The effect of the insecticide dichlorvos on esterase activity extracted from the psocids, *Liposcelis bostrychophila* and *L. entomophila*

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**Abstract**

The inhibition kinetics of dichlorvos on carboxylesterase and acetylcholinesterase (AChE) activity extracted from *Liposcelis bostrychophila* and *L. entomophila* (Psocoptera: Liposcelididae) were compared. The results showed that *L. entomophila* had significantly greater specific activity of carboxylesterase than *L. bostrychophila* (0.045 versus 0.012 µmoles/mg/min). Moreover, the carboxylesterase of *L. entomophila* showed higher affinity (i.e. lower Km value) to the substrate 1-naphthyl acetate than *L. bostrychophila* (0.29 versus 0.67 mM). The specific activity and affinity of AChE of the two species were not significantly different. The carboxylesterase of *L. bostrychophila* was more sensitive to the insecticide dichlorvos than that of *L. entomophila*. The I50s values of dichlorvos to carboxylesterase for *L. bostrychophila* and *L. entomophila* were 1.43 and 3.28 µM, respectively, and to AChE were 324 and 612 nM, respectively. Inhibition kinetics revealed that AChE from *L. bostrychophila* was 5.8-fold more sensitive to inhibition than AChE from *L. entomophila*.

**Keywords:** psocid; susceptibility; carboxylesterase, AChE, DDVP

**Abbreviation:**
- 1-NA 1-naphthyl acetate
- AChE Acetylcholinesterase
- ATChI Acetylthiocholine iodide
- E3 carboxylesterase isozyme (E.C.3.1.1)

**Introduction**

The psocids, *Liposcelis bostrychophila* Badonnel and *L. entomophila* (Enderlein) are worldwide and commonly found in various processed and unprocessed dry foods in households, granaries, and warehouses (Turner, 1994). Outbreaks of *L. bostrychophila* and *L. entomophila* have been reported in humid tropical countries such as Indonesia, Malaysia, Singapore, The Philippines, Thailand, The People’s Republic of China and India (Wang et al., 1999). Information on the management of psocid pests, however, is very limited (Rees, 1994). Routine fumigations of warehouses and storage facilities with methyl bromide have failed to control these pests (Ho and Winks, 1995). In addition, the rapid development of resistance to chemical and physical treatments by the psocids has also been reported (Santoso et al., 1996; Wang and Zhao, 2003).

Metabolic resistance to organophosphorus insecticides has been associated with changes in the activity of carboxylesterases in many insect species (Devonshire and Field 1991). In two well-studied cases in which resistance to organophosphorus insecticides is associated with an increase in carboxylesterase activity, sequestration and slow turnover of the phosphate by an over-expressed esterase are responsible for resistance (Devonshire 1977; Karunarathne et al., 1993; Ketterman et al., 1993; Jayawardena et al., 1994). On the other hand, in some insects, resistance to organophosphorus insecticides is associated with the decrease in carboxylesterase activity, such as in the flies, *Musca domestica*, *Lucilia cuprina*, and *Drosophila melanogaster*, where strains resistant to organophosphorus insecticides have high ali-eaterase, low organophosphorus hydrolase, and intermediate malathion carboxylesterase (MCE) activities (Campbell et al., 1997).

Acetylcholinesterase (AChE) is a key enzyme that terminates nerve impulses by catalyzing the hydrolysis of the neurotransmitter acetylcholine in the nervous system. Organophosphorous insecticides, such as diazinon, target AChE and irreversibly inhibit the enzyme by phosphorylating a serine hydroxyl group within the enzyme active site. In China, dichlorvos is commonly used to control the insect pests not only in the field but also in stored products. As in the case of other organophosphate insecticides, dichlorvos exerts its effects by inhibiting esterases, especially AChE. In addition, the production of different forms of carboxylesterases is also reported to be the cause of dichlorvos resistance in several insect species.
As little is known about the mechanisms of insecticide resistance in psocids insects, information on the pesticide biochemistry of Liposcelis esterases will certainly prove valuable in formulating strategies in the control of these rapidly proliferating pests (Leong and Ho, 1995). This study was initiated to understand the kinetics of carboxylesterase and AChE inhibition by dichlorvos of two Liposcelis species. This is an initial step in elucidating the molecular basis of resistance to organophosphorus insecticides in these species.

