Bull. Environ. Contam. Toxicol. (2004) 72:639–646

DOI: 10.1007/s00128-004-0291-6

Environmental Contamination and Toxicology

Effects of Aluminum in Acidic Water on Hematological and Physiological Parameters of the Neotropical Fish *Leporinus macrocephalus* (Anostomidae)

I. F. Barcarolli, C. B. R. Martinez

Departamento de Ciências Fisiológicas, Universidade Estadual de Londrina, C.P. 6001, CEP: 86051-990, Londrina, Paraná, Brazil

Received: 15 August 2003/Accepted: 4 December 2003

Aluminum in acidic waters has been shown to be highly toxic to fish (Exley et al. 1991). Some investigations have reported ionoregulatory and respiratory disturbances in fish, especially salmonids, under acid/aluminum exposure (Wood et al. 1988; Withers et al. 1990, 1992, 1996; Waring and Brown 1995; Brown and Waring 1996). Despite the importance of aluminum toxicity to fish, little is known about the physiological effects of this metal on neotropical fish species. Gil et al. (1993) examined the influence of aluminum in acidic water on oxygen consumption of the characin fish species *Astyanax bimaculatus*. Except for this study, information about aluminum effects on freshwater neotropical fish is still lacking. Actually, toxicological guidelines for metals, in general, in most tropical countries are mainly derived from data collected in nontropical ecosystems (Oliveira Ribeiro et al. 1996). There is thus a need to assess the validity of such a practice by comparing toxicological effects of various pollutants on fish from tropical and boreal regions (Oliveira Ribeiro et al. 2000).

The present study was conducted in order to evaluate acute effects of Al in acidic water on the Brazilian fish species *Leporinus macrocephalus* (Anostomidae). Considering that this fish is one of the most cultivated freshwater species in Brazil (Martins and Yoshitoshi 2003), where it corresponds to an important source of food, *L. macrocephalus* represents a suitable neotropical fish for the assessment of pollutant effects. Hematocrit, hemoglobin content, NaCl plasma concentrations, blood glucose, lipids and proteins were determined in juvenile *L. macrocephalus*, under laboratory conditions with pH 5.0 and Al concentration (15 μg Al. L^{-1}) similar to field conditions (Yabe and Oliveira 1998).

MATERIALS AND METHODS

Juveniles of *Leporinus macrocephalus* Garavello and Britski 1988 (mean mass 35.66 g, range 25.07 – 47.53 g) were obtained from the Universidade Estadual de Londrina hatchery station. They were held in a 600 L tank, with continuously aerated well water (T = 23°C, pH = 7.3, total hardness = 80 mg. L^{-1} $CaCO_3$), with a 14h/10h light/dark cycle, for at least 7 days prior to experiments. Fish were fed

Correspondence to: C. B. R. Martinez

ad libitum with pellet food each 48 h, except during and on the day preceding the experiments.

Three treatments were tested: pH 7.3 + 0 μg $Al.L^{-1}$ (control group), pH 5.0 + 0 μg Al. L^{-1} (pH 5 group) and pH 5.0 + 15 μg Al. L^{-1} (Al group). For each treatment, two subgroups of six animals were held in 100 L glass aquaria. One group was terminally sampled after 24 and the other after 96 h of exposure to each treatment. The pH was reduced to 5.0 units by adding HCl 10% and monitored at 6 hours interval. Aluminum was added to the water as $Al_2(SO_4)_3$ reagent grade to a nominal concentration of 15 μg Al. L^{-1}. Water temperature, hardness and day/light regime were the same as reported above.

Immediately after removal of the fish from the water, blood samples were taken from the caudal vein by means of heparinized plastic syringes. Hematocrit values were determined by blood centrifugation (5 min, 5000 ***g***) in glass capillaries, using a microhematocrit centrifuge. Total hemoglobin blood concentration was measured by the cyanomethaemoglobin method using a commercially available Kit (Analisa, Brasil). Blood samples were then centrifuged (5 min, 12000 ***g***) and plasma samples were stored frozen (-20°C) until the moment for chemical analyses. Plasma sodium concentration was measured by flame photometry (Analyser, Model 900, Brazil). Chloride and total lipids concentrations in blood plasma were determined by spectrophotometric enzymatic method using commercial Kit (Analisa, Brazil). Plasma glucose concentration was measured by spectrophotometric method using a glucose/peroxidase enzymatic assay. Total proteins in blood plasma were measured using the spectrophotometric determination according to Lowry et al. (1951) with bovine serum albumin as standard. All samples were analyzed in duplicate in a spectrophotometer (Shimadzu UV 1203, Japan).

