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Comparison of Na^+K^+-ATPase Activities and Malondialdehyde Contents in Liver Tissue for Three Fish Species Exposed to Azinphosmethyl

E. Ozcan Oruc,[1] N. Uner,[1] L. Tamer[2]

[1] Department of Biology, Faculty of Arts and Science, Cukurova University, Balcalı, Adana, 01330 Turkey
[2] Department of Biochemistry, Medical Faculty, Mersin University, Mersin, Turkey

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Adenosine triphosphatase (ATPase) is a membrane bound enzyme and responsible for the transport of ions through the membrane and thus regulates, among others, cellular volume, osmotic pressure and membrane permeability (Sancho et al. 1997). Detection of ATPase inhibition could prove to be an important index for tolerable levels of a large group of environmental contaminants, and measurable levels of inhibition should reflect physiological impairment of functions which require these enzyme systems. However, there is no clear tendency on the behaviour of liver Na^+K^+-ATPase in the literature as its activity showed considerable variations among different fish species, pesticides, and exposure conditions. Pesticides possess a neurotoxic action by modifying ionic selectivity of lipid bilayer membranes and inhibiting enzymes (Smetjtek and Paulis-Illangasekare 1979). In vitro and in vivo studies on ATPase activity have shown the inhibition of Na^+K^+-ATPase in some tissues of the teleost fish *Channa gachua* (Dalela et al. 1978) and *Cyprinus carpio* under fenvalerate toxicity (Reddy and Harold-Philip 1991). The wide applicability of organophosphorus pesticides for plant protection causes their entry into the aquatic environment, affecting fish in particular. Earlier studies on these effects were restricted to anticholinesterase activity of organophosphates and little attention was paid to the effect on the ATPase activity. Furthermore, evidence for tissue injury induced by certain pesticides has been shown to be associated with increased fragility of various biological membranes and the structural lipids of biomembranes that have undergone peroxidation decomposition (Chvapil 1972).

Since understanding sublethal effects is necessary to evaluate metabolic changes of azinphosmehtyl toxic stress, the present investigation was undertaken to study the dose- and duration-dependent effects of azinphosmethyl to inhibit Na^+K^+-ATPase activity and also the level of malondialdehyde (MDA), one of the most frequently used indicators of lipid peroxidation (LPO) in the liver of three freshwater fish, *Tilapia zillii, Cyprinus carpio*, *Oreochromis niloticus*.

MATERIALS AND METHODS

The animals were taken from the culture pools and transferred to the laboratory and maintained under standard conditions of 12 hrs light/dark cycle, at 20±2 °C.

Correspondence to: E. Ozcan Oruc

Throughout the experiments, tap water with a pH value of 7.60, an alkalinity of 326 ppm $CaCO_3$, and an oxygen concentration of 7.02 mg/L was used. The fish were acclimatised in these conditions at least for two weeks and were fed with a commercial fish food. The fish were randomly divided into three groups of 8 fish. Two groups were treated with 0,03 ppm and 0,05 ppm doses in which no mortality was observed during preliminary experiments of azinphosmethyl {S-(3,4-dihydro-4-oxobenzo [d] [1,2,3] triazin-3-ylmethyl) O-dimethyl phosphorodithioate, Bayer, Guthion 20 EC, 230 g/L} dissolved in water. The third group were kept in tap water serving as a control. The water was changed daily to reduce the build up of metabolic wastes and to keep concentrations of azinphosmethyl at the nominal level. After 24, 48, 72 and 96 hours of exposure the liver tissues of both control and treated fish were dissected and washed with physiological saline solution (0.9% NaCl).

The activity of ATPase was measured by determination of the inorganic phosphate (P_i) liberated from the hydrolysis of the substrate adenosin triphosphate (ATP) at 37 °C. For the measurement of ATPase activity, liver tissues were homogenised for 90 seconds in 0.3 M sucrose buffer (pH 7.4) containing 1 mM magnesium using a teflon pestle (Heidolph S0110R2R0) at 1000 rpm. Homogenates were centrifuged (Eppendorph Centrifuge 5403) at 1000 rpm to remove the debris for 15 minutes. ATPase activity was carried out immediately with supernatant on the resulting supernatants by measuring rate of liberation of inorganic phosphate from disodium ATP (Reading and İsbir 1979). Incubation media were made up as described previously (Ames and Dubin 1956). Final assay concentrations of chemicals used here (Analar grade or Sigma) were (in mmol/L) tris-HCl (pH 7.4) 135, NaCl 100, KCl 10, $MgCl_2$ 6, ATP (Vanadium free) 3, EDTA 0.1 and ouabain 3.