Materials and methods

Insects

Stock colonies of L. bostrychophila and L. entomophila were started with nymphs collected from a wheat warehouse in Chongqing, the People’s Republic of China in 1990. The colonies were maintained on an artificial diet consisting of whole wheat flour, skim milk and yeast powder (10:1:1) in a room maintained at 28±1 °C and a scotoperiod of 24 h. Cultures were set up in glass bottles (250 ml) with a nylon screen cover and kept in desiccators (5 liter), in which the humidity was controlled with saturated NaCl solution at 75-80%. After several generations, insects from the stock colonies were used for the tests. All experiments were conducted under the conditions described above with 2- to 5-day old female adults.

Chemicals and insecticide

Acetylthiocholine iodide (ATChI, Sigma, www.sigmaaldrich.com), 5,5’-dithiobis-2-nitrobenzoic acid (DTNB, Sigma, www.sigmaaldrich.com), eserine (Sigma), and 1-naphthyl acetate (1-NA) and other biochemical reagents were of reagent grade or better. The insecticide used was 80% dichlorvos (Shalungda Ltd., Changsha, China).

Bioassay

The efficacy of dichlorvos against the two different liposcelids was determined using the small glass tubes (~6mm x 40mm). Various concentrations of dichlorvos were tested until a satisfactory range (10% - 90% mortality) was ascertained. Six concentrations were used in the final analysis. All the concentrations were diluted with acetone. 30µl of insecticide was pipetted onto the inside of the tubes homogeneously and allowed to dry for 30 min before exposing the insects to it.

Each dichlorvos bioassay consisted of 100 adults per concentration and six concentrations (0.36-367 µg/m 2). Control groups received acetone alone. Mortality was assessed after 24 h. Psocids that did not move after stimulation from a camel’s hair brush were scored as dead. All tests were run at 25 °C and replicated at least three times on three different days. Mortality data were corrected with Abbott’s (1925) formula and analyzed by probit analysis (Raymond, 1985) to determine the lethal concentrations (LC50).

Enzyme preparation

For carboxylesterase, fifty female adults were ground in 3 ml of ice-cold 0.1 M, pH 8.0 phosphate buffer containing 0.1% Triton X-100. The crude homogenates were centrifuged at 20,000g at 4 °C for 60 min. The resulting supernatants were used as the enzyme sources.

Carboxylesterase assay

Van Asperen’s (1962) method was adapted for the determination of esterase activity. The general buffer was 0.04 M, pH 7.0 phosphate buffer. 1-NA (3 x 10^-3M) was used as substrate. In determining the Michaelis constants for 1-NA, the substrate concentrations of 1.5, 3, 6, 15, 30 and 60 mM were made up in phosphate buffer (0.04 M, pH 7.0 ). The mixtures were incubated at 37 °C for 30 min in a water-bath. The reaction was terminated by adding 1 ml of Fast Blue B salt- sodium dodecylsulphate solution. Absorbance was read in the spectrophotometer after 30 min at 600 nm. The kinetic parameters (Km and Vmax) were determined graphically by Lineweaver-Burk plots (Wilkinson, 1961).

The in vitro inhibition of carboxylesterase activity was ascertained using 3 x 10^-4 M 1-NA as substrate. Concentrations of dichlorvos ranging from 1 x 10^-9 M to 1 x 10^-3 M in phosphate buffer were tested. Inhibitor (0.1 ml) was added to 5 ml of substrate and the reaction initiated by adding 0.1 ml of enzyme. The relative potency of the inhibitors was investigated by examining their I50 values.