For each parameter analyzed differences among control group, pH 5 group and Al group, for each exposure period (24 and 96 h), were tested for significance by one-way ANOVA and multiple range test (Student-Newman-Keuls procedure) where appropriate. Means were considered significantly different where $P < 0.05$.

RESULTS AND DISCUSSION

L. macrocephalus responses to pH 5.0 without Al were negligible. There was neither hematological nor ionic nor metabolic response during acid exposure (Figures 1, 2, 3). When Wilson et al. (1999) exposed three Amazonian fish species to a graded reduction in water pH, from 6 to 3.5, they noticed a strong relationship between the magnitude of ion losses and the toxicity of exposure to low pH. As *L. macrocephalus* did not show any ionic disturbance following short-term acidic exposure it would be expected a high tolerance to pH 5.0 for this fish species.

Hematocrit was significantly increased in fish exposed to pH 5 + Al for 24 h (Fig. 1). This increase might be explained by the osmotic swelling of red blood cells, as

it was also observed Na^+ and Cl^- decreased plasma concentrations (Fig. 2). It is probably not related to decreased plasma volume nor increased circulating red blood cells once it was not observed significant increase in plasma protein (Fig. 3) nor significant increase in hemoglobin content (Fig. 1). Elevated values of hematocrit were also observed in rainbow trout during combined acid and aluminum exposure (Witters et al. 1990).

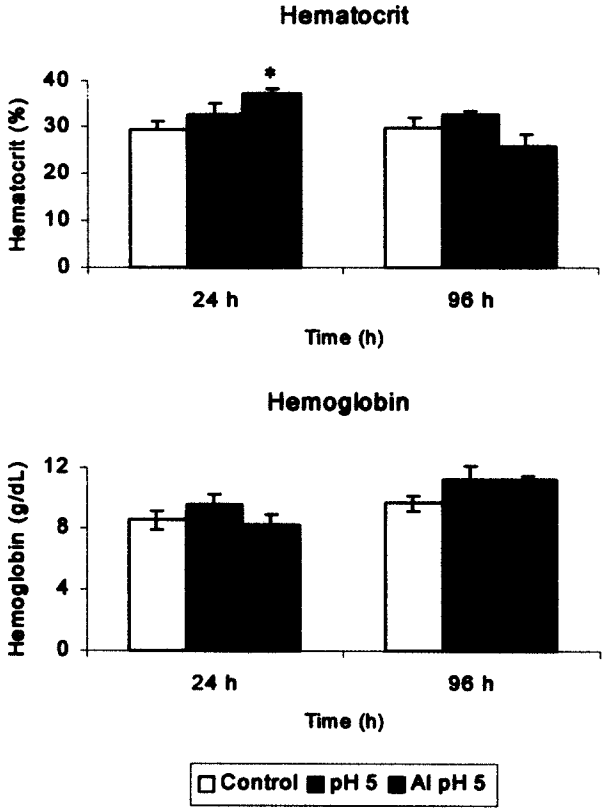

Figure 1. Hematocrit and hemoglobin content of *L. macrocephalus* exposed to pH 7.3 + 0 μg Al.L^{-1} (control group), pH 5.0 + 0 μg Al. L^{-1} (pH 5 group) and pH 5.0 + 15 μg Al. L^{-1} (Al pH 5 group) for 24 or 96 h. The values are mean ± S.E (n=6). (*) indicate significance difference from the respective control ($P<0.05$).

Plasma concentrations of sodium and chloride were significantly reduced after 24 h exposure to pH 5.0 plus 15 μg Al. L^{-1} (Fig.2), while plasma glucose levels, on the other hand, were elevated (Fig. 3). These transient blood alterations are typical of fish exposed to acidic water and aluminum, and indicate the presence of sublethal physiological alterations (Brodeur et al. 2001).

