IN VIVO EFFECTS OF TRIVALENT AND HEXAVALENT CHROMIUM ON RENAL AND HEPATIC ATPASES OF A FRESHWATER TELEOST *ANABAS SCANDENS*

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(Received: March 1992; revised: December 1992)

Abstract. The *in vivo* toxic effect of trivalent and hexavalent chromium (25 mg/L) on the renal and hepatic tissue ATPases of an edible teleost *Anabas scandens* was studied. In an exposure span of 30 days Na^+-K^+ ATPase activity exhibited a progressive inhibition in the kidney, but marked inhibition in Na^+-K^+ ATPase activity was observed in the liver. Mg^{2+} ATPase activity, however, exhibited an elevation on early exposures, with a later inhibition.

Key words: Cr^{+3}, Cr^{+6}, Na^+-K^+ ATPase, Mg^{2+} ATPase, *Anabas scandens*.

1. Introduction

The discharge of heavy metals by industries poses serious water pollution problems due to the toxic properties of these metals and their adverse effect on aquatic life. Chromium is one of the few trace elements that is essential for life, and reviews of the occurrence, function and toxicity of Cr in biological systems have recently been published (Mertz, 1982). The environmental effects of chromium have been well documented (Langard, 1978; International Agency for Research on carcinogenesis Monograph, 1980). Hexavalent chromium most often appears as a water-soluble chromate or bichromate, both powerful oxidants that can easily penetrate biologic membranes and irritate cells (Mertz, 1969). It is one of the constituents of effluents from a large number of industries, particularly of the tanning industry (Dad *et al.*, 1980). The contamination of irrigation reservoirs by tannery effluents in the state of Tamil Nadu has already been reported (Guru Prasad Rao and Nanda Kumar, 1981). In another instance of water pollution from the chromate chemical industry in the state of Tamil Nadu (Nanda Kumar and Rajendra Babu, 1984), a high pollution load by chromium salts contaminated irrigation wells (160–780 mg/L of Cr reported) and reservoir waters (25–100 mg/L of Cr), apart from damaging soil quality and crop pattern. Among the organisms, aquatic animals are targeted heavily by the chromium pollutants (Venugopal *et al.*, 1990).

Little information exists on toxic effects produced by chromium in teleosts (Sastry and Sunita, 1983). The freshwater edible teleost *Anabas scandens* dwells in lakes and irrigation canals and is a sensitive indicator for understanding the

Environmental Monitoring and Assessment **28:** 131–136, 1993.

effect of Cr^{3+} and Cr^{6+} (Venugopal and Reddy, 1992).

ATPases are the enzymes concerned with the immediate release of useful energy for all types of physiological activities. ATPase activity is a measure of the intricacies of energy metabolism and forms a useful toxicological tool. Sodium-potassium ATPase is a key enzyme in the cellular water balance and it plays a central role in ion regulation throughout the body. Hence the present study reports the sublethal toxic effect of chromium on renal and hepatic ATPases (Na^+-K^+ ATPase and Mg^{2+} ATPase) of a freshwater edible teleost *Anabas scandens*, an important component of aquatic ecosystems that deserves an understanding of the effect of environmental pollutant like chromium, in its two chemical species.

2. Materials and Methods

Freshwater fishes *Anabas scandens*, weighing 35 ± 5 g (length ranging from 14–16 cm) were collected from the ponds around Kolleru lake (where they are cultured), West Godavari district, Andhra Pradesh. The fishes were brought to the laboratory and acclimated to laboratory conditions. They were fed with groundnut-oil cake *ad libitum*. Only active, healthy and uninjured male fishes were selected for the experiment. The fishes were transferred to plastic tubs and maintained with dechlorinated tap water. The physicochemical characteristics of water were as follows: pH 7.2–7.4; dissolved oxygen 7.8–8.0 mg/L; carbon dioxide 2.08 mg/L; salinity 0.190 gm/L; alkalinity 102 mg/L (as $CaCO_3$); hardness of water 112 mg/L; and O_2 saturation, 8%. The temperature of the water was $27 \pm 0.5°$ C.

