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Effect of Cypermethrin, Mexacarbate, and Phorate on Phospholipid and Lipid Peroxidation in the Snail *Lymnaea acuminata*

Ajay Singh, D. K. Singh, and R. A. Agarwal

Department of Zoology, University of Gorakhpur-273 009 (U.P.), India

Cypermethrin (pyrethroid), mexacarbate (carbamate) and phorate (organophosphate) are potent molluscicides (Godan 1983; Singh and Agarwal 1983a,b, 1986, 1991; Agarwal and Singh 1988). It is known that the primary target of these pesticides is the nervous system (Singh and Agarwal 1983a,b). It was observed that these synthetic pesticides affect acetycholine, dopamine and norepinephrine levels in different tissues of exposed animals (Spehar et al. 1980; Singh and Agarwal 1983a). 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 undergo peroxidation decomposition (Chvapil et al. 1972). Tissue with low mitotic rates such as the brain, which contains many or much lipid peroxide is most susceptible to lipid peroxidation (Rehman 1984). In the present study the effect of cypermethrin, mexacarbate and phorate on the rate of lipid peroxidation and levels of phospholipids was observed in the nervous tissue of the snanil Lymnaea acuminata. This snail is the vector of the liver flukes Fasciola hepatica and Fasciola gigantica which cause endemic fascioliasis of cattle in northern India (Singh and Agarwal 1981).

MATERIALS AND METHODS

Snails were maintained and treated with cypermethrin [(S,R)-Cyano-3-phenoxybenzyl (1R, IS, cis-trans)-2, 2-dimethyl-3-(2,2-dichlorovinyl) cyclopropane carboxylate], mexacarbate [4-dimethylamino)3-5-dimethylphenyl methyl-carbamate] and phorate [O,O-diethyl S-[(ethylthio)methyl] phosporodithioate] according to the method of Singh and Agarwal (1986). Adult Lymnaea acuminata (2.0±0.3 cm in length) were kept in glass aquaria containing 3 L dechlorinated tap water. Each aquarium contained 20 experimental animals. Snails were exposed to 40% (0.14 mg/l, 0.68 mg/l and 6 mg/l) and 80% (0.28 mg/l, 1.36 mg/l and 12 mg/l) of LC50 doses of cypermethrin, mexacarbate and phorate respectively for 96h. These doses were based on 96h LC50 values reported by Singh and Agarwal (1983a,

Send reprint requests to D.K. Singh at the above address.

1986). After 96h, snails were removed from the aquaria and rinsed with water. Rate of lipid peroxidation (Utely et al. 1967) and the level of phospholipid (Matrinetti 1962) were determined in nervous tissue.

For the estimation of the lipid peroxidation, nervous tissues were homogenized in chilled 0.15 M KCl using a teflon pestle to give a 10% w/v homogenate. To each sample, 1 ml of homogenate was incubated at 37°C for 2h, and 1 ml. of 10% w/v trichloroacetic acid was added. After thorough mixing, the reaction mixture was centrifuged at 2000 rev./min for 10 min. 1 ml of supernatant liquid was then taken with an equal volume of 0.67% w/v 2-thiobarbituric acid (Sigma Chemical Company St. Louis, M.O., U.S.A.) and kept in a boiling water bath for 10 min, cooled and diluted with 1 ml of distilled water. The absorbance was read at 535 nm and results were expressed as μ mol of malonaldialdehyde formed per 30 min. The extinction coefficient was 1.56×10^5 as described by Utely et al (1967).

Phospholipids were extracted in a chloroform-menthanol 2:1 v/v mixture and phosphate contents were measured according to method of Marinetti (1962).

Chemicals used in this study were of analytical grade (M/S Bharat Pulversing Mills Ltd. India, All India Medical Corp., Dow Chemical, N.Y. and British Drug House, India). Data were subjected to statistical analysis using student's 't'-test. Significant differences were evaluated considering P at least < 0.05.

RESULTS AND DISCUSSION

In vivo 96h exposure of 40% and 80% of LC50 of cypermethrin, mexacarbate and phorate caused significant change in the rate of lipid peroxidation and the level of phospholipid in the nervous tissue of Lymnaea acuminata (Table-1). Significant enhancement in the rate of lipid peroxidation, 125%, 118% and 115% of controls was observed at 40% LC50 doses of cypermethrin, mexacarbate and phorate, respectively. Treatment of 80% of LC50 of cypermethrin, mexacarbate and phorate increases the rate of lipid peroxidation, up to 195%, 190% and 134% of the controls, respectively (Table-1). On the other hand, the phospholipid contents were significantly decreased in the nervous tissue of Lymnaea acuminata. The 80% LC50 of cypermethrin, mexacarbate and phorate decreased the phospholipid level up to 46%, 52% and 55% of controls, respectively (Table-1).

It is evident from the results that the synthetic pesticides (cypermethrin, mexacarbate, phorate), which are potent molluscicides (Singh and Agarwal 1983b; 1986, 1991) led to significant degradation of phospholipid levels and increases in lipid peroxidation in nervous tissue of snail Lymnaea acuminata. Lipid peroxidation is a deteriorative reaction involved in many disease processes and has been implicated in the cell damage due to many environmental chemicals and metals (Mudd and Freeman

Table 1. In vivo effect of 40% and 80% LC50 of cypermethrin, mexacarbate and phorate on rate of lipid peroxidation and level of phospholipid in nervous tissues of Lymnaea acuminata after 96h treatment.

	Control	Cypermethrin		Mexacarbate		Phorate	
		40% LC50	80% LC50	40% LC50	80% LC50	40% LC50	80% LC50
Lipid Peroxidation (μ mol malonaldialdihyde formed/30 min.)	3.7±0.4 (100)	4.6±0.3* (125)	7.0±0.6* (195)	4.3±0.4* (118)	6.7±0.2* (190)	4.2±0.4* (115)	5.0±0.3* (134)
Phospholipid (mg/g fresh tissue)	27.8±0.7 (100)	16.9±1.0* (60)	13.0±0.1* (46)	16.1±0.1* (58)	14.4±0.1* (52)	21.0±0.9* (75)	15.3±0.1* (55)

Values are means ± SE of six replicates.
Values in parenthesis indicate percentage with control values taken as 100%.
*, ($p<0.05$), Student's t-test shows significant difference between control and treated groups.

1977; Rehman 1984; Hussain et al 1985). It appears that these pesticides (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. Peroxidation degradation of phospholipids would lead to alteration in the configuration and function of membranes and may thus alter their permeability characteristics. These observations suggest that these pesticides cause degradation of phospholipids and perhaps agument lipid peroxidation in nervous tissue of snail.

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