⁻³ Heavy metal pollution has been implicated as one of the possible causes. Metal accumulation in the tissues of amphibians is dependent upon exposure concentra-

tions, time, temperature, age of amphibian, interaction with other metals, metabolic activity and water chemistry [Enuneku AA, unpublished data].

Cadmium is a toxic heavy metal widely used in industrial processes like the production of nickel-cadmium batteries, as a stabilizer in PVC products, in metallic pipes, as a neutron absorber in nuclear power plants, and as a color pigment and an anticorrosive agent and in phosphate fertilizers.⁴ Dumping and incineration of cadmium polluted waste causes pollution of terrestrial and aquatic ecosystems where amphibians reside. Effluents from these activities are sources of cadmium in aquatic environments.⁵ These have predisposed animal and human populations to both subtle and direct exposure pathways with an attendant increase in cadmium related pathologies.⁶ Cadmium is one of the PBTs (persistent, bioaccumulative and toxic) chemicals identified as a primary toxicant.⁷ Cadmium is considered to have toxic effects on hematologic parameters, prostate, kidney, lungs and testes.⁸ Recent research findings reveal that the monitoring of environmental toxicants using biological organisms is more realistic than physico-chemical measures of concentrations in water, soil or sediment. Maheswaran *et al.*⁹ have reported that the main components of blood, *i.e.* hemoglobin concentration (Hb), total erythrocyte count (TEC), total leukocyte count (TLC) and hematocrit (Hct), have been used as indicators of heavy metal pollution in the aquatic environment.

This study was conducted to evaluate the toxicity of cadmium on the flat backed toad *Bufo maculatus*, native to the Niger Delta Region of Nigeria and other West African countries. This species was selected because it is ubiquitous, sensitive to heavy metals, and easy to handle and raise in the laboratory.

Materials and Methods

The initial mean weight of toads was 27.14 ± 0.34 g. There was no significant difference ($P > 0.05$) between the mean weights of toads used in the experiments. Because metabolic activity changes with size and affects the parameters to be measured, individuals of similar size were used.¹⁰ The toads were acclimatized to laboratory conditions in dechlorinated tap water for two weeks prior to the assay in plastic tanks measuring 49 cm $\times$ 29 cm $\times$ 24 cm. During acclimation, the mean values for the test water quality were: temperature $22 \pm 1^\circ\text{C}$; pH 5.7 ± 0.4 ; dissolved oxygen 4.7 ± 0.7 ppm hardness 36 ± 1.24 ppm. The toads were fed daily *ad libitum* with termites. Toads maintained a photoperiod of 10 h light, 14 h dark regime. Stock solutions of $\text{CdCl}_2 \cdot \text{H}_2\text{O}$ were pre-

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Key words: hematologic parameters, toxicity, amphibian, cadmium.

Contributions: Both authors contributed to study design and concept, sublethal exposure, laboratory analysis, collation of results and statistical analysis. A greater part of the funds used was provided by the second author. Both authors wrote the manuscript and edited the final version.

Conflicts of interests: the authors declare no potential conflicts of interests.

Received for publication: 11 October 2011.

Revision received: 22 November 2011.

Accepted for publication: 22 November 2011.

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Journal of Xenobiotics 2011; 1:e7
doi:10.4081/xeno.2011.e7

pared by dissolving toxicant in distilled water. Treatment concentrations were achieved by serial dilutions.³ The toxicant in the test chamber was completely renewed with fresh solution of the same concentration every 24 h.

Four sublethal concentrations (0.25, 0.50, 1.00 and 2.00 mg/L) of cadmium were prepared and 3 toads were introduced in each concentration per tank. For this sublethal exposure, 3 replicates were used. The toads were fed with termites *ad libitum* as in controls. At the end of 28 days, toads were decapitated and blood collected by heart puncture by means of a hypodermic syringe and needle. The blood collected from the heart was transferred to ethylenediaminetetraacetic acid (EDTA) containers for hematologic analysis.¹¹ The treatment and control blood samples were used to estimate the hematologic parameters.