The stocks of the toxicant solutions $K_2Cr_2O_7$ and $CrCl_3$ (Merck) were prepared in deionized water and appropriate amounts of toxicant solutions were added to each tub to obtain serial concentrations of the toxicants ($CrCl_3$ and $K_2Cr_2O_7$). Fishes in batches of 10 were taken in six 50 L plastic troughs and were exposed for 30 days to selected concentrations. The toxicity of trivalent and hexavalent chromium to *Anabas scandens* was evaluated in static medium according to the method of Doudoroff *et al.* (1951). The mortality of fishes to each concentration was recorded. The bioassay experiments were repeated thrice and average values were taken. LC_{50} calculations were made following the probit method (Finney, 1964) by drawing plots between log concentrations and percent mortality and between log concentrations and probit mortality. The LC_{50} values of *Anabas scandens* to hexavalent and trivalent chromium were 74.88 ppm and 110 ppm, respectively.

The fishes were divided into three groups. The animals of groups I and II were exposed to a sublethal concentration of 25 mg/L of Cr, as potassium dichromate and chromium chloride, respectively, for a subchronic period of 30 days. Group III served as control. During treatment with chromium the fish were fed every 24 h before renewing the toxicant water. The fish were starved 24 h prior to the experiment to avoid metabolic differences, if any, due to differential feeding and food reserves. Fish were sacrificed on days 1, 7, 15 and 30 of exposure and the liver and kidneys were isolated from normal and exposed fish and transferred to a deep

freeze store at −80° C for enzyme assays. Each biochemical parameter was assayed in six individual animals, in duplicate, in both control and chromium-treated groups. Parallel controls were maintained, to avoid possible variations in percent responses that might occur on account of the poikilothermic status of these animals. ATPase (adenosine triphosphate; ATPase phosphorylase E.C.3.6.1.3.) activity was assayed by the method of Kaplay (1978). The inorganic phosphate liberated was measured by the method of Taussaky and Shorr (1953). The difference in the activity of enzyme in the absence and presence of ouabain was taken as Na^+-K^+ ATPase activity. Total activity in the presence of ouabain was Mg^{2+} ATPase activity. The protein content in the enzyme source was estimated according to the method of Lowry *et al.* (1951). Enzyme activity was expressed as micromoles of inorganic phosphate formed/mg protein/hour. The results were subjected to statistical analysis and student's '*t*' test was used since each treatment is compared only to a control and not to another treatment.

3. Results and Discussion

The present investigation to assess the toxicological effect of a sublethal concentration of trivalent and hexavalent chromium on the energy metabolism of renal and hepatic tissues of fish shows significant alterations in ATPase activities, reflecting time-bound organ-sensitivity to chromium toxicity.

Na^+-K^+ ATPase is a key enzyme in cellular water balance and in osmoregulation. It plays an important role in aquatic organisms which maintain hemolymph ion concentrations which are different from the ion concentration of their environment (Degani and Warburg, 1984). There was an increase in the activity of Na^+-K^+ ATPase on the first day of exposure in the liver (Table I). The apparent increase in the Na^+-K^+ ATPase activity on early exposure suggests enhanced active transport across tissue membranes, facilitating the exchange of nutrients (Dange, 1986). It may also suggest the stimulation of anaerobic metabolism at the expense of aerobic processes (Schwartz *et al.*, 1975). However, there was a decrease in the activity of Na^+-K^+ ATPase activity in the liver after other exposure spans (Table I). Kidney tissue demonstrated an inhibition in Na^+-K^+ ATPase activity throughout the exposure period (Table I); this suggests its sensitivity to hexavalent chromium. Kuhnert and Kuhnert (1976) showed that Na^+-K^+ ATPase activity was significantly inhibited in the kidney and intestine of rainbow trout exposed to Cr(VI). Metal ions also may bring about changes in the concentration of cofactors of reactants by altering membrane permeability, including that of mitochondria, and indirectly affect enzyme activity (Passow, 1970). Some part of the Na^+-K^+ ATPase activity is mitochondrial, and a disruption in vital energy-yielding phosphorylation processes (Verma *et al.*, 1978; Waston and Bemich, 1980) agrees with the observed inhibition in ATPase system in the present study. The profiles of other parameters also indicate an altered energy metabolism. An inhibition in the activities of mitochondrial dehydrogenases (Venugopal and Reddy, 1991) and a depletion in

TABLE I

Na^+-K^+ ATPase activity in the tissues of freshwater teleost *Anabas scandens* exposed to a sublethal concentration of trivalent and hexavalent chromium.