Acetylcholinesterase assay

AChE activity and its inhibition by dichlorvos were determined according to the method of Ellman et al. (1961) using ATChI as substrate. Briefly, the reaction mixture consisting of ATChI (1.5 mM), DTNB (1 mM) and enzyme preparation (200 µl) was prepared in a final volume of 2.4 ml with phosphate buffer (0.1 M, pH 8.0). The inhibition of dichlorvos was determined by adding various concentrations (from 1 x 10^-9 M to 1 x 10^-3 M in phosphate buffer) inhibitor (0.1 ml) to the substrate. Absorbance was recorded at 412 nm after 30 min water-bath.

Values of Km and Vmax of AChE were determined at 30 °C, with 5 ATChI concentrations (ranging from 15 µM to 6.0 mM). The changes of absorbance were observed at 412 nm for 5 min.

The bimolecular rate constants (ki = kp/Ka), phosphorylation constant kp and affinity constant Ka, with dichlorvos as inhibitor were determined by pre-incubation of the supernatants with varying inhibitor concentrations. Progressive inhibition of AChE activity over time was continuously recorded for 5 min. The activity of AChE at each 30 second interval was measured for fitting the inhibition curve. The ki value was calculated according to the method of Main (1964). The ki was determined from the gradient of the linear regression:

\[
\frac{1}{i} = \frac{\Delta t}{2.303a\Delta \log v} - \frac{1}{Ka}
\]

where i is the initial concentration of inhibitor. Values of (Δt / 2.303Δlog v) were obtained from a plot of log v against t at constant i. The slope is ki, the intercept on the (1/i) axis is (-1/Ka), and the intercept on the (Δt / 2.303Δlog v) axis is (1/kp).

Assays of protein contents

Protein contents of the enzyme homogenate were...
determined according to the method of Bradford (1976) using bovine serum albumin as standard. The measurement was performed with the spectrophotometer at 595 nm.

Results

Bioassays

The exposure concentrations of dichlorvos required obtaining LC50 values for L. bostrychophila and L. entomophila adults are summarized in Tables 1. The data show that L. bostrychophila is more tolerant of dichlorvos than L. entomophila based on LC50 values. However, the difference of tolerance between two species is not significant considering the 95% confidence limit (P > 0.05).

Activities of carboxylesterase

There was a strong linear relationship between homogenate concentrations and carboxylesterase activity for both L. bostrychophila ($R^2 = 0.998$) and L. entomophila ($R^2 = 0.999$). Homogenate concentrations of 5 and 1.67 insects equivalents per assay were in the middle portion of the linear regression and were used throughout this study.

L. bostrychophila and L. entomophila differed significantly in the amount of protein per individual. It averaged 37.4 and 66.1 µg/insect for L. bostrychophila and L. entomophila, respectively (Table 2).

Carboxylesterase from L. entomophila showed a significantly higher affinity (i.e. lower $K_m$ value) to the substrate 1-NA than that of L. bostrychophila (P < 0.05). In contrast, the catalytic activity of 1-NA towards carboxylesterase in L. entomophila was higher (i.e. higher $V_{max}$ value) than that in L. bostrychophila (Table 4). The higher activity of L. entomophila esterase towards 1-NA than that of L. bostrychophila is observed (Table 2).

The relative susceptibility of the esterase from the two liposcelids to dichlorvos is shown in Fig 1A. Based on the $I_{50}$ values (the concentration required to inhibit 50% of esterase activity), L. bostrychophila esterase (1.43 µM) showed higher susceptibility to dichlorvos than L. entomophila (3.28 µM). Inhibition kinetics of carboxylesterase indicated that the inhibition type of dichlorvos were competitive for both L. bostrychophila and L. entomophila.

Activity of AChE

A strong linear relationship between homogenate concentrations and AChE activities for both L. bostrychophila ($R^2 = 0.99$) and L. entomophila ($R^2 = 0.997$) was obtained. Homogenate concentrations of 4.8 and 6.4 insects per assay were in the middle portion of the linear regression and so 5 insects per assay were used throughout this study.