After pre-incubation of the medium for 5 minutes at 37 °C, reaction was started by adding the samples and ATP appropriately. The reaction was continued for 30 minutes. It was stoped after putting the samples on ice and addition of a lubrol-molybdate mixture (1:1). Samples were then vortexed and kept at room temperature for 10 minutes. Inorganic phosphate was determined at 340 nm (Shimadzu-UV 260) using 1 mL aliquots of the incubated mixtures. All assays were carried out in triplicate and run with enzyme and reaction blank. ATPase activity was normalised by protein concentration in crude homogenate and expressed as μmol Pi/mg protein/hr.

LPO was estimated by thiobarbituric acid reaction with MDA, a product form due to peroxidation of lipids, according to the method of Ohkawa et al. (1979). The protein content in the sample was determined according to the method by Lowry et al. (1951).

Data analyses were carried out using the SPSS statistical package. Data are expressed as arithmethic means ± standart deviation. Oneway analysis of variance (ANOVA) was used to determine the treatment toxic effects and Duncan's Significant Difference Test was used for mean separation (Duncan 1955).

The values were considered as significant at $p<0,05$ level.

RESULTS AND DISCUSSION

The effects of azinphosmethyl on the Na^+K^+-ATPase activity and MDA level in the liver tissues of *T. zillii*, *C. carpio* and *O. niloticus* are shown in Table 1, 2, 3 respectively, together with the statistical comparisons. When compared to control values, azinphosmethyl exposure caused the inhibition of liver Na^+K^+-ATPase activity, whereas increasing MDA level in *T. zillii* in all exposure concentrations and times. Na^+K^+-ATPase activity and MDA level in *C. carpio* did not show any significant difference from that of control animals after pesticide exposure. The activity of Na^+K^+-ATPase in *O. niloticus* did not change following exposure to azinphosmethyl for 24 h and 48 h. It decreased after 72 h and 96 h pesticide exposure. There were no differences among the groups in MDA content.

The results indicate that exposure of azinphosmethyl caused inhibition of liver Na^+K^+-ATPase and increase in MDA level in *T. zillii* and *O. niloticus*. In support of the findings of the present study, it has been reported that Na^+K^+-ATPase activity in liver of *C. gachua* exposed to endosulfan was significantly decreased (Sharma 1988). Exposure to fenitrothion decreased ATPase activity of *Anguilla anguilla* (Sancho et al. 1997). Na^+K^+-ATPase activity was decreased in rats exposed to a new phosphorothionate (RPR-II) (Rahman et al. 2000). LPO is a deteriorative reaction involved in many disease processes and has been implicated in the cell damaged due to many environmental chemicals (Mudd and Freeman 1977). Increased lipid peroxide formation could disturb the anatomical integrity of the biomembrane and diminish its fluidity leading to inhibition of several membrane-bound enzymes including Na^+K^+-ATPase. It is reported that cypermethrin, mexacarbate and phorate impair the stability of the cell membrane by damaging its structural lipid by peroxidation decomposition, which may lead to subsequent cell necrosis and functional derangement (Singh et al. 1993). Shaheen et al. (1996) suggested that free radicals generated by those disorders attack the membrane phospholipids, causing their peroxidation. These peroxidative processes are surely contributory to the inactivation of membrane-bound biomolecules such as enzymes, since phospholipids are important for the optimum activity of many enzymes.

The observations made here clearly indicate that azinphosmethyl is a potent inhibitor of Na^+K^+-ATPase activity. This study also showed that *T. zillii* is the most sensitive species to azinphosmethyl stress because Na^+K^+-ATPase activity was significantly inhibited following exposure to this pesticide. Considering the exposure concentrations of azinphosmethyl in this study, results suggest that the liver Na^+K^+-ATPase activity of *C. carpio* is less sensitive to pesticide than the other fish species. This is probably due to differences between metabolic activities of *C. carpio* and the other fish species as a result of adaptation to different ecological conditions in the nature.