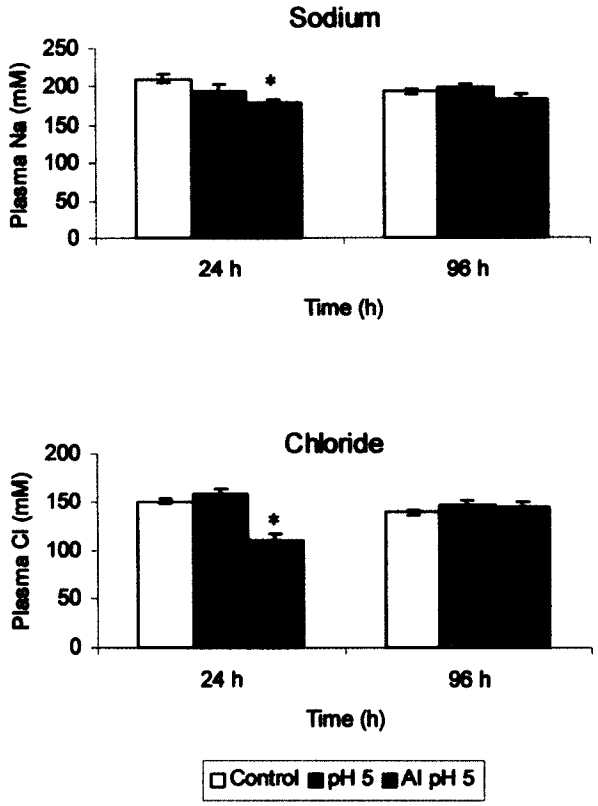

Figure 2. Sodium and chloride plasma concentrations of *L. macrocephalus* exposed to pH 7.3 + 0 μg $Al.L^{-1}$ (control group), pH 5.0 + 0 μg Al. L^{-1} (pH 5 group) and pH 5.0 + 15 μg Al. L^{-1} (Al pH 5 group) for 24 or 96 h. The values are mean ± S.E (n=6). (*) indicate significance difference from the respective control ($P<0.05$).

The toxicity of Al to salmonid fish in the pH range 4.2 to 5.5 has been partially attributed to a failure of ionoregulation caused by a combination of Al and H^+ effects, primarily localized at the gill site (Witters et al. 1992, 1996). In the present study ionoregulatory changes, as shown by plasma Na^+ and Cl^- levels, were observed in fish exposed to Al in acid water for 24 h (Fig. 2). Considering that there was no indication of any changes in blood volume, as plasma protein concentrations remained stable, this ion reduction might be the result of increased ion efflux or decreased ion influx, or both. Similar results have been noted by Waring and Brown (1995) in brown trout exposed to pH 5.0 plus 12.5 μg Al. L^{-1}. In rainbow trout, mainly Na^+ efflux was stimulated due to Al exposure, while the reduction of the Na^+ influx contributed to a lesser extend to the net branchial Na^+ loss (Witters et al. 1992).

