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Alterations in Enzymes in an Indian Catfish, *Clarias batrachus* (Linn.), Exposed to Vanadium

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The increase in air and water borne concentrations of vanadium, a physiologically and pharmacologically active substance (Jandhyala and Hom 1983), has caused renewed interest in the toxicology of this metal. Toxicity studies of vanadium on fish are comparatively scarce (Holdway and Sprague 1979; Miramand et al.).

Cytochrome P-450 monoxygenases and conjugating enzymes are important in fish metabolism and elimination of endogenous as well as foreign lipophilic compounds (Zhang et al. 1990). These enzymes are also known to participate in activation and inactivation of carcinogenic and environmental chemicals. (In vitro) investigations have shown that fish metabolize xenobiotics by mechanisms very similar to those found in mammals (Vodicnik et al. 1981). Impairment of drug-metabolizing enzymes is thus deleterious to the organism. Heavy metals are known to influence this group of enzymes (Dierickx 1982). This prompted us to investigate the effect of vanadate, in a dose-responsive and time-dependent manner, on the relative rates and patterns of hepatic glutathione transferase (GST) activity, UDP-glucuronyl transferase (UDPGT) activity and cytochrome P-450 (Cyt P-450) level in an Indian catfish, Clarias batrachus. We chose an aquatic species for our experiment since the environment where aquatic animals live is the main destination of industrial and agricultural wastes. The variations recorded in the level and activity of cytochrome P-450, glutathione transferase and UDP-glucuronyl transferase are discussed in relation to carcinogenic risk depending on the environment of the fish. Largely, the support of the above hypothesis comes from the report of Payne et al. (1987) where petroleum has been found to induce cytochrome P-450 and subsequently produce high levels of neoplasia in fish. Furthermore, GST, UDPGT and Cyt P-450 have been reported as reliable markers for preneoplastic lesions in liver (Sato 1990).

MATERIALS AND METHODS

Mature Clarias batrachus (16-18 cm in standard length) were obtained from a local pond and acclimatized in the laboratory for two weeks before they were exposed to various concentrations of ammonium monovanadate salt (NH_4VO_3) (2,5, and 10 mg/L). The fish were held in groups of five in glass aquaria (70x30x30 cu cm) containing 30L of pond water (pH 7.4 to 7.6, temperature 22-24^{o}C and DO content

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8-10 ppm. The working concentrations were selected on the basis of a prior dose-response study and the determination of tolerance limit and 28-d LC_{50} (14.5 mg/L) of the salt. The exposure period was 28 days. The concentrations of vanadium were maintained by daily renewal with fresh water and salt in the experimental sets and without it in the control. Fish were removed for enzyme assay every 7 days during the exposure period.

Liver was excised and homogenized with 4 volumes of isotonic (1.15%) KCl solution. The homogenate was centrifuged at 12,000xg for 25 min in a refrigerated centrifuge and the precipitate was discarded. The microsomal fraction was sedimented by centrifugation at 78,000xg for 90 min. The firmly packed microsomal pellet was resuspended in isotonic KCl solution and again centrifuged as above. The washed microsomes were finally suspended in isotonic KCl and cytochrome P-450 content was assessed according to Omura and Sato (1964) on an Aminco DW-2a uv/vis spectrophotometer.

For the assay of UDP-glucuronyl transferase and glutathione transferase, livers were removed and washed with ice-cooled 0.1M phosphate buffer (pH 7.4). Microsomes were prepared according to Förlin and Haux (1985) and the microsomal pellets were suspended in 0.1M phosphate buffer (pH 7.4) containing 0.15M KCl and 20% glycerol and then stored at -80°C.

Glutathione-S-transferase (GST) activity was measured according to Habig et al. (1974) using 1-chloro-2,4-dinitrobenzene as substrate. The assay prepared in the cuvette was carried out in a 2.5 ml mixture of 0.1M phosphate buffer (pH 7.4) and 20 mM 1-chloro-2,4-dinitrobenzene. The reaction was initiated by addition of 5 µl 10,000xg supernatant (75 mg protein). The increase in absorbance at 344 nm was recorded at 20°C for 1 min.

UDP-glucuronyl transferase (UDPGT) activity towards p-nitrophenol was determined as described by Andersson et al.(1985). The reaction mixture consisted of 0.1 µM of p-nitrophenol, 0.1 µM of UDP glucuronic acid, 0.2 ml of 0.5M phosphate buffer at pH 7.4 and 0.2 ml of microsomal enzyme, in a total volume of 2 ml. Protein was estimated according to Lowry et al. (1951).

Student's t-test was used for statistical analyses.

RESULTS AND DISCUSSION

Cytochrome P-450 serves as the terminal electron acceptor and substrate-binding site of the mixed-function oxidase system and is probably the most versatile and unique biological catalyst known. Although this enzyme system was formerly believed to function primarily in detoxification, it is now known to metabolically activate many protoxins and procarcinogens to reactive metabolites that initiate toxic and carcinogenic events (Conney 1982).

Our results show that unlike many heavy metals (Dierickx 1982), vanadium caused a steady increase in the cytochrome P-450 level with time (Table 1). A similar increase in levels of P-450 was

also observed in goldfish (Gooch and Matsumara 1983) and rainbow trout (Zhang et al. 1990) treated with β-naphthoflavone and codfish, haddock and whiting exposed to environmental contaminants around oil rigs (Payne et al.1987). It has been established that fish are capable of increasing microsomal monoxygenase activities as a result of exposure to some of the xenobiotics like pesticides and other environmental pollutants that cause induction to mammals (Vodicnik et al.1981). This increase in cytochrome P-450 level may be of considerable significance because several inducers of liver microsomal enzymes are promoters of liver tumors in different fish species as well as in humans (Payne et al.1987). The mechanism by which vanadate increases cytochrome P-450 level is not clear. It is possible that vanadate induces cytochrome P-450 level by regulating the transcriptional activation of the cytochrome P-450 gene (Hardwick et al. 1987).