EDTA was used as anticoagulant and non-coagulated blood was tested shortly after collection for Hb, TEC, TLC and Hct. Hb concentration was estimated as cyanomethemoglobin according to Ochei and Kolhakter.¹² TEC and TLC were determined according to the classical method using the Neubauer hemacytometer. Hematocrit was determined using microhematocrit tubes and a hematocrit centrifuge (Juan Model A 13). Blood was centrifuged at $1200 \times g$ for 5 min and the hematocrit value was obtained.¹³

Statistical Analysis

Data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's Multisample Range post hoc test using SPSS 15 software (SPSS Inc. Chicago, USA). $P < 0.05$ was considered level of statistical significance.

Results

There was no significant difference in water quality parameters (pH, temperature, dissolved oxygen, hardness and turbidity) monitored during the exposure periods for sublethal assays between various concentrations of the heavy metal and the control groups, or within concentrations ($P > 0.05$). The parameters were almost uniform throughout the study, irrespective of the treatment or duration.

The effects of cadmium on hematologic parameters are presented in Figures 1-4. The mean values of hematocrit, total erythrocyte count, total leukocyte count and hemoglobin were seen to increase with an increase in concentration of cadmium compared to control groups. However, total leukocyte count decreased as concentration of heavy metal increased at levels of $P < 0.05$.

Discussion

In this study, the administration of cadmium to *B. maculatus* induced a reduction in total erythrocyte counts, hematocrit and hemoglobin levels with an increase in the concentration of cadmium. The observed decrease in these blood indices is indicative of cadmium-induced anemia and is consistent with previous studies of anemia in fish, rats and rabbits exposed to cadmium, mercury, lead, nickel, copper and zinc.¹⁴⁻¹⁶ Various mechanisms have been proposed for cadmium-induced anaemia. These include iron deficiency anemia due to competition of cadmium with iron in the iron transfer system of the intestinal mucosa, hypoplastic anemia derived from the inhibitory effect of cadmium on the growth of erythroid progenitor cells¹⁷ or bone marrow cells,¹⁸ and hemolytic anemia due to red cell sequestration in the spleen.¹⁹

Lowe-Jinde and Niimi²⁰ observed a reduction in the number of erythroblasts-proerythrocytes in goldfish, *Carassius auratus* and rainbow trout *Salmo gairdneri* exposed to cadmium. They suggested that cadmium caused a reduction in erythropoiesis and impeded the formation of red blood cells. Again the accumulation of cadmium in various organs particularly the kidney and liver has been observed²¹⁻²³ and it is

conceivable that the activity of these hematopoietic tissues may be suppressed or compromised. However, it cannot be ruled out that a decline in hemoglobin and hematocrit in the toad, *Bufo maculatus* could be due to a decrease in the synthesis or release of erythrocytes into the circulation or an increase in the rate of erythrocyte destruction.

The increase in total leukocyte count observed in this study could be attributed to a stimulation of the immune system in response

to tissue damage caused by cadmium toxicity. Tissue damage or injury could induce stimulation of the immune system manifested by an increase in lymphocytes.²⁴ Various authors have reported an increase in leukocyte count due to heavy metal exposure in experimental organisms. Lowe-jinde and Niimi²⁰ also reported that the rainbow trout exposed to 4 µg/L cadmium sulphate for 14 days had an increase in leukocyte production when compared to the control fish but that after 96 days they showed

Figure 1. Variation in hematocrit in *B. maculatus* exposed to cadmium. Values are mean $\pm$ standard deviation (n=9).

Figure 2. Variation in total erythrocyte count in *B. maculatus* exposed to cadmium. Values are mean $\pm$ standard deviation (n=9).

Figure 3. Variation in total leukocyte count in *B. maculatus* exposed to cadmium. Values are mean $\pm$ standard deviation (n=9).

Figure 4. Variation in hemoglobin concentration in *B. maculatus* exposed to cadmium. Values are mean $\pm$ standard deviation (n=9).

a significant decrease.

The release of cadmium into the environment could possibly affect the wellbeing of the organism as well as result in a decline in the numbers of these very sensitive anurans that make a significant contribution to the food chain.

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