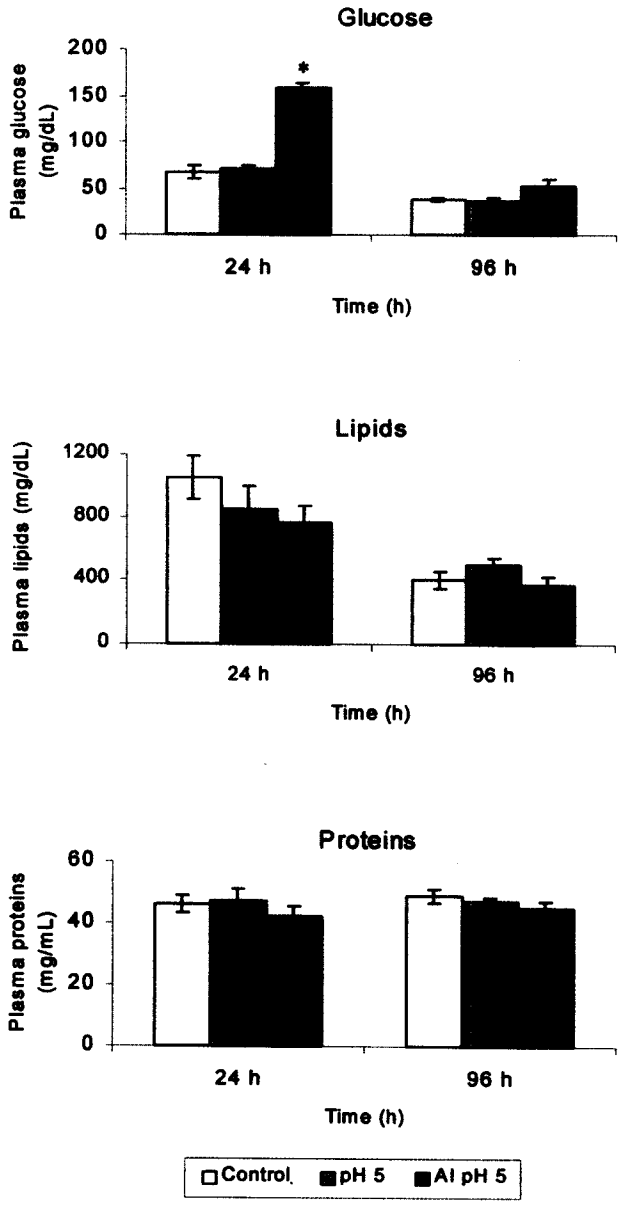

Figure 3. Glucose, total lipids and total proteins plasma concentrations of *L. macrocephalus* exposed to pH 7.3 + 0 µg Al.L^{-1} (control group), pH 5.0 + 0 µg Al. L^{-1} (pH 5 group) and pH 5.0 + 15 µg Al. L^{-1} (Al pH 5 group) for 24 or 96 h. The values are mean ± S.E (n=6). (*) indicate significance difference from the respective control ($P<0.05$).

The recovery of plasma Na^+ and Cl^- to control levels, after 96 h (Fig. 2), indicates that *L. macrocephalus* were able to correct ionoregulatory disturbances caused by acidic water and Al. This physiological recovery is probably the result of a damage-repair phenomenon involving physiological, biochemical, and structural changes at the gills (McDonald and Wood 1993).

There was a blood glucose increase in *L. macrocephalus* exposed to pH 5 + Al for 24 h. Similar increase due to Al exposure was also mentioned by Goss and Wood (1988) and Witters et al. (1990) for rainbow trout and by Brodeur et al. (2001) for Atlantic salmon. The increased level of glucose could have a double function: firstly an osmoregulatory role in maintaining plasma osmolality during ionoregulatory failure, and secondly as an energy source for elevated metabolic costs during stress (Witters et al. 1990). A rise in the plasma glucose concentration indicates an activated carbohydrate metabolism, which in fish is normally under the control of cortisol (Brown and Whitehead 1995). An increase in plasma cortisol concentrations has already been reported in aluminum-stressed freshwater fish (Goss and Wood 1988; Brown and Waring 1996).

Aluminum in acidic water has been reported to stimulate interrenal activity and plasma corticosteroid and glucose levels in brown trout (Brown and Whitehead 1995). Hypersecretion of adrenalin and cortisol are considered primary stress responses. These effects in turn trigger a broad suite of biochemical and physiological alterations called secondary stress response. Metabolic effects include hyperglycemia and altered blood levels of protein, cholesterol and free fatty acids (Wendelaar Bonga 1997). In this study, *L. macrocephalus* presented hyperglycemia associated to lowered lipids after 24 h exposure to Al in acidic water (Fig.3), illustrating a "classical general adaptation syndrome to stress".

In summary, this study shows that despite being tolerant to low pH, *Leporinus macrocephalus* is very sensitive to the presence of low Al concentrations (15 μg Al. L^{-1}) in acidic water. The results indicated that the mechanisms of acute Al toxicity in acidic waters for *L. macrocephalus* are similar to those described for salmonid fish species.

Acknowledgments. The authors thank the Universidade Estadual de Londrina hatchery station (EPUEL) for the supply of fish. This study was supported by a grant from FINEP (Financiadora de Estudos e Projetos – Brazil).

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