A similar trend was observed with GST activity at all doses, although the increase in activity was only moderate (Table 2). The glutathione-S-transferases are enzymes involved in the detoxification of a wide variety of electrophilic chemicals of both exogenous and endogenous origin via conjugation with glutathione (GSH). However, recent studies have shown that glutathione-S-conjugate formation may also serve as a bioactivation mechanism producing either cytotoxic or carcinogenic metabolites that may be involved in cell damage and death in rat and bovine renal tissues (Dekant et al. 1987). Thus, enhanced activation of GST in our experiment might have modulated the GSH conjugate-dependent toxicity, thereby leading to sufficient toxic stress in the organism.

In contrast to cytochrome P-450 and GST,UDPGT activity decreased at all the tested doses with time (Table 3). UDPGT is a tightly-bound microsomal enzyme and is sensitive to changes in protein-lipid interaction because it is an oligomer whose activity is dependent on the proper association of monomeric polypeptides (Castuma and Brenner 1989). Since stimulation of lipid peroxidation has already been reported to be induced by vanadium (Haider and Elfakhri 1991) and noted in our personal observations, it is likely that alteration in the membrane environment is affected, resulting in the disaggregation of UDPGT and a reduction in catalytic activity.

Glucuronic acid conjugation, catalyzed by UDPGT, is quantitatively the most important Phase II reaction. Phase II reactions are catalyzed by the conjugation enzymes joining the Phase I metabolites (often more toxic), to an endogenous conjugating agent forming more water soluble derivatives that are eliminated from the body (Merill and Bray 1982). Hence, decrease in UDPGT activity could result in enhanced toxicity of environmental chemicals, pharmaceutical agents or endogenous compounds (Neuman and Zannoni 1990).

In view of the above, with a significant decrease in UDPGT activity, along with an increase in cytochrome P-450 level and GST activity at the higher doses (5 mg/L) and 10 mg/L) of vanadate, it is quite possible that some forms of neoplasms may be induced by vanadate, a chemical pollutant.

Table 1. Hepatic cytochrome P-450 levels in Clarias batrachus after 7,14,21 and 28 days exposure to ammonium monovanadate (Mean ± SE of 5 replicates)

Control	Time (days)	2 mgL^{-1}	% increase	5 mgL^{-1}	% increase	10 mgL^{-1}	% increase
0.2 ± 0.003	7	0.216 ± 0.001[a]	8	0.220 ± 0.007[b]	10	0.224 ± 0.002[a]	12
	14	0.224 ± 0.001[a]	22	0.250 ± 0.001[a]	25	0.256 ± 0.002[a]	28
	21	0.300 ± 0.007[a]	50	0.326 ± 0.003[a]	63	0.340 ± 0.002[a]	70
	28	0.306 ± 0.006[a]	53	0.349 ± 0.001[a]	74.8	0.350 ± 0.003[a]	75

a Statistically different from controls ($p<0.01$)

b Statistically different from controls ($p<0.05$)

Table 2. Hepatic glutathione-S-transferase activity in Clarias batrachus after 7,14,21 and 28 days exposure to different concentrations of ammonium monovanadate (Mean ± SE of 5 replicates).

Control	Time (days)	2 mgL^{-1}	% increase	5 mgL^{-1}	% increase	10 mgL^{-1}	% increase
0.24 ± 0.004	7	0.252 ± 0.002[b]	5	0.252 ± 0.003[b]	5	0.254 ± 0.002[b]	6
	14	0.259 ± 0.001[a]	8	0.266 ± 0.001[a]	11	0.268 ± 0.003[a]	12
	21	0.290 ± 0.001[a]	21	0.307 ± 0.003[a]	28	0.312 ± 0.004[a]	30
	28	0.298 ± 0.002[a]	24.5	0.321 ± 0.002[a]	33.7	0.324 ± 0.002[a]	35

a Statistically different from controls ($p<0.01$)

b Statistically different from controls ($p<0.05$)

Table 3. Hepatic UDP-glucuronyl transferase activity in Clarias batrachus after 7,14,21 and 28 days exposure to different concentrations of ammonium monovanadate (Mean ± SE of 5 replicates).

Control	Time (days)	2 mgL^{-1}	% decrease	5 mgL^{-1}	% decrease	10 mgL^{-1}	% decrease
0.152 ± 0.002	7	0.147 ± 0.001[b]	3	0.139 ± 0.002[a]	8	0.136 ± 0.003[a]	10
	14	0.143 ± 0.002[a]	6	0.129 ± 0.001[a]	15	0.124 ± 0.004[a]	18
	21	0.139 ± 0.002[a]	8	0.114 ± 0.002[a]	25	0.109 ± 0.002[a]	28
	28	0.137 ± 0.001[a]	10	0.109 ± 0.002[a]	28	0.106 ± 0.003[a]	30

a Statistically different from controls ($p<0.01$)

b Statistically insignificant difference from control ($p<0.05$)

Fish live in an environment where they are exposed to various amounts of toxic chemicals that are metabolized by the various drug-metabolizing enzymes. In this context, the induction or inhibition of drug-metabolizing enzymes acquires significance from a toxicological standpoint. However, the answer to whether the induction or inhibition of these drug metabolizing enzymes is good, bad or unimportant depends on the chemical environment of the fish.

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