Tissue		Exposure period in days			
		1	7	15	30
Liver	Control	1.80 ±0.344	1.748±0.156	1.926±0.121	1.848±0.189
	Cr^{+3}	2.206±0.207	1.508±0.316	1.406±0.417	1.594±0.129*
	Difference	+22.48	−13.17	−26.99	−35.38
	Cr^{+6}	2.241±0.286*	1.372±0.161**	1.168±0.211***	0.759±0.277***
	%Difference	+24.43	−21.51	−39.35	−58.92
Kidney	Control	3.662±0.360	3.924±0.329	3.816±0.379	3.821±0.406
	Cr^{+3}	2.721±0.318	2.644±0.281	2.328±0.301	1.955±0.171
	%Difference	−25.69	−32.61	−38.99	−48.83
	Cr^{+6}	2.527±0.262***	2.508±0.198***	2.105±0.311***	1.855±0.271***
	%Difference	−30.99	−36.08	−44.83	−51.45

Each value is a mean of six individual observations ±S.D. Value expresses as μmoles of Pi/mg Protein/h; Experimental value significantly different from control with statistical significance at * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

glycogen and glucose content in the tissues of the same fish model (Venugopal and Reddy, 1992), substantially support the observed inhibition of ATPases in liver and kidney in the present study. The interference of xenobiotics with ionic homeostasis may be reflected as inhibited Na^+-K^+ ATPase activity (Haya and Waiwood, 1983). The supposition of Cr-ATP complex formation, which slowly hydrolyses and inactivates the enzyme, will explain the inhibition of Na^+-K^+ ATPase in liver and kidney in the present study.

The Mg^{2+} ATPase operates in a reverse direction as a coupling enzyme in the oxidative phosphorylation of ADP to ATP. The enzyme mediates an ATP-driven uptake of various extracellular solutes against an electrochemical gradient. Mg^{2+} ATPase activity in the present study was elevated on the 1st and 7th day of the exposure (Table II) in liver and kidney. The apparent increase in Mg^{2+} ATPase activity on the 1st and 7th day of exposure suggests the stimulation of anaerobic metabolism at the expense of aerobic processes, and it may also point to an enhanced active transport across tissue membranes to combat the metal-imposed stress, facilitating the exchange of nutrients. Such tissue-specific toxic responses in ATPase of aquatic animals exposed to heavy metals have been recorded earlier (Tucker, 1979; Tucker and Matte, 1980). Chromium is known to activate the cytochrome system up to a limiting concentration. Increase in Mg^{2+} ATPase activity at lower concentrations of chromate, followed by an inhibition at high

TABLE II

Mg^{2+} ATPase activity in the tissues of freshwater teleost *Anabas scandens* exposed to a sublethal concentration of trivalent and hexavalent chromium.

Tissue		Exposure period in days			
		1	7	15	30
Liver	Control	1.418±0.114	1.348±0.135	1.582±0.191	1.439±0.186
	Cr^{+3}	1.586±0.129*	1.569±0.101**	1.198±0.211**	1.189±0.169*
	%Difference	+11.84	(+16.39)	−24.27	−17.37
	Cr^{+6}	1.842±0.345*	1.827±0.255**	0.928±0.086	1.068±0.110**
	%Difference	+29.90	+35.53	−41.34	−25.78
Kidney	Control	2.237±0.193	2.312±0.331	2.392±0.323	2.228±0.193
	Cr^{+3}	2.501±0.189*	2.644±0.443 Ns	1.941±0.231*	1.725±0.156
	%Difference	+11.80	+14.35	−18.85	−22.57
	Cr^{+6}	2.647±0.291*	2.835±0.361*	1.642±0.281**	1.644±0.167
	%Difference	+22.62	+22.62	−31.35	−26.21

Each value is a mean of six individual observations ± S.D. Values expressed as μmoles of inorganic phosphate/mg protein/h; Experimental value significantly different from control with statistical significance at * $P < 0.05$; ** $P < 0.01$.

concentrations of (100 μg/ml), was recorded in the gastrocnemius muscle of frog (Rajendra Babu and Nanda Kumar, 1987). The inhibition observed in the present investigation might be attributed to the inhibition caused at the phosphate receptor site of Mg^{2+} ATPase.

Acknowledgements

The first author (NBRK) is grateful to CSIR, New Delhi for providing a research fellowship. Thanks are also due to Professor M.A. Khan, Head, Dept. of Zoology, for providing facilities.

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