There was no significant difference (P > 0.05) between the affinities ($K_m$) of the AChE from either Liposcelis spp. The catalytic activities ($V_{max}$) of AChE toward ATChI were also similar. This implies that similar degrees of substrate protection was afforded in both species in the inhibition studies.

The activity of AChE per insect in L. entomophila was significantly higher than that of L. bostrychophila, but the specific activity (nanomoles/min) from both species was similar. The protein content differed in both insects (P < 0.05) (Table 3).

The effects of dichlorvos on AChE activity are shown in Fig. 1B. The inhibition of enzyme activity was between 10% and 90%. The efficiencies of the AChE inhibitors were compared based on their $I_{50}$. Liposcelis bostrychophila AChE was more sensitive to the inhibitory action of dichlorvos than that of L. entomophila.

Table 3. AChE activity in Liposcelis bostrychophila and L. entomophila.

| Insects          | Protein (µg/insect) | Nanomoles/min |
|------------------|---------------------|---------------|
| L. bostrychophila| 25.2±0.7a           | 24.5±0.5a     |
| L. entomophila   | 35.9±0.8b           | 25.2±0.7a     |

Within the same row, means followed by the different letters are significantly different (P < 0.05).

Table 1. The toxicity of DDVP to Liposcelis bostrychophila and L. entomophila.

| Insects          | Insects Slope±SE | LC50 (µg/m2) (95%CL) | 2b       |
|------------------|------------------|----------------------|----------|
| L. bostrychophila| 1.1±0.2          | 54.4 (73.1~35.7)     | 2.7      |
| L. entomophila   | 0.9±0.1          | 45.3 (62.0~28.7)     | 2.4      |

Within the same row, means followed by the different letters are significantly different (P < 0.05).

Discussion

In the survey of possible interactions between insecticides and carboxylesterase from Aphis gossypii, Owusu (1996) found significant inhibition of the enzyme by a number of organophosphates...
higher esterase sensitivity to inhibition in \textit{L. bostrychophila} than in \textit{L. entomophila}. The similar result was also obtained in the present study. However, based on the bioassay, \textit{L. bostrychophila} is more tolerant to dichlorvos than \textit{L. entomophila}. This may be due to the sensitive esterase of \textit{L. bostrychophila} that may preferentially bind dichlorvos, thereby protecting AChE, resulting in greater tolerance to dichlorvos.

Acetylcholinesterase is of interest because it is the target site for organophosphate and carbamate insecticides in the central nervous system, and its role in cholinergic synapses is essential for life (Fournier and Mutero, 1994). Based on the values of $I_{50}$s (the concentration required to inhibit 50% of AChE activity) and $k_i$ (bimolecular rate constants for AChE inhibition), \textit{L. bostrychophila}'s AChE was more sensitive to the inhibitory action of dichlorvos than that of \textit{L. entomophila}. In contrast, Leong and Ho (1995) reported that dichlorvos is a more potent inhibitor of AChE in \textit{L. entomophila} than in \textit{L. bostrychophila}. They also found that \textit{L. entomophila} is more tolerant of dichlorvos than \textit{L. bostrychophila} based on bioassay data. The difference might be due to the different geographic population used. Price (1988) pointed out that the sensitivity of AChE does not necessarily mean that insects will be susceptible to the particular chemical when it is used as an insecticide as toxicity of a particular compound depends on many factors including cuticle penetration and metabolic processes (Matsumura, 1985; Hassall, 1990). This suggests that the higher sensitivity of \textit{L. bostrychophila} AChE to inhibition implies that the underlying mechanism of tolerance in insecticide bioassays in the this species is largely due to metabolic activities which either detoxify or limit the intoxicating ability of dichlorvos.

The present study has provided some basic information on the esterases of these two psocids that will be useful to understand the mechanisms of insecticide resistance in the psocids. As strains of lipocelid psocids that exhibit varying tolerance to insecticides become available they will be useful for further comparative toxicology and biochemical studies.

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