Table 1. Effects of 0.05 ppm and 0.03 ppm azinphosmethyl exposures on the activity of Na^+,K^+-ATPase (μmol Pi/mg protein/hr) and the level of MDA (nmol/mg protein) in the liver of *Tilapia zillii,* for 24, 48, 72, 96 hours.

	Duration							
	24 h		48 h		72 h		96 h	
Concentration	Na^+,K^+-ATPase	MDA	Na^+,K^+-ATPase	MDA	Na^+,K^+-ATPase	MDA	Na^+,K^+-ATPase	MDA
0.05 ppm	8.33±0.15	1.20±0.14	8.00±0.36	2.06±0.14	7.31± 0.18	2.70± 1.12	7.06±041	3.03± 0.17
	ax	ax	ax	ay	ay	ay	ay	ay
0.03 ppm	8.16±0.47	1.20±0.11	7.20± 0.38	2.25± 0.27	7.88± 0.50	2.63± 0.22	6.85±0.47	3.00± 0.32
	ax	ax	ax	ay	ay	az	az	at
Control	13.28±1.65	0.165±0.15	12.96±1.12	0.165±0.13	14.58± 0.90	0.173± 0.10	3.08±0.78	0.63±0.17
	bx	bx	bx	bx	bx	bx	bx	bx

Results are given as mean ± standard deviation. Letters a, and b show the differences among exposure concentrarions, letters x, y,z and t show the differences among exposure duration. Data shown with different letters are significantly different at the $p<0.05$ level.

Table 2.Effects of 0.05 ppm and 0.03 ppm azinphosmethyl exposures on the activity of Na^+,K^+-ATPase (μmol Pi/mg protein/hr) and the level of MDA (nmol/mg protein) in the liver of *Cyprinus carpio,* for 24, 48, 72, 96 hours.

	Duration							
	24 h		48 h		72 h		96 h	
Concentration	Na^+,K^+-ATPase	MDA	Na^+,K^+-ATPase	MDA	Na^+,K^+-ATPase	MDA	Na^+,K^+-ATPase	MDA
0.05 ppm	7.00±0.22	0.90±0.05	7.60±0.22	1.04±0.07	7.45±0.21	1.20±0.03	7.08±0.25	1.46± 0.18
0.03 ppm	7.68±0.34	0.91±0.03	7.35±0.34	1.09± 0.07	7.03±0.47	1.18± 0.02	7.10±0.35	1.48± 0.17
Control	7.73±0.32	0.93±0.04	7.18±0.33	1.14±0.02	6.63±0.62	1.43± 0.10	7.05±0.42	1.51±0.08

Results are given as mean ± standard deviation. No statistically significant differences were found among exposure groups in all exposure time.

Table 3. Effects of 0.05 ppm and 0.03 ppm azinphosmethyl exposures on the activity of Na^+,K^+-ATPase (μmol Pi/mg protein/hr) and the level of MDA (nmol/mg protein) in the liver of *Oreochromis niloticus,* for 24, 48, 72, 96 hours.

	Duration							
	24 h		48 h		72 h		96 h	
Concentration	Na^+,K^+-ATPase	MDA	Na^+,K^+-ATPase	MDA	Na^+,K^+-ATPase	MDA	Na^+,K^+-ATPase	MDA
0.05 ppm	8.13±0.63	1.13±0.02	8.60±0.36	1.13±0.04	6.83± 0.24	1.17± 0.17	6.42±0.33	1.12± 0.0 7
	ax	ax	ax	ax	ay	ax	ay	ax
0.03 ppm	8.55±0.42	1.18±0.04	8.55± 0.42	1.16± 0.04	6.75± 0.21	1.15± 0.08	6.88±0.30	1.18± 0.10
	ax	ax	ax	ax	ay	ax	ay	ax
Control	8.30±0.68	1.08±0.17	8.25±0.65	1.14±0.11	7.98± 0.17	1.15± 0.09	8.73±0.53	1.17±0.09
	ax	abx	ax	abx	bx	ax	bx	ax

Results are given as mean ± standard deviation. Letters a, and b show the differences among exposure concentrarions, letters x and y show the differences among exposure duration. Data shown with different letters are significantly different at the $p<0.05$ level.

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